THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097

TO: Haiyan Tong
Environmentally Protection Agency
7315

FROM: Biomedical IRB

Authorized signature on behalf of IRB

APPROVAL DATE: 3/21/2007

EXPIRATION DATE OF APPROVAL: 2/25/2008

RE: Notice of IRB Approval by Full Board Review
Submission Type: Initial
Study #: 07-0190
Other #: GCRC - GCRC-2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated.

Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Should any adverse event or unanticipated problem involving risks to subjects or others occur it must be reported immediately to the IRB using the adverse event form at the same web site.

Details:

Call the IRB at 966-3113 if you have any questions. You can now access IRB status information at https://my.research.unc.edu/.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), and 21 CFR 50 & 56 (FDA), where applicable.

The University of North Carolina at Chapel Hill holds a Federal Wide Assurance approved by the Office for Human Research Protections, Department of Health and Human Services (FWA # 4801).

CC:
James Samet, Environmentally Protection Agency, , Faculty Advisor
Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 3/21/07 AND 2/25/08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill
Consent for Screening of GSTM1 Genotype With Identifying Information—
MAIN STUDY

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: 2 March 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Maryann Bassett, RN; Andrew Ghio MD; Debbie Levin, BSN; Tracey Montilla, RN; Michael Schmitt MS
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. The purpose of this

Initial/Date _____

study is to look at the cardioprotective effects of fish oil when people exposed to diesel exhaust. We will have equal numbers of subjects with GSTM1 sufficient gene or GSTM1 null gene enrolled in this study. The purpose of this screening session is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 60 participants in this study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 15 minutes.

What will happen if you participate in this study?
For this portion of the study, you will be required to provide about 50 ml of blood sample.

How will the blood sample be collected?
You will have about 50 ml (about 5 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample collected will be used to look at the GSTM1 gene. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol) and lipid lowering statins. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your genotype of GSTM1 gene and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

Initial/Date _____

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $60 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Initial/Date _____

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance

Initial/Date _____

company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or Dr. Richard Hermann (919-966-6217), EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects
Biomedical Form—MAIN STUDY

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 3/21/07 AND 2/25/08 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: 2 March, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, and chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.

Initial/Date _____

- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Use olive oil for cooking, dressings, and sauces during the study.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 60 people in this research study.

How long will your participation in this study last?

You will have up to 4 visits to the research facility over approximately 5-6 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) two-week of fish oil/olive oil treatments part, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 4 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participated in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Initial/Date _____

Today' visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a 15-day fish oil or olive oil supplementation and the exposure session. You will be given a 15-day supply of either fish oil or olive oil tablets. Please take 2 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next two weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instruction on how to complete the record, including instruction on how o estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this 15-day of supplementation period. We will give you another 15-day supply of either fish oil or olive oil tablets and you will need to restart the pills again.

In the morning of your last pill of supplements, you will come to the HSF for the baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF in the next morning for the exposure and the monitor will be removed.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any

Initial/Date _____

activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat before midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we will perform on your last pill day).
- Have about 80 ml blood drawn (about 8 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- We will bring you back to the HSF where we may also measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT) at HSF and we will compare the findings with ultrasound measurement of the artery in your arm. We will place two small PAT probes to both of your index fingers. The procedure is similar to that of ultrasound measurement and we will only place a blood pressure cuff on one of your arm. We will obtain recordings for 5 minutes at rest, 5 minutes during the blood pressure cuff inflated, and 5 minutes after the blood pressure cuff released.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

Initial/Date _____

During the exposure, you will:

- Have an exposure to diesel exhaust (the concentration will be determined based on the pilot study) for 2 hours in a small exposure chamber. This particle concentration is representative of diesel exhaust particle (DEP) levels which you would inhale if you were occupationally exposed to DEP, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over a period at a busy intersection in a polluted city (such as Los Angeles) where DEP concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will take you back to the GCRC at the NCMH for another ultrasound of the artery in your arm.
- We will obtain another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 cc or about 8 tablespoons).
- Fill out a symptom score questionnaire.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 4 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will take you back to the GCRC at the NCMH for another ultrasound of the artery in your arm.
- You will have another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 cc or about 8 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, ad ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: The concentration of diesel exhaust in this study has been chosen based on a pilot study in susceptible older subjects. Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause

Initial/Date _____

people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from healthy food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Finger Endo-PAT: Similar to the measurement by brachial artery ultrasound, there are no significant risks associated with index fingers arteries measurement.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be

Initial/Date _____

exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the no-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28

Initial/Date _____

U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1087.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total =	$60
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36

Initial/Date _____

Exposure sessions (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Brachial artery ultrasound (2@ $50 each)	$100
Finger Endo-PAT	$20
Time (8h @$12/h)	$96
Lunch	$5
Food record (2 times)	$300
Total for completion of exposure = $753	
18 hours after exposure (4 hour)	
Venipuncture (~80ml)	$30
Time (4 hrs @$12/h)	$48
Brachial artery ultrasound	$50
Finger Endo-PAT	$10
Day 3 total for completion of post-exposure = $138	
Protocol Completion Bonus	$100
Approximate TOTAL for completion of exposure = $1087	

Subjects will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Initial/Date _____

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact Dr. Richard Hermann, the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office, at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ________________

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

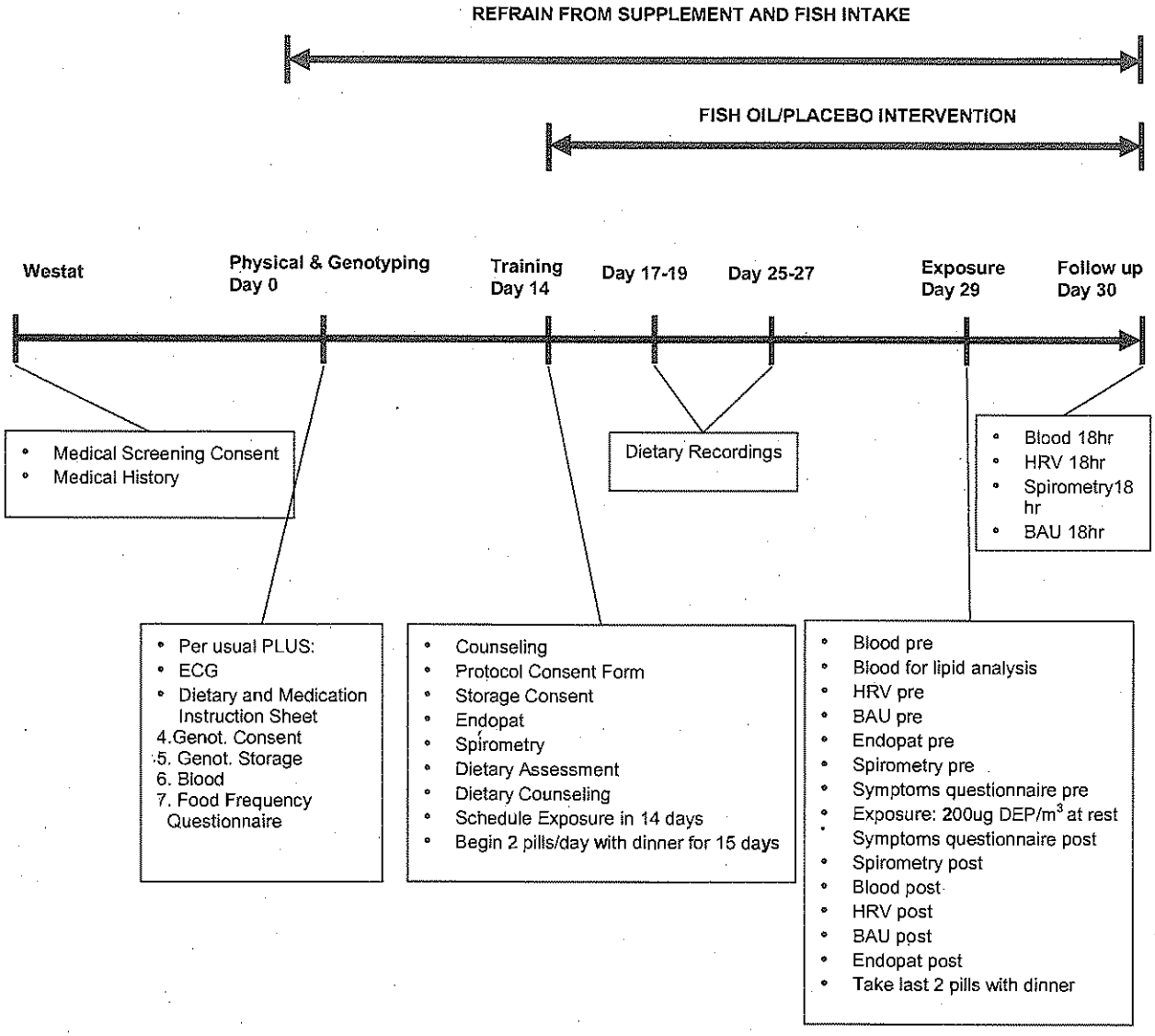
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



MAIN STUDY FLOW DIAGRAM

Initial/Date _____

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects
Biomedical Form—PILOT STUDY

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 3/21/07 AND 2/25/08
APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 2 March, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify a safe concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.

Initial/Date _____

- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 5-6 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 6 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participated in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today' visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate for the study at any time. Our goal of this pilot study is to find a safe concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we

Initial/Date _____

determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 9 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.
- Have about 50 ml blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store**

Initial/Date _____

some of your blood we obtained during the study for yet-to-be-determined tests in the future.

- We might measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.

Initial/Date _____

- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?
You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:

Initial/Date _____

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the no-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal

Initial/Date _____

remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $1461.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
	Pre-study qualification total = $60

Pre-exposure screening/training (3 hours)

Initial/Date _____

Time (3h @$12/h)	$36
Exposure sessions (6 hours)	
Venipuncture (~80ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~80ml, post; 3@$ 30 each)	$90
Finger Endo-PAT (6@$10 each)	$60
Time (3X6h @$12/h)	$216
Lunch (3@$5 each)	$15

Total for completion of exposure = $987

18 hours after exposure (3 hour)	
Venipuncture (~80ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus	$150

Approximate TOTAL for completion of exposure = $1461

Subjects will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

Initial/Date _____

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact Dr. Richard Hermann, the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office, at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ________________

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

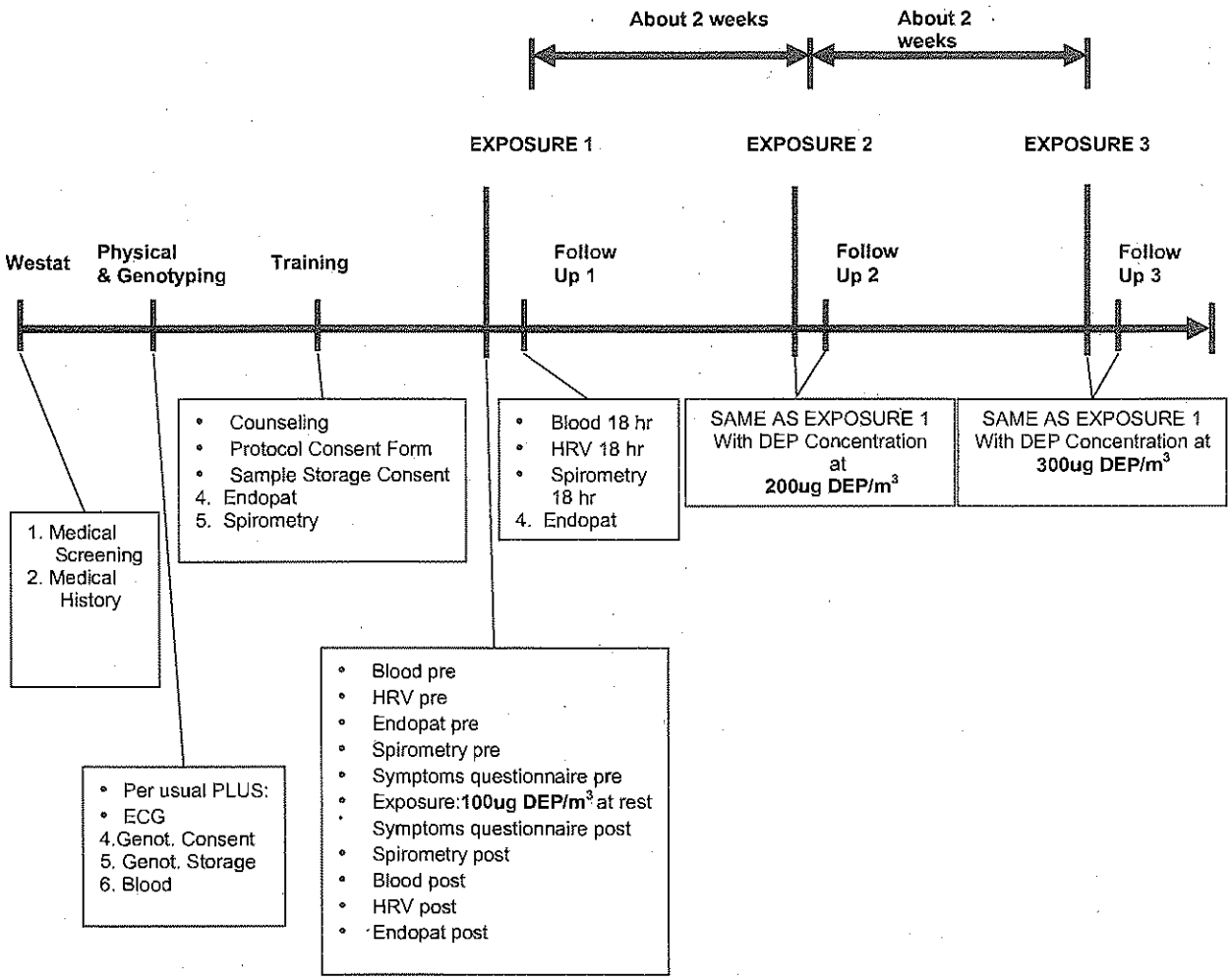
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 3/21/07 AND 2/25/08 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill
Consent for Screening of GSTM1 and GSTP1 Genotypes With Identifying Information--
PILOT STUDY

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: 2 March 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Maryann Bassett, RN; Andrew Ghio MD; Debbie Levin, BSN; Tracey Montilla, RN; Michael Schmitt MS
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

Initial/Date _____

Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 15 minutes.

What will happen if you participate in this study?
For this portion of the study, you will be required to provide about 20 ml of blood sample.

How will the blood sample be collected?
You will have about 20 ml (about 2 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers and lipid lowering statins. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you

Initial/Date _____

will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $60 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has

Initial/Date _____

limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the

Initial/Date _____

risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or Dr. Richard Hermann (919-966-6217), EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 3/21/07 AND 2/25/08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill
Consent for Storing Biological Specimens With Identifying Information

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IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: 2 November 2006

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Maryann Bassett, RN; Andrew Ghio MD; Debbie Levin, BSN; Tracey Montilla, RN; Michael Schmitt MS
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to the effect of air pollutants such as ozone and diesel exhaust. The purpose of this research study is to examine the role of the GSTM1 gene in mediating the cardiovascular

Initial/Date _____

effects following acute diesel exhaust exposure in GSTM1 null and GSTM1 sufficient healthy older adults.

Diesel exhaust exposure has been found to cause a range of lung and systemic changes in normal subjects. We will be collecting blood samples which will help us to further study this condition. In order to do so, we will need to collect and store blood. Blood samples will be collected in order to look for but not limited to indicators of inflammation due to the diesel exhaust exposure. We may further isolate RNA from blood for genetic analysis. RNA may be isolated for genetic analysis to see if there is a difference between pre- and post diesel exhaust exposures.

All samples will be stored where only project members will have access to the samples. There is a need to store samples in such a repository because this will be an ongoing study where samples from subjects will be collected over an extended period of time. Storing of samples allows for all samples to be processed at the same time and also allows our scientist the opportunity to further study these samples with as yet unknown questions and techniques.

How will the specimen be collected?
The description of the samples to be collected and the manner in which this will be done have been described in the main study consent.

What will happen to the specimen?
Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the diesel exhaust exposure. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens (or data) for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens (or data) will be provided without identifiers to these other researchers by employing a data use agreement.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding the cardiovascular effects of diesel exhaust exposure in older population.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:

1. Blood sampling will be performed by medical personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise.

Initial/Date _____

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you for storage of the specimens?
There will be no cost to you for the storage and use of the specimens for research purposes.

Will you receive anything for being in this study?
You will not receive anything for taking part in this study with regard to storage of excess specimens. Reimbursement for participation in the main study is addressed in the detailed consent for that study.

Who owns the specimens?
Any blood, body fluids, or tissue specimens obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Initial/Date _____

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the specimens from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Initial/Date _____

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or Dr. Richard Hermann (919-966-6217), EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

The US Environmental Protection Agency is seeking

ADULT VOLUNTEERS

Ages 50 to 75 for Research

This is a pilot study about genetics and exposure to diesel exhaust.

We are looking for healthy older adults with a very specific genotype to study the effects of diesel exhaust exposure on heart and lung function.

If you qualify for this study, you will complete up to 7 visits over about 5-6 weeks. Total time commitment after screening is about 30 hours. You will receive payment for screening, the study, and out of town travel. Parking is provided.

1-888-279-9353 or
919-966-0604
www.epastudies.org

WESTAT • EPA Recruits • In care of the environment

The Human Studies Facility is located on the UNC-CH campus

APPROVED - IRB, UNC-CH

MAR 21 2007

OMEGA PILOT STUDY
E-mail Announcement

SUBJECT: INFORMATIONAL: Pilot Research study for Healthy Older Adults

The main purpose of this pilot study is to identify a safe concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This pilot research will study the responses to diesel exhaust in healthy older subjects with the specific GSTM1 and GSTP1 genotype. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Committee on the Protection of the Rights of Human Subjects Biomedical Institutional Review Board. IRB # _____(GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

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MAR 21 2007

Web Site Advertising: www.epastudies.org

OMEGA PILOT: Elder Diesel Pilot Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this pilot study is to identify a safe concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of particular genes you carry. The genes, glutathione-S-transferase (GSTM1 and GSTP1) are genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying an inactive form of GSTM1 and a variance of GSTP1 may be more susceptible to the effects of air pollutants. Therefore, in this pilot study, we will study the responses to diesel exhaust in healthy older subjects with very specific genetic characteristics.

If you are eligible for the remainder of the study you will have up to 7 visits over approximately 5 to 6 weeks. Total time commitment after eligibility screening is about 30 hours. Procedures involved include breathing tests, heart rate monitoring, blood tests, and exposures to controlled amounts of diesel exhaust. Even at the highest level of exposure, you will not be exposed to more particles than you might encounter if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day. Compensation is provided.

APPROVED - IRB. UNC-CH
MAR 21 2007

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA PILOT**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA Pilot Study Guidelines
For All Study Visits

Illness. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores.

No active allergies. If you have questions about allergies, please call the EPA medical station at 966-6232.

Medications

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- No caffeine for 12 hours prior to all study visits.

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Food

- Do not eat or drink anything for 2 hours prior to the training day visit.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.

Exercise. Clothing

- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the exposure and follow-up visits.

If you are unable to keep your appointment, please call Recruitment. Please be on time!

Thank You!
Westat EPA Support Services

The US Environmental Protection Agency is seeking

ADULT VOLUNTEERS

Ages 50 to 75 for Research

This is a research study about genetics, diet supplementation and exposure to diesel exhaust.

We are looking for healthy older adults to study diet supplementation and the effects of diesel exhaust exposure on heart and lung function.

Study participants will complete 4 visits over about 5-6 weeks. Total time commitment after screening is about 16 hours. You will receive payment for screening, the study, and out of town travel. Parking is provided.

1-888-279-9353 or
919-966-0604
www.epastudies.org

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The Human Studies Facility is located on the UNC-CH campus

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Web Site Advertising: www.epastudies.org

OMEGA: Elder Diesel Full Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

Participation in this study will include two-weeks of fish oil or olive oil treatments and one exposure to a controlled amount of diesel exhaust, no more than you might encounter if you visited a large city such as Las Angeles, New York, or Mexico City on a smoggy day. Study procedures include breathing tests, heart rate monitoring, blood tests, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a does of nitroglycerine, which is a short-acting vasodilator. Total time commitment is 4 visits after eligibility screening, about 16 hours, over 5-6 weeks. Compensation is provided.

APPROVED - IRB. UNC-CH

MAR 21 2007

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA**

DATE:

TIME:

APPROVED - IRB, UNC-CH
MAR 21 2007

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA Full Study Guidelines
For All Study Visits

Illness and Allergies. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores. If you have questions about allergies, please call the EPA medical station at 966-6232.

Medications

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits. No red wine throughout the study.
- No caffeine for 12 hours prior to all study visits.

Food and Food Supplements

- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for 2 weeks before and during the study.
- No antioxidants (beta-carotene, selenium, vitamin C, vitamin E, zinc) for 2 weeks before and during the study.
- Use olive oil only for cooking, dressings, and sauces during the study.
- Do not eat or drink anything for 2 hours prior to the training day visit.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.

Exercise, Clothing

- No strenuous exercise for 24 hours prior to and after all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the exposure and follow-up visits.

If you are unable to keep your appointment, please call Recruitment. Please be on time!
Thank You!
Westat EPA Support Services

OMEGA FULL STUDY
E-mail Announcement

SUBJECT: INFORMATIONAL: Research study for Healthy Older Adults

The main purpose of this research study is to determine if a component of diesel exhaust elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Volunteers ages 50 to 75 will be tested to determine the type of a particular gene they carry, one of several genes responsible for protecting the body against oxidants such as diesel exhaust. Participation will include two-weeks of fish oil or olive oil treatments and one exposure to a controlled amount of diesel exhaust. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Committee on the Protection of the Rights of Human Subjects Biomedical Institutional Review Board. IRB # _____(GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

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OMEGA INSTRUCTIONS

EXPOSURE DAY

1-PLEASE EAT A LIGHT BREAKFAST (SEE BREAKFAST SELECTION FORM) AND DRINK PLENTY OF FLUIDS.

2-NO STRENUOUS EXERCISE 24 HOURS PRIOR TO EXPOSURE AND 24 HOURS AFTER EXPOSURE.

3- NO INGESTION OF PAN FRIED OR GRILLED MEATS AFTER MIDNIGHT AND PRIOR TO THE STUDY.

4- AVOID DRINKING ALCOHOL 24 HOURS PRIOR TO EXPOSURE

5- AVOID SMOKE AND FUMES FOR AT LEAST 24 HOURS BEFORE STUDY.

6- NO CAFFEINE FOR 12 HOURS PRIOR TO STUDY.

7- IF YOU ARE SICK PLEASE CALL THE MEDICAL STATION AT 966-6232. YOU WILL BE RESCHEDULED.

8-WEAR COMFORTABLE CLOTHES THAT OPEN IN FRONT. YOU MAY WANT TO WEAR SOCKS AND BRING A SWEATER.

9-YOU WILL BE WEARING A HEART MONITOR OVERNIGHT AND WILL NOT BE ABLE TO BATHE.

10- GENTLEMEN- IF YOU HAVE A HAIRY CHEST -YOU WILL HAVE YOUR CHEST SHAVED. YOU CAN SHAVE YOUR OWN CHEST OR IT CAN BE DONE AT THE MEDICAL STATION.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH & ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
Human Studies Division
104 Mason Farm Road MD 58-A
Chapel Hill, NC 27599

OMEGA
Brachial Artery Ultrasound Information Sheet

Instructions for Medication Use and preparation for screening procedure.

The following medications should be withheld 2 **WEEKS** prior to testing:

1- High dose niacin

2- Decongestants i.e. sudaphed and similar type of medications

3- Vitamin C and E

4- Fish Oil

5- Arginine (This is an amino acid found in protein supplements).

6- Ibuprofen (Motrin), naproxsyn (Aleve) or aspirin to treat an ache. However, if you normally take an aspirin daily, please continue to take it. You may use Tylenol for aches or pains.

7- Do not exercise VIGOROUSLY 12 hours prior to your participation in the screening portion.

The following medication should be withheld as indicated:

1- Erectile dysfunction medications, i.e. Viagara, Cialis, Levitra, for 4 days prior to study.

MAR 21 2007

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH & ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
Human Studies Division
104 Mason Farm Road MD 58-A
Chapel Hill, NC 27599

OMEGA STUDY INFORMATION SHEET

1-No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to your participation in the study. Low dose aspirin and Tylenol are permitted. No antioxidants (eg. Beta-carotene, selenium, vitamin C, vitamin E,) for 2 weeks before the study and during the study.

2- Two weeks prior to study, avoid Omega 3 fatty acids or having more than one 4-6 oz.serving of all tupes of fish and shellfish, walnuts, flaxseds and flaxseed oil, rapeseed oil, anola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil and during the study.

3-Please use olive oil for cooking, dressings, and sauces during the study.

4-Avoid red wine during study. No alcohol for 24 hours prior to visit.

5- No caffeine for 12 hours prior to study. We will supply lunch for you.

6- Do not eat pan fried and/or grilled foods after midnight prior to study day.

7- Do not put on lotions, perfumes, or powders on your chest. Deodorant is okay.

8- Do not exercise VIGOROUSLY 24 hours prior to your participation in the study and 24 hours after exposure

9- No illness for 4 weeks prior to your admission into the study.

If you have any questions please call the Medical Station at 966-6232 (we accept collect calls) and we can help settle any questions.

Thank you for your participation.

U:MAB\omegainfosheet..wpd1/07

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Breakfast Choices for OMEGA STUDY

1 lowfat granola bar	#1
1 medium banana	
1 cup fruit yogurt, fat free no sugar added	
1 cup orange juice	
1 cup fat free milk	

1 cup whole grain oat rings cereal Cheerios	#2
1 medium banana	
1 cup fruit yogurt, fat free, no sugar added	
1 cup fat free milk	

3/4 cup frosted shredded wheat	#3
2 slices whole wheat bread	
1 medium banana	
1 cup fat free milk	
1 cup orange juice	

2/3 cup bran cereal	#4
1 slice whole wheat bread	
1 medium banana	
1 cup fruit yogurt, fat free, no sugar added	
1 cup fat free milk	
2 tsp jelly	

3/4 cup whole wheat flakes cereal	#5
1 slice whole wheat bread	
1 medium banana	
1 cup fat free milk	
1 cup orange juice	
1 tsp soft margarine	

½ cup instant oatmeal, flavored	#6
1 mini whole wheat bagel	
1 medium banana	
1 cup fat free milk	
1 tbsp cream cheese, fat free	

3/4 cup cornflakes	#7
½ cup fruit yogurt, fat free, no sugar added	
1 medium apple	
1 cup grape juice	
1 cup fat free milk	

U.S. ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
HUMAN STUDIES DIVISION (MD-58-A)
CHAPEL HILL, NC 27514
(919) 966-6232

POST DIESEL EXPOSURE INFORMATION

You have just completed one of your exposures in our diesel chamber. Your exposure has been to diesel exhaust. As explained in the consent form, the exposure is similar to that of a truck driver. You may or may not experience some symptoms after exposure to this pollutant. The most common symptoms may be: headache or dizziness. You may also experience none of those symptoms.

If you have any concerns or are experiencing any other symptoms, please give me a call. Many matters can be resolved over the phone.

I can be reached in several ways:

1- EPA Medical Station -966-6232 (between 8 AM and 5 PM). We accept collect calls.

2- Home (after 5 PM) 408-0018

3- Pager 216-1378

_____MD
Andrew Ghio MD

U: omega pstdieselinst 1/07

APPROVED - IRB. UNC-CH
MAR 21 2007

Serving Size Booklet

Thank you for participating in this important research study.

Over the next two weeks, please record everything you consume (food, beverage, and/or supplements) on the notepad provided for three consecutive days each week (total of six days of records during the two-week period), with two of the three days being weekdays, and one of the three days being a weekend day.

For each eating occasion, please write down the 1) date, 2) time of day, and 3) location (if not at home, please provide name of restaurant or eating establishment), then list all items consumed in as much detail as possible. Next to each item, write down the estimated portion size. Use this portion size booklet to guide you in estimating the serving size of each food and beverage item. For mixed dishes, please list all ingredients and describe the preparation method (e.g., baked or fried entrée?; cooked or raw vegetables?). Remember to list all beverages, including water. Please write as clearly as possible.

We appreciate your help with this research project.

APPROVED - IRB, UNC-CH

MAR 21 2007

APPROVED - IRB, UNC-CH
MAR 21 2007

OMEGA FULL STUDY
GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1087.

Procedures:

Volunteers initially undergo routine medical screening at the EPA Human Studies Facility, which includes one visit to complete medical history forms and another for a physical exam and blood draw for genotyping. You will be paid $60 for completing these two screening appointments. If it is determined that your genotype is not needed for this study, no further visits will be scheduled. If you are eligible for the remainder of the study you will have up to 4 visits over approximately 5 to 6 weeks.

Participation in this study will include two-weeks of fish oil or olive oil treatments (taken as capsules) which will begin on the day of your physical exam. A training visit, lasting about 3 hours, will be scheduled 14 days later, and a diesel exposure visit and follow up day will occur two weeks after that. The exposure day lasts 8 hours and the follow up day, about 4. You will also be asked to record your food intake twice for 3 days each between the training session and exposure.

Procedures involved in this study include breathing tests, heart rate monitoring, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a does of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of diesel exhaust, no more than you might encounter if you visited a large city such as Las Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be available for training 14 days after the physical exam and for the exposure 15 days after that. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Must not eat or drink anything for 2 hours prior to the training day visit.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Medical History Screening, days/times as requested (1 hour)

Physical Exam, Genotyping, Dietary Instructions: days and times TBD (Day 1, 3 hours)

Training: 14 days after the Physical Exam Day (3 hours):

Exposure Day: 8:00 AM on Day 29 after Physical Exam (8 hours)

Follow-up Day: 18 hours after Exposure Day (4 hours)

OMEGA PILOT STUDY
GCRC #2579

APPROVED - IRB, UNC-CH
MAR 21 2007

Purpose:

The main purpose of this pilot study is to identify a safe concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will be used to determine the type of particular genes you carry. The genes, called glutathione-S-transferase, are responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying an inactive form of one of these genes and a variance of another may be more susceptible to the effects of air pollutants. Therefore, in this pilot research we will study the responses to diesel exhaust in healthy older subjects with very specific genetic characteristics.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1461.00 if all study visits are completed.

Procedures:

Volunteers initially undergo routine medical screening at the EPA Human Studies Facility, which includes one visit to complete medical history forms and another for a physical exam and blood draw for genotyping. You will be paid $60 for completing these two screening appointments. If it is determined that your genotype is not needed for this study, no further visits will be scheduled for the pilot study, but you may be eligible for the full study at a later time.

If you are eligible for the remainder of the pilot study you will have up to 7 visits over approximately 5 to 6 weeks. The goal of this pilot study is to find a safe concentration of diesel exhaust to be used in the future study. Results will be analyzed after finishing each proposed exposure to decide whether to continue on to the next level of proposed exposure. You may not have all these 3 exposures if this pilot study is terminated at some point based on interim results.

Your participation in this pilot study will include one training session for about 3 hours, up to 3 exposure sessions, each of which will last approximately 6 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours. Exposures are separated by about 2 weeks.

Study procedures will include breathing tests, heart rate monitoring, blood draws, and exposures to controlled amounts of diesel exhaust. Even at the highest level of exposure, you will not be exposed to more particles than you might encounter if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In order to participate you must be willing to observe several important restrictions. They are:

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Do not eat or drink anything for 2 hours prior to the training day visit.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposures and follow-ups.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Medical History Screening, days/times as requested (1 hour)
Physical Exam and Genotyping: days and times TBD (2 hours)

Training: days/times TBD (3 hours)

Exposure Day 1: 9:00 AM days TBD (6 hours)
Follow-up 1: 18 hours after Exposure 1 (3 hours)

Exposure Day 2: 9:00 AM days TBD, about 2 weeks after Exposure 1 (6 hours)
Follow-up 2: 18 hours after Exposure 2 (3 hours)

Exposure Day 3: 9:00 AM days TBD, about 2 weeks after Exposure 2 (6 hours)
Follow-up 3: 18 hours after Exposure 3 (3 hours)

This is a sample form. Do not use for scanning.

APPROVED - IRB, UNC-CH

MAR 21 2007

NATIONAL INSTITUTES OF HEALTH

Diet History Questionnaire

GENERAL INSTRUCTIONS

- Answer each question as best you can. Estimate if you are not sure. A guess is better than leaving a blank.
- Use only a black ball-point pen. Do not use a pencil or felt-tip pen. Do not fold, staple, or tear the pages.
- Put an X in the box next to your answer.
- If you make any changes, cross out the incorrect answer and put an X in the box next to the correct answer. Also draw a circle around the correct answer.
- If you mark NEVER, NO, or DON'T KNOW for a question, please follow any arrows or instructions that direct you to the next question.

BEFORE TURNING THE PAGE, PLEASE COMPLETE THE FOLLOWING QUESTIONS.

Today's date:

MONTH	DAY		YEAR
☐ Jan	☐0	☐0	☐ 2002
☐ Feb	☐1	☐1	☐ 2003
☐ Mar	☐2	☐2	☐ 2004
☐ Apr	☐3	☐3	☐ 2005
☐ May		☐4	☐ 2006
☐ Jun		☐5	
☐ Jul		☐6	
☐ Aug		☐7	
☐ Sep		☐8	
☐ Oct		☐9	
☐ Nov			
☐ Dec			

In what month were you born?

☐ Jan
☐ Feb
☐ Mar
☐ Apr
☐ May
☐ Jun
☐ Jul
☐ Aug
☐ Sep
☐ Oct
☐ Nov
☐ Dec

In what year were you born?

19 ___ ___

☐0	☐0
☐1	☐1
☐2	☐2
☐3	☐3
☐4	☐4
☐5	☐5
☐6	☐6
☐7	☐7
☐8	☐8
☐9	☐9

Are you male or female?

☐Male
☐Female

BAR CODE LABEL OR SUBJECT ID HERE

This is a sample form. Do not use for scanning.

1. Over the past 12 months, how often did you drink **tomato juice** or **vegetable juice**?

☐ NEVER (GO TO QUESTION 2)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

1a. Each time you drank **tomato juice** or **vegetable juice**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1¼ cups (6 to 10 ounces)
☐ More than 1¼ cups (10 ounces)

2. Over the past 12 months, how often did you drink **orange juice** or **grapefruit juice?**

☐ NEVER (GO TO QUESTION 3)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

2a. Each time you drank **orange juice** or **grapefruit juice**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1¼ cups (6 to 10 ounces)
☐ More than 1¼ cups (10 ounces)

3. Over the past 12 months, how often did you drink **other 100% fruit juice** or **100% fruit juice mixtures** (such as apple, grape, pineapple, or others)?

☐ NEVER (GO TO QUESTION 4)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

3a. Each time you drank **other fruit juice** or **fruit juice mixtures**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1½ cups (6 to 12 ounces)
☐ More than 1½ cups (12 ounces)

Question 4 appears in the next column

Over the past 12 months...

4. How often did you drink other **fruit drinks** (such as cranberry cocktail, Hi-C, lemonade, or Kool-Aid, diet or regular)?

☐ NEVER (GO TO QUESTION 5)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

4a. Each time you drank **fruit drinks**, how much did you usually drink?

☐ Less than 1 cup (8 ounces)
☐ 1 to 2 cups (8 to 16 ounces)
☐ More than 2 cups (16 ounces)

4b. How often were your fruit drinks **diet** or **sugar-free drinks**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

5. How often did you drink **milk as a beverage** (NOT in coffee, NOT in cereal)? (Please include chocolate milk and hot chocolate.)

☐ NEVER (GO TO QUESTION 6)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

5a. Each time you drank **milk as a beverage**, how much did you usually drink?

☐ Less than 1 cup (8 ounces)
☐ 1 to 1½ cups (8 to 12 ounces)
☐ More than 1½ cups (12 ounces)

5b. What kind of **milk** did you usually drink?

☐ Whole milk
☐ 2% fat milk
☐ 1 % fat milk
☐ Skim, nonfat, or ½% fat milk
☐ Soy milk
☐ Rice milk
☐ Other

Question 6 appears on the next page

Over the past 12 months...

6. How often did you drink **meal replacement, energy, or high-protein beverages** such as Instant Breakfast, Ensure, Slimfast, Sustacal or others?

- ☐ NEVER (GO TO QUESTION 7)
- ☐ 1 time per month or less
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

6a. Each time you drank **meal replacement beverages**, how much did you usually drink?

- ☐ Less than 1 cup (8 ounces)
- ☐ 1 to 1½ cups (8 to 12 ounces)
- ☐ More than 1½ cups (12 ounces)

7. Over the past 12 months, did you drink **soft drinks, soda,** or **pop**?

- ☐ NO (GO TO QUESTION 8)
- ☐ YES

7a. How often did you drink **soft drinks, soda,** or **pop IN THE SUMMER**?

- ☐ NEVER
- ☐ 1 time per month or less
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

7b. How often did you drink **soft drinks, soda,** or **pop DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1 time per month or less
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

7c. Each time you drank **soft drinks, soda,** or **pop,** how much did you usually drink?

- ☐ Less than 12 ounces or less than 1 can or bottle
- ☐ 12 to 16 ounces or 1 can or bottle
- ☐ More than 16 ounces or more than 1 can or bottle

7d. How often were these soft drinks, soda, or pop **diet** or **sugar-free**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

7e. How often were these soft drinks, soda, or pop **caffeine-free**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

8. Over the past 12 months, did you drink **beer**?

- ☐ NO (GO TO QUESTION 9)
- ☐ YES

8a. How often did you drink **beer IN THE SUMMER**?

- ☐ NEVER
- ☐ 1 time per month or less
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

8b. How often did you drink **beer DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1 time per month or less
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

8c. Each time you drank **beer**, how much did you usually drink?

- ☐ Less than a 12-ounce can or bottle
- ☐ 1 to 3 12-ounce cans or bottles
- ☐ More than 3 12-ounce cans or bottles

Question 8 appears in the next column

Question 9 appears on the next page

Over the past 12 months...

9. How often did you drink **wine** or **wine coolers**?

- ☐ NEVER (GO TO QUESTION 10)

- ☐ 1 time per month or less
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

9a. Each time you drank **wine** or **wine coolers**, how much did you usually drink?

- ☐ Less than 5 ounces or less than 1 glass
- ☐ 5 to 12 ounces or 1 to 2 glasses
- ☐ More than 12 ounces or more than 2 glasses

10. How often did you drink **liquor** or **mixed drinks**?

- ☐ NEVER (GO TO QUESTION 11)

- ☐ 1 time per month or less
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

10a. Each time you drank **liquor** or **mixed drinks**, how much did you usually drink?

- ☐ Less than 1 shot of liquor
- ☐ 1 to 3 shots of liquor
- ☐ More than 3 shots of liquor

11. Over the past 12 months, did you eat **oatmeal, grits,** or **other cooked cereal?**

- ☐ NO (GO TO QUESTION 12)
- ☐ YES

11a. How often did you eat **oatmeal, grits,** or **other cooked cereal IN THE WINTER**?

- ☐ NEVER

- ☐ 1–6 times per winter
- ☐ 7–11 times per winter
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

11b. How often did you eat **oatmeal, grits,** or **other cooked cereal DURING THE REST OF THE YEAR**?

- ☐ NEVER

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

11c. Each time you ate **oatmeal, grits,** or **other cooked cereal,** how much did you usually eat?

- ☐ Less than ¾ cup
- ☐ ¾ to 1¼ cups
- ☐ More than 1¼ cups

12. How often did you eat **cold cereal**?

- ☐ NEVER (GO TO QUESTION 13)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

12a. Each time you ate **cold cereal**, how much did you usually eat?

- ☐ Less than 1 cup
- ☐ 1 to 2½ cups
- ☐ More than 2½ cups

12b. How often was the cold cereal you ate **Total, Product 19,** or **Right Start**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

12c. How often was the cold cereal you ate **All Bran, Fiber One, 100% Bran,** or **Bran Buds?**

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 12 appears in the next column

Question 13 appears on the next page

Over the past 12 months...

12d. How often was the cold cereal you ate **some other bran** or **fiber cereal** (such as Cheerios, Shredded Wheat, Raisin Bran, Bran Flakes, Grape-Nuts, Granola, Wheaties, or Healthy Choice)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

12e. How often was the cold cereal you ate any **other type of cold cereal** (such as Corn Flakes, Rice Krispies, Frosted Flakes, Special K, Froot Loops, Cap'n Crunch, or others)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

12f. Was **milk** added to your cold cereal?

- ☐ NO (GO TO QUESTION 13)
- ☐ YES

12g. What kind of **milk** was usually added?

- ☐ Whole milk
- ☐ 2% fat milk
- ☐ 1% fat milk
- ☐ Skim, nonfat, or ½% fat milk
- ☐ Soy milk
- ☐ Rice milk
- ☐ Other

12h. Each time **milk was added to your cold cereal**, how much was usually added?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

13. How often did you eat **applesauce**?

- ☐ NEVER (GO TO QUESTION 14)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

Question 14 appears in the next column

13a. Each time you ate **applesauce**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

14. How often did you eat **apples**?

- ☐ NEVER (GO TO QUESTION 15)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

14a. Each time you ate **apples**, how many did you usually eat?

- ☐ Less than 1 apple
- ☐ 1 apple
- ☐ More than 1 apple

15. How often did you eat **pears** (fresh, canned, or frozen)?

- ☐ NEVER (GO TO QUESTION 16)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

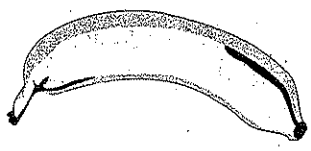
15a. Each time you ate **pears**, how many did you usually eat?

- ☐ Less than 1 pear
- ☐ 1 pear
- ☐ More than 1 pear

16. How often did you eat **bananas?**

- ☐ NEVER (GO TO QUESTION 17)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day



Question 17 appears on the next page

Over the past 12 months...

16a. Each time you ate **bananas**, how many did you usually eat?

- ☐ Less than 1 banana
- ☐ 1 banana
- ☐ More than 1 banana

17. How often did you eat **dried fruit**, such as prunes or raisins (not including dried apricots)?

- ☐ NEVER (GO TO QUESTION 18)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

17a. Each time you ate **dried fruit**, how much did you usually eat (not including dried apricots)?

- ☐ Less than 2 tablespoons
- ☐ 2 to 5 tablespoons
- ☐ More than 5 tablespoons

18. Over the past 12 months, did you eat **peaches, nectarines,** or **plums**?

- ☐ NO (GO TO QUESTION 19)
- ☐ YES

18a. How often did you eat **fresh peaches, nectarines,** or **plums WHEN IN SEASON**?

- ☐ NEVER

- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

18b. How often did you eat **peaches, nectarines,** or **plums** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR**?

- ☐ NEVER

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 19 appears in the next column

18c. Each time you ate **peaches, nectarines,** or **plums,** how much did you usually eat?

- ☐ Less than 1 fruit or less than ½ cup
- ☐ 1 to 2 fruits or ½ to ¾ cup
- ☐ More than 2 fruits or more than ¾ cup

19. How often did you eat **grapes**?

- ☐ NEVER (GO TO QUESTION 20)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

19a. Each time you ate **grapes**, how much did you usually eat?

- ☐ Less than ½ cup or less than 10 grapes
- ☐ ½ to 1 cup or 10 to 30 grapes
- ☐ More than 1 cup or more than 30 grapes

20. Over the past 12 months, did you eat **cantaloupe**?

- ☐ NO (GO TO QUESTION 21)
- ☐ YES

20a. How often did you eat **fresh cantaloupe WHEN IN SEASON**?

- ☐ NEVER

- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

20b. How often did you eat **fresh** or **frozen cantaloupe DURING THE REST OF THE YEAR**?

- ☐ NEVER

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 21 appears on the next page

Over the past 12 months...

20c. Each time you ate **cantaloupe**, how much did you usually eat?

- ☐ Less than ¼ melon or less than ½ cup
- ☐ ¼ melon or ½ to 1 cup
- ☐ More than ¼ melon or more than 1 cup

21. Over the past 12 months, did you eat **melon, other than cantaloupe** (such as watermelon or honeydew)?

- ☐ NO (GO TO QUESTION 22)
- ☐ YES

21a. How often did you eat **fresh melon, other than cantaloupe** (such as watermelon or honeydew) **WHEN IN SEASON**?

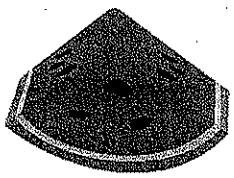
- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

21b. How often did you eat **fresh** or **frozen melon, other than cantaloupe** (such as watermelon or honeydew) **DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

21c. Each time you ate **melon other than cantaloupe**, how much did you usually eat?

- ☐ Less than ½ cup or 1 small wedge
- ☐ ½ to 2 cups or 1 medium wedge
- ☐ More than 2 cups or 1 large wedge



Question 22 appears in the next column

22. Over the past 12 months, did you eat **strawberries**?

- ☐ NO (GO TO QUESTION 23)
- ☐ YES

22a. How often did you eat **fresh strawberries WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

22b. How often did you eat **fresh** or **frozen strawberries DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

22c. Each time you ate **strawberries**, how much did you usually eat?

- ☐ Less than ¼ cup or less than 3 berries
- ☐ ¼ to ¾ cup or 3 to 8 berries
- ☐ More than ¾ cup or more than 8 berries

23. Over the past 12 months, did you eat **oranges, tangerines,** or **tangelos**?

- ☐ NO (GO TO QUESTION 24)
- ☐ YES

23a. How often did you eat **fresh oranges, tangerines, or tangelos WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 24 appears on the next page

Over the past 12 months...

23b. How often did you eat **oranges, tangerines,** or **tangelos** (fresh or canned) **DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

23c. Each time you ate **oranges, tangerines,** or **tangelos,** how many did you usually eat?

- ☐ Less than 1 fruit
- ☐ 1 fruit
- ☐ More than 1 fruit

24. Over the past 12 months, did you eat **grapefruit**?

- ☐ NO (GO TO QUESTION 25)
- ☐ YES

24a. How often did you eat **fresh grapefruit WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

24b. How often did you eat **grapefruit** (fresh or canned) **DURING THE REST OF THE YEAR?**

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

24c. Each time you ate **grapefruit**, how much did you usually eat?

- ☐ Less than ½ grapefruit
- ☐ ½ grapefruit
- ☐ More than ½ grapefruit

25. How often did you eat **other kinds of fruit**?

- ☐ NEVER (GO TO QUESTION 26)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

25a. Each time you ate **other kinds of fruit**, how much did you usually eat?

- ☐ Less than ¼ cup
- ☐ ¼ to ¾ cup
- ☐ More than ¾ cup

26. How often did you eat **COOKED greens** (such as spinach, turnip, collard, mustard, chard, or kale)?

- ☐ NEVER (GO TO QUESTION 27)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

26a. Each time you ate **COOKED greens**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

27. How often did you eat **RAW greens** (such as spinach, turnip, collard, mustard, chard, or kale)? *(We will ask about lettuce later.)*

- ☐ NEVER (GO TO QUESTION 28)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

27a. Each time you ate **RAW greens**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

Question 25 appears in the next column

Question 28 appears on the next page

Over the past 12 months...

28. How often did you eat **coleslaw**?

- ☐ NEVER (GO TO QUESTION 29)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

28a. Each time you ate **coleslaw**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ¾ cup
☐ More than ¾ cup

29. How often did you eat **sauerkraut** or **cabbage** (other than coleslaw)?

- ☐ NEVER (GO TO QUESTION 30)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

29a. Each time you ate **sauerkraut** or **cabbage**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1 cup
☐ More than 1 cup

30. How often did you eat **carrots** (fresh, canned, or frozen)?

- ☐ NEVER (GO TO QUESTION 31)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

30a. Each time you ate **carrots**, how much did you usually eat?

☐ Less than ¼ cup or less than 2 baby carrots
☐ ¼ to ½ cup or 2 to 5 baby carrots
☐ More than ½ cup or more than 5 baby carrots

Question 31 appears in the next column

31. How often did you eat **string beans** or **green beans** (fresh, canned, or frozen)?

- ☐ NEVER (GO TO QUESTION 32)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

31a. Each time you ate **string beans** or **green beans**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

32. How often did you eat **peas** (fresh, canned, or frozen)?

- ☐ NEVER (GO TO QUESTION 33)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

32a. Each time you ate **peas**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ¾ cup
☐ More than ¾ cup

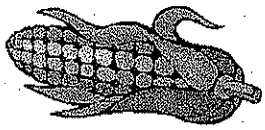
33. Over the past 12 months, did you eat **corn**?

- ☐ NO (GO TO QUESTION 34)
- ☐ YES

33a. How often did you eat **fresh corn WHEN IN SEASON**?

☐ NEVER

☐ 1–6 times per season
☐ 7–11 times per season
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day



Question 34 appears on the next page

Over the past 12 months...

33b. How often did you eat **corn** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

33c. Each time you ate **corn**, how much did you usually eat?

☐ Less than 1 ear or less than ½ cup
☐ 1 ear or ½ to 1 cup
☐ More than 1 ear or more than 1 cup

34. Over the past 12 months, how often did you eat **broccoli** (fresh or frozen)?

☐ NEVER (GO TO QUESTION 35)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

34a. Each time you ate **broccoli**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1 cup
☐ More than 1 cup

35. How often did you eat **cauliflower** or **Brussels sprouts** (fresh or frozen)?

☐ NEVER (GO TO QUESTION 36)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

35a. Each time you ate **cauliflower** or **Brussels sprouts**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ½ cup
☐ More than ½ cup

Question 36 appears in the next column

36. How often did you eat mixed **vegetables**?

☐ NEVER (GO TO QUESTION 37)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

36a. Each time you ate **mixed vegetables**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

37. How often did you eat **onions**?

☐ NEVER (GO TO QUESTION 38)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

37a. Each time you ate **onions**, how much did you usually eat?

☐ Less than 1 slice or less than 1 tablespoon
☐ 1 slice or 1 to 4 tablespoons
☐ More than 1 slice or more than 4 tablespoons

38. Now think about all the **cooked vegetables** you ate in the past 12 months and how they were prepared. How often were your vegetables **COOKED WITH** some sort of **fat**, including oil spray? *(Please do not include potatoes.)*

☐ NEVER (GO TO QUESTION 39)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

Question 39 appears on the next page

Over the past 12 months...

38a. Which fats were usually added to your vegetables **DURING COOKING**? *(Please do not include potatoes. **Mark all that apply.**)*

- ☐ Margarine (including low-fat)
- ☐ Butter (including low-fat)
- ☐ Lard, fatback, or bacon fat
- ☐ Olive oil
- ☐ Corn oil
- ☐ Canola or rapeseed oil
- ☐ Oil spray, such as Pam or others
- ☐ Other kinds of oils
- ☐ None of the above

39. Now, thinking again about all the **cooked vegetables** you ate in the past 12 months, how often was some sort of fat, sauce, or dressing added **AFTER COOKING OR AT THE TABLE**? *(Please do not include potatoes.)*

- ☐ NEVER (GO TO QUESTION 40)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 times per day
- ☐ 3 or more times per day

39a. Which fats, sauces, or dressings were usually added **AFTER COOKING OR AT THE TABLE**? *(Please do not include potatoes. **Mark all that apply.**)*

- ☐ Margarine (including low-fat)
- ☐ Butter (including low-fat)
- ☐ Lard, fatback, or bacon fat
- ☐ Salad dressing
- ☐ Cheese sauce
- ☐ White sauce
- ☐ Other

39b. If margarine, butter, lard, fatback, or bacon fat was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- ☐ Did not usually add these
- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

39c. If salad dressing, cheese sauce, or white sauce was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- ☐ Did not usually add these
- ☐ Less than 1 tablespoon
- ☐ 1 to 3 tablespoons
- ☐ More than 3 tablespoons

Question 40 appears in the next column

40. Over the past 12 months, how often did you eat **sweet peppers** (green, red, or yellow)?

- ☐ NEVER (GO TO QUESTION 41)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

40a. Each time you ate **sweet peppers**, how much did you usually eat?

- ☐ Less than ⅛ pepper
- ☐ ⅛ to ¼ pepper
- ☐ More than ¼ pepper

41. Over the past 12 months, did you eat **fresh tomatoes** (including those in salads)?

- ☐ NO (GO TO QUESTION 42)
- ☐ YES

41a. How often did you eat **fresh tomatoes** (including those in salads) **WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

41b. How often did you eat **fresh tomatoes** (including those in salads) **DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

41c. Each time you ate **fresh tomatoes**, how much did you usually eat?

- ☐ Less than ¼ tomato
- ☐ ¼ to ½ tomato
- ☐ More than ½ tomato

Question 42 appears on the next page

Over the past 12 months...

42. How often did you eat **lettuce salads** (with or without other vegetables)?

- ☐ NEVER (GO TO QUESTION 43)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

42a. Each time you ate **lettuce salads**, how much did you usually eat?

- ☐ Less than ¼ cup
- ☐ ¼ to 1 /4 cups
- ☐ More than 1 /4 cups

43. How often did you eat **salad dressing** (including low-fat) on salads?

- ☐ NEVER (GO TO QUESTION 44)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

43a. Each time you ate **salad dressing** on salads, how much did you usually eat?

- ☐ Less than 2 tablespoons
- ☐ 2 to 4 tablespoons
- ☐ More than 4 tablespoons

44. How often did you eat **sweet potatoes** or **yams**?

- ☐ NEVER (GO TO QUESTION 45)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

44a. Each time you ate **sweet potatoes** or **yams**, how much did you usually eat?

- ☐ 1 small potato or less than ¼ cup
- ☐ 1 medium potato or ¼ to ¾ cup
- ☐ 1 large potato or more than ¾ cup

45. How often did you eat **French fries, home fries, hash browned potatoes**, or **tater tots**?

- ☐ NEVER (GO TO QUESTION 46)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

45a. Each time you ate **French fries, home fries, hash browned potatoes**, or **tater tots** how much did you usually eat?

- ☐ Less than 10 fries or less than ½ cup
- ☐ 10 to 25 fries or ½ to 1 cup
- ☐ More than 25 fries or more than 1 cup

46. How often did you eat **potato salad**?

- ☐ NEVER (GO TO QUESTION 47)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

46a. Each time you ate **potato salad**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

47. How often did you eat **baked, boiled,** or **mashed potatoes**?

- ☐ NEVER (GO TO QUESTION 48)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

47a. Each time you ate **baked, boiled,** or **mashed potatoes**, how much did you usually eat?

- ☐ 1 small potato or less than ½ cup
- ☐ 1 medium potato or ½ to 1 cup
- ☐ 1 large potato or more than 1 cup

Question 45 appears in the next column

Question 48 appears on the next page

Over the past 12 months...

47b. How often was **sour cream** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- ☐ Almost never or never (GO TO QUESTION 47d)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

47c. Each time **sour cream** was added to your potatoes, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 3 tablespoons
- ☐ More than 3 tablespoons

47d. How often was **margarine** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

47e. How often was **butter** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

47f. Each time **margarine** or **butter** was added to your potatoes, how much was usually added?

- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

47g. How often was cheese or cheese sauce added to your potatoes, EITHER IN COOKING OR AT THE TABLE?

- ☐ Almost never or never (GO TO QUESTION 48)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 48 appears in the next column

47h. Each time **cheese** or **cheese sauce** was added to your potatoes, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 3 tablespoons
- ☐ More than 3 tablespoons

48. How often did you eat **salsa**?

- ☐ NEVER (GO TO QUESTION 49)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

48a. Each time you ate **salsa**, how much did you usually eat?

- ☐ Less than 1 tablespoon
- ☐ 1 to 5 tablespoons
- ☐ More than 5 tablespoons

49. How often did you eat **catsup**?

- ☐ NEVER (GO TO QUESTION 50)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

49a. Each time you ate **catsup**, how much did you usually eat?

- ☐ Less than 1 teaspoon
- ☐ 1 to 6 teaspoons
- ☐ More than 6 teaspoons

50. How often did you eat **stuffing, dressing,** or **dumplings**?

- ☐ NEVER (GO TO QUESTION 51)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

50a. Each time you ate **stuffing, dressing,** or **dumplings**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

Question 51 appears on the next page

Over the past 12 months...

51. How often did you eat **chili**?

☐ NEVER (GO TO QUESTION 52)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

51a. Each time you ate **chili**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 3/4 cups
☐ More than 1 3/4 cups

52. How often did you eat **Mexican foods** (such as tacos, tostados, burritos, tamales, fajitas, enchiladas, quesadillas, and chimichangas)?

☐ NEVER (GO TO QUESTION 53)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

52a. Each time you ate **Mexican foods**, how much did you usually eat?

☐ Less than 1 taco, burrito, etc.
☐ 1 to 2 tacos, burritos, etc.
☐ More than 2 tacos, burritos, etc.

53. How often did you eat **cooked dried beans** (such as baked beans, pintos, kidney, blackeyed peas, lima, lentils, soybeans, or refried beans)? *(Please don't include bean soups or chili.)*

☐ NEVER (GO TO QUESTION 54)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

53a. Each time you ate **beans**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

53b. How often were the beans you ate **refried beans, beans prepared with any type of fat**, or **with meat added**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

54. How often did you eat **other kinds of vegetables**?

☐ NEVER (GO TO QUESTION 55)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

54a. Each time you ate **other kinds of vegetables**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ½ cup
☐ More than ½ cup

55. How often did you eat **rice** or **other cooked grains** (such as bulgur, cracked wheat, or millet)?

☐ NEVER (GO TO QUESTION 56)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

55a. Each time you ate **rice or other cooked grains**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 /2 cups
☐ More than 1 /2 cups

55b. How often was **butter, margarine**, or **oil** added to your rice **IN COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 54 appears in the next column

Question 56 appears on the next page

Over the past 12 months...

56. How often did you eat **pancakes, waffles,** or **French toast**?

- ☐ NEVER (GO TO QUESTION 57)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

56a. Each time you ate **pancakes, waffles,** or **French toast**, how much did you usually eat?

- ☐ Less than 1 medium piece
- ☐ 1 to 3 medium pieces
- ☐ More than 3 medium pieces

56b. How often was **margarine** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

56c. How often was **butter** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

56d. Each time **margarine** or **butter** was added to your pancakes, waffles, or French toast, how much was usually added?

- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

56e. How often was **syrup** added to your pancakes, waffles, or French toast?

- ☐ Almost never or never (GO TO QUESTION 57)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 57 appears in the next column

56f. Each time **syrup** was added to your pancakes, waffles, or French toast, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 4 tablespoons
- ☐ More than 4 tablespoons

57. How often did you eat **lasagna, stuffed shells, stuffed manicotti, ravioli,** or **tortellini**? *(Please do not include spaghetti or other pasta.)*

- ☐ NEVER (GO TO QUESTION 58)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

57a. Each time you ate **lasagna, stuffed shells, stuffed manicotti, ravioli,** or **tortellini**, how much did you usually eat?

- ☐ Less than 1 cup
- ☐ 1 to 2 cups
- ☐ More than 2 cups

58. How often did you eat **macaroni and cheese**?

- ☐ NEVER (GO TO QUESTION 59)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

58a. Each time you ate **macaroni and cheese**, how much did you usually eat?

- ☐ Less than 1 cup
- ☐ 1 to 1 /2 cups
- ☐ More than 1 /2 cups

59. How often did you eat **pasta salad or macaroni salad**?

- ☐ NEVER (GO TO QUESTION 60)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 60 appears on the next page

Over the past 12 months...

59a. Each time you ate **pasta salad** or **macaroni salad**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

60. Other than the pastas listed in Questions 57, 58, and 59, how often did you eat **pasta, spaghetti**, or **other noodles**?

- ☐ NEVER (GO TO QUESTION 61)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

60a. Each time you ate **pasta, spaghetti, or other noodles**, how much did you usually eat?

- ☐ Less than 1 cup
- ☐ 1 to 3 cups
- ☐ More than 3 cups

60b. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITH meat**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

60c. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITHOUT meat**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

60d. How often did you eat your pasta, spaghetti, or other noodles with **margarine, butter, oil,** or **cream sauce**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 61 appears in the next column

61. How often did you eat **bagels** or **English muffins**?

- ☐ NEVER (GO TO INTRODUCTION TO QUESTION 62)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

61a. Each time you ate **bagels** or **English muffins**, how many did you usually eat?

- ☐ Less than 1 bagel or English muffin
- ☐ 1 bagel or English muffin
- ☐ More than 1 bagel or English muffin

61b. How often was **margarine** (including low-fat) added to your bagels or English muffins?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

61c. How often was **butter** (including low-fat) added to your bagels or English muffins?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

61d. Each time **margarine** or **butter** was added to your bagels or English muffins, how much was usually added?

- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 2 teaspoons
- ☐ More than 2 teaspoons

61e. How often was **cream cheese** (including low-fat) spread on your bagels or English muffins?

- ☐ Almost never or never (GO TO INTRODUCTION TO QUESTION 62)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Introduction to Question 62 appears on the next page

Over the past 12 months...

61f. Each time **cream cheese** was added to your bagels or English muffins, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 2 tablespoons
- ☐ More than 2 tablespoons

The next questions ask about your intake of breads other than bagels or English muffins. First, we will ask about bread you ate as part of sandwiches only. Then we will ask about all other bread you ate.

62. How often did you eat **breads** or **rolls AS PART OF SANDWICHES** (including burger and hot dog rolls)?

- ☐ NEVER (GO TO QUESTION 63)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

62a. Each time you ate **breads** or **rolls AS PART OF SANDWICHES**, how many did you usually eat?

- ☐ 1 slice or ½ roll
- ☐ 2 slices or 1 roll
- ☐ More than 2 slices or more than 1 roll

62b. How often were the breads or rolls that you used for your sandwiches **white bread** (including burger and hot dog rolls)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

62c. How often was **mayonnaise** or **mayonnaise-type dressing** (including low-fat) added to your sandwich bread or rolls?

- ☐ Almost never or never (GO TO QUESTION 62e)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 62e appears in the next column

Question 63 appears in the next column

62d. Each time **mayonnaise** or **mayonnaise-type dressing** was added to your sandwich breads or rolls, how much was usually added?

- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

62e. How often was **margarine** (including low-fat) added to your sandwich bread or rolls?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

62f. How often was **butter** (including low-fat) added to your sandwich bread or rolls?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

62g. Each time **margarine** or **butter** was added to your sandwich breads or rolls, how much was usually added?

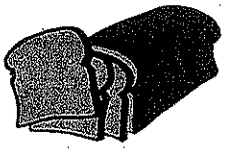
- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 2 teaspoons
- ☐ More than 2 teaspoons

63. How often did you eat **breads** or **dinner rolls, NOT AS PART OF SANDWICHES**?

- ☐ NEVER (GO TO QUESTION 64)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

63a. Each time you ate **breads** or **dinner rolls, NOT AS PART OF SANDWICHES**, how much did you usually eat?

- ☐ 1 slice or 1 dinner roll
- ☐ 2 slices or 2 dinner rolls
- ☐ More than 2 slices or 2 dinner rolls



Question 64 appears on the next page

Over the past 12 months...

63b. How often were the breads or rolls you ate **white bread**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

63c. How often was **margarine** (including low-fat) added to your breads or rolls?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

63d. How often was **butter** (including low-fat) added to your breads or rolls?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

63e. Each time **margarine** or **butter** was added to your breads or rolls, how much was usually added?

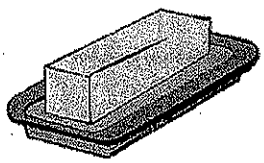
- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 2 teaspoons
- ☐ More than 2 teaspoons

63f. How often was **cream cheese** (including low-fat) added to your breads or rolls?

- ☐ Almost never or never (GO TO QUESTION 64)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

63g. Each time **cream cheese** was added to your breads or rolls, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 2 tablespoons
- ☐ More than 2 tablespoons



Question 64 appears in the next column

64. How often did you eat **jam, jelly,** or **honey** on bagels, muffins, bread, rolls, or crackers?

- ☐ NEVER (GO TO QUESTION 65)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

64a. Each time you ate **jam, jelly,** or **honey**, how much did you usually eat?

- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

65. How often did you eat **peanut butter** or **other nut butter**?

- ☐ NEVER (GO TO QUESTION 66)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

65a. Each time you ate **peanut butter** or **other nut butter**, how much did you usually eat?

- ☐ Less than 1 tablespoon
- ☐ 1 to 2 tablespoons
- ☐ More than 2 tablespoons

66. How often did you eat **roast beef** or **steak IN SANDWICHES**?

- ☐ NEVER (GO TO QUESTION 67)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

66a. Each time you ate **roast beef** or **steak IN SANDWICHES**, how much did you usually eat?

- ☐ Less than 1 slice or less than 2 ounces
- ☐ 1 to 2 slices or 2 to 4 ounces
- ☐ More than 2 slices or more than 4 ounces

Question 67 appears on the next page

Over the past 12 months...

67. How often did you eat **turkey** or **chicken COLD CUTS** (such as loaf, luncheon meat, turkey ham, turkey salami, or turkey pastrami)? *(We will ask about other turkey or chicken later.)*

- ☐ NEVER (GO TO QUESTION 68)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

67a. Each time you ate **turkey or chicken COLD CUTS**, how much did you usually eat?

- ☐ Less than 1 slice
- ☐ 1 to 3 slices
- ☐ More than 3 slices

68. How often did you eat **luncheon** or **deli-style ham**? *(We will ask about other ham later.)*

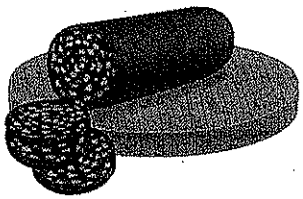
- ☐ NEVER (GO TO QUESTION 69)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

68a. Each time you ate **luncheon** or **deli-style ham**, how much did you usually eat?

- ☐ Less than 1 slice
- ☐ 1 to 3 slices
- ☐ More than 3 slices

68b. How often was the luncheon or deli-style ham you ate **light**, **low-fat**, or **fat-free**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always



Question 69 appears in the next column

69. How often did you eat **other cold cuts** or **luncheon meats** (such as bologna, salami, corned beef, pastrami, or others, including low-fat)? *(Please do not include ham, turkey, or chicken cold cuts.)*

- ☐ NEVER (GO TO QUESTION 70)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

69a. Each time you ate **other cold cuts** or **luncheon meats**, how much did you usually eat?

- ☐ Less than 1 slice
- ☐ 1 to 3 slices
- ☐ More than 3 slices

69b. How often were the other cold cuts or luncheon meats you ate **light, low-fat**, or **fat-free cold cuts or luncheon meats**? *(Please do not include ham, turkey, or chicken cold cuts.)*

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

70. How often did you eat **canned tuna** (including in salads, sandwiches, or casseroles)?

- ☐ NEVER (GO TO QUESTION 71)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

70a. Each time you ate **canned tuna**, how much did you usually eat?

- ☐ Less than ¼ cup or less than 2 ounces
- ☐ ¼ to ½ cup or 2 to 3 ounces
- ☐ More than ½ cup or more than 3 ounces

70b. How often was the canned tuna you ate **water-packed tuna**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 71 appears on the next page

Over the past 12 months...

70c. How often was the canned tuna you ate **prepared with mayonnaise** or **other dressing** (including low-fat)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

71. How often did you eat **GROUND chicken** or **turkey**? *(We will ask about other chicken and turkey later.)*

- ☐ NEVER (GO TO QUESTION 72)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

71a. Each time you ate **GROUND chicken** or **turkey**, how much did you usually eat?

- ☐ Less than 2 ounces or less than ½ cup
- ☐ 2 to 4 ounces or ½ to 1 cup
- ☐ More than 4 ounces or more than 1 cup

72. How often did you eat **beef hamburgers** or **cheeseburgers**?

- ☐ NEVER (GO TO QUESTION 73)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

72a. Each time you ate **beef hamburgers** or **cheeseburgers**, how much did you usually eat?

- ☐ Less than 1 patty or less than 2 ounces
- ☐ 1 patty or 2 to 4 ounces
- ☐ More than 1 patty or more than 4 ounces

72b. How often were the beef hamburgers or cheeseburgers you ate made with **lean ground beef**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 73 appears in the next column

73. How often did you eat **ground beef in mixtures** (such as meatballs, casseroles, chili, or meatloaf)?

- ☐ NEVER (GO TO QUESTION 74)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

73a. Each time you ate **ground beef in mixtures**, how much did you usually eat?

- ☐ Less than 3 ounces or less than ½ cup
- ☐ 3 to 8 ounces or ½ to 1 cup
- ☐ More than 8 ounces or more than 1 cup

74. How often did you eat **hot dogs** or **frankfurters**? *(Please do not include sausages or vegetarian hot dogs.)*

- ☐ NEVER (GO TO QUESTION 75)

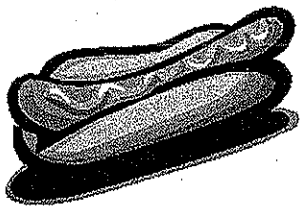
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

74a. Each time you ate **hot dogs** or **frankfurters**, how many did you usually eat?

- ☐ Less than 1 hot dog
- ☐ 1 to 2 hot dogs
- ☐ More than 2 hot dogs

74b. How often were the hot dogs or frankfurters you ate **light** or **low-fat hot dogs**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always



Question 75 appears on the next page

Over the past 12 months...

75. How often did you eat beef mixtures such as **beef stew, beef pot pie, beef and noodles,** or **beef and vegetables**?

- ☐ NEVER (GO TO QUESTION 76)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

75a. Each time you ate **beef stew, beef pot pie, beef and noodles,** or **beef and vegetables,** how much did you usually eat?

- ☐ Less than 1 cup
- ☐ 1 to 2 cups
- ☐ More than 2 cups

76. How often did you eat **roast beef** or **pot roast**? *(Please do not include roast beef or pot roast in sandwiches.)*

- ☐ NEVER (GO TO QUESTION 77)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

76a. Each time you ate **roast beef** or **pot roast** (including in mixtures), how much did you usually eat?

- ☐ Less than 2 ounces
- ☐ 2 to 5 ounces
- ☐ More than 5 ounces

77. How often did you eat **steak** (beef)? *(Do not include steak in sandwiches)*

- ☐ NEVER (GO TO QUESTION 78)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

77a. Each time you ate **steak** (beef), how much did you usually eat?

- ☐ Less than 3 ounces
- ☐ 3 to 7 ounces
- ☐ More than 7 ounces

77b. How often was the steak you ate **lean steak**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

78. How often did you eat **pork** or **beef spareribs**?

- ☐ NEVER (GO TO QUESTION 79)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

78a. Each time you ate **pork** or **beef spareribs**, how much did you usually eat?

- ☐ Less than 4 ribs
- ☐ 4 to 12 ribs
- ☐ More than 12 ribs

79. How often did you eat **roast turkey, turkey cutlets,** or **turkey nuggets** (including in sandwiches)?

- ☐ NEVER (GO TO QUESTION 80)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

79a. Each time you ate **roast turkey, turkey cutlets,** or **turkey nuggets**, how much did you usually eat? *(Please note: 4 to 8 turkey nuggets = 3 ounces.)*

- ☐ Less than 2 ounces
- ☐ 2 to 4 ounces
- ☐ More than 4 ounces

80. How often did you eat **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures**?

- ☐ NEVER (GO TO QUESTION 81)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 78 appears in the next column

Question 81 appears on the next page

Over the past 12 months...

80a. Each time you ate **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures,** how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 /2 cups
- ☐ More than 1 /2 cups

81. How often did you eat **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets)? *(Please do not include chicken in mixtures.)*

- ☐ NEVER (GO TO QUESTION 82)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

81a. Each time you ate **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets), how much did you usually eat?

- ☐ Less than 2 drumsticks or wings, less than 1 breast or thigh, or less than 4 nuggets
- ☐ 2 drumsticks or wings, 1 breast or thigh, or 4 to 8 nuggets
- ☐ More than 2 drumsticks or wings, more than 1 breast or thigh, or more than 8 nuggets

81b. How often was the chicken you ate **fried chicken** (including deep fried) or **chicken nuggets**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

81c. How often was the chicken you ate **WHITE meat**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

81d. How often did you eat chicken **WITH skin**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 82 appears in the next column

82. How often did you eat **baked ham** or **ham steak**?

- ☐ NEVER (GO TO QUESTION 83)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

82a. Each time you ate **baked ham** or **ham steak**, how much did you usually eat?

- ☐ Less than 1 ounce
- ☐ 1 to 3 ounces
- ☐ More than 3 ounces

83. How often did you eat **pork** (including chops, roasts, and in mixed dishes)? *(Please do not include ham, ham steak, or sausage.)*

- ☐ NEVER (GO TO QUESTION 84)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

83a. Each time you ate **pork**, how much did you usually eat?

- ☐ Less than 2 ounces or less than 1 chop
- ☐ 2 to 5 ounces or 1 chop
- ☐ More than 5 ounces or more than 1 chop

84. How often did you eat **gravy** on meat, chicken, potatoes, rice, etc.?

- ☐ NEVER (GO TO QUESTION 85)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

84a. Each time you ate **gravy** on meat, chicken, potatoes, rice, etc., how much did you usually eat?

- ☐ Less than ⅛ cup
- ☐ ⅛ to ½ cup
- ☐ More than ½ cup

Question 85 appears on the next page

Over the past 12 months...

85. How often did you eat **liver** (all kinds) or **liverwurst**?

- ☐ NEVER (GO TO QUESTION 86)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

85a. Each time you ate **liver** or **liverwurst**, how much did you usually eat?

- ☐ Less than 1 ounce
- ☐ 1 to 4 ounces
- ☐ More than 4 ounces

86. How often did you eat **bacon** (including low-fat)?

- ☐ NEVER (GO TO QUESTION 87)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

86a. Each time you ate **bacon**, how much did you usually eat?

- ☐ Fewer than 2 slices
- ☐ 2 to 3 slices
- ☐ More than 3 slices

86b. How often was the bacon you ate **light, low-fat,** or **lean bacon**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

87. How often did you eat **sausage** (including low-fat)?

- ☐ NEVER (GO TO QUESTION 88)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

87a. Each time you ate **sausage**, how much did you usually eat?

- ☐ Less than 1 patty or 2 links
- ☐ 1 to 3 patties or 2 to 5 links
- ☐ More than 3 patties or 5 links

87b. How often was the sausage you ate **light, low-fat**, or **lean sausage**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

88. How often did you eat **fish sticks** or **fried fish** (including fried seafood or shellfish)?

- ☐ NEVER (GO TO QUESTION 89)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

88a. Each time you ate **fish sticks** or **fried fish**, how much did you usually eat?

- ☐ Less than 2 ounces or less than 1 fillet
- ☐ 2 to 7 ounces or 1 fillet
- ☐ More than 7 ounces or more than 1 fillet

89. How often did you eat **fish** or **seafood that was NOT FRIED** (including shellfish)?

- ☐ NEVER (GO TO INTRODUCTION TO QUESTION 90)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

89a. Each time you ate eat **fish** or **seafood that was NOT FRIED**, how much did you usually eat?

- ☐ Less than 2 ounces or less than 1 fillet
- ☐ 2 to 5 ounces or 1 fillet
- ☐ More than 5 ounces or more than 1 fillet

Question 88 appears in the next column

Introduction to Question 90 appears on the next page

Over the past 12 months...

Now think about all the meat, poultry, and fish you ate in the past 12 months and how they were prepared.

90. How often was **oil, butter, margarine, or other fat** used to **FRY, SAUTE, BASTE, OR MARINATE** any meat, poultry, or fish you ate? *(Please do not include deep frying.)*

- ☐ NEVER (GO TO QUESTION 91)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

90a. Which of the following **fats** were regularly used to prepare your meat, poultry, or fish? ***(Mark all that apply.)***

- ☐ Margarine (including low-fat)
- ☐ Butter (including low-fat)
- ☐ Lard, fatback, or bacon fat
- ☐ Olive oil
- ☐ Corn oil
- ☐ Canola or rapeseed oil
- ☐ Oil spray, such as Pam or others
- ☐ Other kinds of oils
- ☐ None of the above

91. How often did you eat **tofu, soy burgers,** or **soy meat-substitutes**?

- ☐ NEVER (GO TO QUESTION 92)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

91a. Each time you ate **tofu, soy burgers,** or **soy meat-substitutes**, how much did you usually eat?

- ☐ Less than ¼ cup or less than 2 ounces
- ☐ ¼ to ½ cup or 2 to 4 ounces
- ☐ More than ½ cup or more than 4 ounces

Question 92 appears in the next column

92. Over the past 12 months, did you eat **soups**?

- ☐ NO (GO TO QUESTION 93)
- ☐ YES

92a. How often did you eat **soup DURING THE WINTER**?

- ☐ NEVER
- ☐ 1–6 times per winter
- ☐ 7–11 times per winter
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

92b. How often did you eat **soup DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

92c. Each time you ate **soup**, how much did you usually eat?

- ☐ Less than 1 cup
- ☐ 1 to 2 cups
- ☐ More than 2 cups

92d. How often were the soups you ate **bean soups**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

92e. How often were the soups you ate **cream soups** (including chowders)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 93 appears on the next page

Over the past 12 months...

92f. How often were the soups you ate **tomato** or **vegetable soups**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

92g. How often were the soups you ate **broth soups** (including chicken) **with** or **without noodles** or **rice**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

93. How often did you eat **pizza**?

☐ NEVER (GO TO QUESTION 94)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

93a. Each time you ate **pizza**, how much did you usually eat?

- ☐ Less than 1 slice or less than 1 mini pizza
- ☐ 1 to 3 slices or 1 mini pizza
- ☐ More than 3 slices or more than 1 mini pizza

93b. How often did you eat pizza with **pepperoni, sausage**, or **other meat**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

94. How often did you eat **crackers**?

☐ NEVER (GO TO QUESTION 95)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 95 appears in the next column

94a. Each time you ate **crackers**, how many did you usually eat?

- ☐ Fewer than 4 crackers
- ☐ 4 to 10 crackers
- ☐ More than 10 crackers

95. How often did you eat **corn bread** or **corn muffins**?

☐ NEVER (GO TO QUESTION 96)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

95a. Each time you ate **corn bread** or **corn muffins,** how much did you usually eat?

- ☐ Less than 1 piece or muffin
- ☐ 1 to 2 pieces or muffins
- ☐ More than 2 pieces or muffins

96. How often did you eat **biscuits**?

☐ NEVER (GO TO QUESTION 97)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

96a. Each time you ate **biscuits**, how many did you usually eat?

- ☐ Fewer than 1 biscuit
- ☐ 1 to 2 biscuits
- ☐ More than 2 biscuits

97. How often did you eat **potato chips, tortilla chips,** or **corn chips** (including low-fat, fat-free, or low-salt)?

☐ NEVER (GO TO QUESTION 98)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 98 appears on the next page

Over the past 12 months...

97a. Each time you ate **potato chips, tortilla chips,** or **corn chips**, how much did you usually eat?

- ☐ Fewer than 10 chips or less than 1 cup
- ☐ 10 to 25 chips or 1 to 2 cups
- ☐ More than 25 chips or more than 2 cups

97b. How often were the chips you ate **Wow chips** or other **chips made with fat substitute** (Olean or Olestra)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

97c. How often were the chips you ate other **low-fat** or **fat-free chips**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

98. How often did you eat **popcorn** (including low-fat)?

☐ NEVER (GO TO QUESTION 99)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

98a. Each time you ate **popcorn**, how much did you usually eat?

- ☐ Less than 2 cups, popped
- ☐ 2 to 5 cups, popped
- ☐ More than 5 cups, popped

99. How often did you eat **pretzels**?

☐ NEVER (GO TO QUESTION 100)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

99a. Each time you ate **pretzels**, how many did you usually eat?

- ☐ Fewer than 5 average twists
- ☐ 5 to 20 average twists
- ☐ More than 20 average twists

100. How often did you eat **peanuts, walnuts, seeds,** or **other nuts**?

☐ NEVER (GO TO QUESTION 101)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

100a. Each time you ate **peanuts, walnuts, seeds,** or **other nuts**, how much did you usually eat?

- ☐ Less than ¼ cup
- ☐ ¼ to ½ cup
- ☐ More than ½ cup

101. How often did you eat **energy, high-protein**, or **breakfast bars** such as **Power Bars, Balance, Clif,** or **others**?

☐ NEVER (GO TO QUESTION 102)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

101a. Each time you ate **energy, high-protein**, or **breakfast bars**, how much did you usually eat?

- ☐ Less than 1 bar
- ☐ 1 bar
- ☐ More than 1 bar

102. How often did you eat **yogurt** (NOT including frozen yogurt)?

☐ NEVER (GO TO QUESTION 103)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 100 appears in the next column

Question 103 appears on the next page

Over the past 12 months...

102a. Each time you ate **yogurt**, how much did you usually eat?

- ☐ Less than ½ cup or less than 1 container
- ☐ ½ to 1 cup or 1 container
- ☐ More than 1 cup or more than 1 container

103. How often did you eat **cottage cheese** (including low-fat)?

- ☐ NEVER (GO TO QUESTION 104)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

103a. Each time you ate **cottage cheese**, how much did you usually eat?

- ☐ Less than ¼ cup
- ☐ ¼ to 1 cup
- ☐ More than 1 cup

104. How often did you eat **cheese** (including low-fat; including on cheeseburgers or in sandwiches or subs)?

- ☐ NEVER (GO TO QUESTION 105)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

104a. Each time you ate **cheese**, how much did you usually eat?

- ☐ Less than ½ ounce or less than 1 slice
- ☐ ½ to 1 /2 ounces or 1 slice
- ☐ More than 1 /2 ounces or more than 1 slice

104b. How often was the cheese you ate **light** or **low-fat cheese**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 105 appears in the next column

104c. How often was the cheese you ate **fat-free cheese**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

105. How often did you eat **frozen yogurt, sorbet,** or **ices** (including low-fat or fat-free)?

- ☐ NEVER (GO TO QUESTION 106)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

105a. Each time you ate **frozen yogurt, sorbet,** or **ices**, how much did you usually eat?

- ☐ Less than ½ cup or less than 1 scoop
- ☐ ½ to 1 cup or 1 to 2 scoops
- ☐ More than 1 cup or more than 2 scoops

106. How often did you eat **ice cream, ice cream bars,** or **sherbet** (including low-fat or fat-free)?

- ☐ NEVER (GO TO QUESTION 107)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

106a. Each time you ate **ice cream, ice cream bars,** or **sherbet**, how much did you usually eat?

- ☐ Less than ½ cup or less than 1 scoop
- ☐ ½ to 1 /2 cups or 1 to 2 scoops
- ☐ More than 1 /2 cups or more than 2 scoops

106b. How often was the ice cream you ate **light, low-fat,** or **fat-free ice cream** or **sherbet**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 107 appears on the next page

Over the past 12 months...

107. How often did you eat **cake** (including low-fat or fat-free)?

- ☐ NEVER (GO TO QUESTION 108)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

107a. Each time you ate **cake**, how much did you usually eat?

- ☐ Less than 1 medium piece
- ☐ 1 medium piece
- ☐ More than 1 medium piece

107b. How often was the cake you ate **light, low-fat,** or **fat-free cake**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

108. How often did you eat **cookies** or **brownies** (including low-fat or fat-free)?

- ☐ NEVER (GO TO QUESTION 109)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

108a. Each time you ate **cookies** or **brownies**, how much did you usually eat?

- ☐ Less than 2 cookies or 1 small brownie
- ☐ 2 to 4 cookies or 1 medium brownie
- ☐ More than 4 cookies or 1 large brownie

108b. How often were the cookies or brownies you ate **light, low-fat,** or **fat-free cookies** or **brownies**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 109 appears in the next column

109. How often did you eat **doughnuts, sweet rolls, Danish,** or **pop-tarts**?

- ☐ NEVER (GO TO QUESTION 110)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

109a. Each time you ate **doughnuts, sweet rolls, Danish,** or **pop-tarts**, how much did you usually eat?

- ☐ Less than 1 piece
- ☐ 1 to 2 pieces
- ☐ More than 2 pieces

110. How often did you eat **sweet muffins** or **dessert breads** (including low-fat or fat-free)?

- ☐ NEVER (GO TO QUESTION 111)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

110a. Each time you ate **sweet muffins or dessert breads**, how much did you usually eat?

- ☐ Less than 1 medium piece
- ☐ 1 medium piece
- ☐ More than 1 medium piece

110b. How often were the sweet muffins or dessert breads you ate **light, low-fat,** or **fat-free sweet muffins** or **dessert breads**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

111. How often did you eat **fruit crisp, cobbler,** or **strudel**?

- ☐ NEVER (GO TO QUESTION 112)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 112 appears on the next page

Over the past 12 months...

111a. Each time you ate **fruit crisp, cobbler,** or **strudel,** how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

112. How often did you eat **pie**?

☐ NEVER (GO TO QUESTION 113)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

112a. Each time you ate **pie**, how much did you usually eat?

- ☐ Less than ⅛ of a pie
- ☐ About ⅛ of a pie
- ☐ More than ⅛ of a pie

The next four questions ask about the kinds of pie you ate. Please read all four questions before answering.

112b. How often were the pies you ate **fruit pie** (such as apple, blueberry, others)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

112c. How often were the pies you ate **cream, pudding, custard,** or **meringue pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

112d. How often were the pies you ate **pumpkin** or **sweet potato pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 113 appears in the next column

112e. How often were the pies you ate **pecan pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

113. How often did you eat **chocolate candy**?

☐ NEVER (GO TO QUESTION 114)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

113a. Each time you ate **chocolate candy**, how much did you usually eat?

- ☐ Less than 1 average bar or less than 1 ounce
- ☐ 1 average bar or 1 to 2 ounces
- ☐ More than 1 average bar or more than 2 ounces

114. How often did you eat **other candy**?

☐ NEVER (GO TO QUESTION 115)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

114a. Each time you ate **other candy**, how much did you usually eat?

- ☐ Fewer than 2 pieces
- ☐ 2 to 9 pieces
- ☐ More than 9 pieces

115. How often did you eat **eggs, egg whites,** or **egg substitutes** (NOT counting eggs in baked goods and desserts)? *(Please include eggs in salads, quiche, and soufflés.)*

☐ NEVER (GO TO QUESTION 116)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 116 appears on the next page

Over the past 12 months...

115a. Each time you ate **eggs**, how many did you usually eat?

- ☐ 1 egg
- ☐ 2 eggs
- ☐ 3 or more eggs

115b. How often were the eggs you ate **egg substitutes**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

115c. How often were the eggs you ate **egg whites only**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

115d. How often were the eggs you ate **regular whole eggs**?

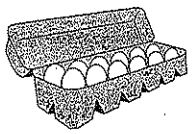
- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

115e. How often were the eggs you ate **cooked in oil, butter,** or **margarine**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

115f. How often were the eggs you ate part of **egg salad**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always



Question 116 appears in the next column

116. How many cups of **coffee**, caffeinated or decaffeinated, did you drink?

- ☐ NEVER (GO TO QUESTION 117)
- ☐ Less than 1 cup per month
- ☐ 1–3 cups per month
- ☐ 1 cup per week
- ☐ 2–4 cups per week
- ☐ 5–6 cups per week
- ☐ 1 cup per day
- ☐ 2–3 cups per day
- ☐ 4–5 cups per day
- ☐ 6 or more cups per day

116a. How often was the coffee you drank **decaffeinated**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

117. How many glasses **of ICED tea,** caffeinated or decaffeinated, did you drink?

- ☐ NEVER (GO TO QUESTION 118)
- ☐ Less than 1 cup per month
- ☐ 1–3 cups per month
- ☐ 1 cup per week
- ☐ 2–4 cups per week
- ☐ 5–6 cups per week
- ☐ 1 cup per day
- ☐ 2–3 cups per day
- ☐ 4–5 cups per day
- ☐ 6 or more cups per day

117a. How often was the iced tea you drank **decaffeinated** or **herbal tea**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

118. How many cups of **HOT tea**, caffeinated or decaffeinated, did you drink?

- ☐ NEVER (GO TO QUESTION 119)
- ☐ Less than 1 cup per month
- ☐ 1–3 cups per month
- ☐ 1 cup per week
- ☐ 2–4 cups per week
- ☐ 5–6 cups per week
- ☐ 1 cup per day
- ☐ 2–3 cups per day
- ☐ 4–5 cups per day
- ☐ 6 or more cups per day

118a. How often was the hot tea you drank **decaffeinated** or **herbal tea**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 119 appears on the next page

Over the past 12 months...

119. How often did you add **sugar** or **honey** to your coffee or tea?

- ☐ NEVER (GO TO QUESTION 120)
- ☐ Less than 1 cup per month
- ☐ 1–3 cups per month
- ☐ 1 cup per week
- ☐ 2–4 cups per week
- ☐ 5–6 cups per week
- ☐ 1 cup per day
- ☐ 2–3 cups per day
- ☐ 4–5 cups per day
- ☐ 6 or more cups per day

119a. Each time **sugar** or **honey** was added to your coffee or tea, how much was usually added?

- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

120. How often did you add **artificial sweetener** to your coffee or tea?

- ☐ NEVER (GO TO QUESTION 121)
- ☐ Less than 1 time per month
- ☐ 1–3 times per month
- ☐ 1 time per week
- ☐ 2–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

120a. What kind of **artificial sweetener** did you usually use?

- ☐ Equal or aspartame
- ☐ Sweet N Low or saccharin

121. How often was **non-dairy creamer** added to your coffee or tea?

- ☐ NEVER (GO TO QUESTION 122)
- ☐ Less than 1 time per month
- ☐ 1–3 times per month
- ☐ 1 time per week
- ☐ 2–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

121a. Each time **non-dairy creamer** was added to your coffee or tea, how much was usually used?

- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

Question 122 appears in the next column

121b. What kind of **non-dairy creamer** did you usually use?

- ☐ Regular powdered
- ☐ Low-fat or fat-free powdered
- ☐ Regular liquid
- ☐ Low-fat or fat-free liquid

122. How often was **cream** or **half and half** added to your coffee or tea?

- ☐ NEVER (GO TO QUESTION 123)
- ☐ Less than 1 time per month
- ☐ 1–3 times per month
- ☐ 1 time per week
- ☐ 2–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

122a. Each time **cream** or **half and half** was added to your coffee or tea, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 2 tablespoons
- ☐ More than 2 tablespoons

123. How often was **milk** added to your coffee or tea?

- ☐ NEVER (GO TO QUESTION 124)
- ☐ Less than 1 time per month
- ☐ 1–3 times per month
- ☐ 1 time per week
- ☐ 2–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2–3 times per day
- ☐ 4–5 times per day
- ☐ 6 or more times per day

123a. Each time **milk** was added to your coffee or tea, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 3 tablespoons
- ☐ More than 3 tablespoons

123b. What kind of **milk** was usually added to your coffee or tea?

- ☐ Whole milk
- ☐ 2% milk
- ☐ 1% milk
- ☐ Skim, nonfat, or ½% milk
- ☐ Evaporated or condensed (canned) milk
- ☐ Soy milk
- ☐ Rice milk
- ☐ Other

Question 124 appears on the next page

Over the past 12 months...

124. How often was **sugar** or **honey** added to foods you ate? (Please do not include sugar in coffee, tea, other beverages, or baked goods.)

- ☐ NEVER (GO TO INTRODUCTION TO QUESTION 125)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

124a. Each time **sugar** or **honey** was added to foods you ate, how much was usually added?

- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

The following questions are about the kinds of margarine, mayonnaise, sour cream, cream cheese, and salad dressing that you eat. If possible, please check the labels of these foods to help you answer.

125. Over the past 12 months, did you eat **margarine**?

- ☐ NO (GO TO QUESTION 126)
- ☐ YES

125a. How often was the margarine you ate **regular-fat margarine** (stick or tub)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

125b. How often was the margarine you ate **light** or **low-fat margarine** (stick or tub)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 126 appears in the next column

125c. How often was the margarine you ate **fat-free margarine**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

126. Over the past 12 months, did you eat **butter**?

- ☐ NO (GO TO QUESTION 127)
- ☐ YES

126a. How often was the butter you ate **light** or **low-fat butter**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

127. Over the past 12 months, did you eat **mayonnaise** or **mayonnaise-type dressing**?

- ☐ NO (GO TO QUESTION 128)
- ☐ YES

127a. How often was the mayonnaise you ate **regular-fat mayonnaise**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

127b. How often was the mayonnaise you ate **light** or **low-fat mayonnaise**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 128 appears on the next page

Over the past 12 months...

127c. How often was the mayonnaise you ate **fat-free mayonnaise**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

128. Over the past 12 months, did you eat **sour cream**?

- ☐ NO (GO TO QUESTION 129)
- ☐ YES

128a. How often was the sour cream you ate **regular-fat sour cream**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

128b. How often was the sour cream you ate **light, low-fat,** or **fat-free sour cream**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

129. Over the past 12 months, did you eat **cream cheese**?

- ☐ NO (GO TO QUESTION 130)
- ☐ YES

129a. How often was the cream cheese you ate **regular-fat cream cheese**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

129b. How often was the cream cheese you ate **light, low-fat,** or **fat-free cream cheese**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

130. Over the past 12 months, did you eat **salad dressing**?

- ☐ NO (GO TO INTRODUCTION TO QUESTION 131)
- ☐ YES

130a. How often was the salad dressing you ate **regular-fat salad dressing** (including oil and vinegar dressing)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

130b. How often was the salad dressing you ate **light** or **low-fat salad dressing**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

130c. How often was the salad dressing you ate **fat-free salad dressing**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

The following two questions ask you to summarize your usual intake of vegetables and fruits. Please do not include salads, potatoes, or juices.

131. Over the past 12 months, how many servings of **vegetables** (not including salad or potatoes) did you eat per week or per day?

- ☐ Less than 1 per week
- ☐ 1–2 per week
- ☐ 3–4 per week
- ☐ 5–6 per week
- ☐ 1 per day
- ☐ 2 per day
- ☐ 3 per day
- ☐ 4 per day
- ☐ 5 or more per day

Question 130 appears in the next column

Over the past 12 months...

132. Over the past 12 months, how many servings of **fruit** (not including juices) did you eat per week or per day?

- ☐ Less than 1 per week
- ☐ 1–2 per week
- ☐ 3–4 per week
- ☐ 5–6 per week
- ☐ 1 per day
- ☐ 2 per day
- ☐ 3 per day
- ☐ 4 per day
- ☐ 5 or more per day

133. Over the past month, which of the following foods did you eat **AT LEAST THREE TIMES?** ***(Mark all that apply.)***

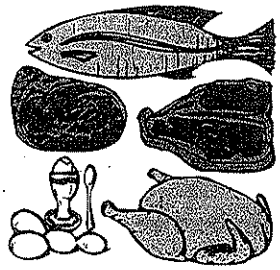
- ☐ Avocado, guacamole
- ☐ Cheesecake
- ☐ Chocolate, fudge, or butterscotch toppings or syrups
- ☐ Chow mein noodles
- ☐ Croissants
- ☐ Dried apricots
- ☐ Egg rolls
- ☐ Granola bars
- ☐ Hot peppers
- ☐ Jello, gelatin
- ☐ Milkshakes or ice-cream sodas
- ☐ Olives
- ☐ Oysters
- ☐ Pickles or pickled vegetables or fruit
- ☐ Plantains
- ☐ Pork neckbones, hock, head, feet
- ☐ Pudding or custard
- ☐ Veal, venison, lamb
- ☐ Whipped cream, regular
- ☐ Whipped cream, substitute
- ☐ NONE

134. For **ALL** of the past 12 months, have you followed any type of **vegetarian diet**?

- ☐ NO (GO TO INTRODUCTION TO QUESTION 135)
- ☐ YES

134a. Which of the following foods did you **TOTALLY EXCLUDE** from your diet? ***(Mark all that apply.)***

- ☐ Meat (beef, pork, lamb, etc.)
- ☐ Poultry (chicken, turkey, duck)
- ☐ Fish and seafood
- ☐ Eggs
- ☐ Dairy products (milk, cheese, etc.)



Introduction to Question 135 appears in the next column

The next questions are about your use of fiber supplements or vitamin pills.

135. Over the past 12 months, did you take any of the following types of **fiber or fiber supplements** on a regular basis (more than once per week for at least 6 of the last 12 months)? ***(Mark all that apply.)***

- ☐ NO, didn't take any fiber supplements on a regular basis (GO TO QUESTION 136)
- ☐ YES, psyllium products (such as Metamucil, Fiberall, Serutan, Perdiem, Correctol)
- ☐ YES, methylcellulose/cellulose products (such as Citrucel, Unifiber)
- ☐ YES, Fibercon
- ☐ YES, Bran (such as wheat bran, oat bran, or bran wafers)

136. Over the past 12 months, did you take any **multivitamins**, such as One-a-Day-, Theragran-, or Centrum-type multivitamins (as pills, liquids, or packets)?

- ☐ NO (GO TO INTRODUCTION TO QUESTION 138)
- ☐ YES

137. How often did you take **One-a-day-, Theragran-, or Centrum-type** multivitamins?

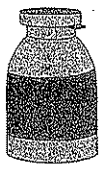
- ☐ Less than 1 day per month
- ☐ 1–3 days per month
- ☐ 1–3 days per week
- ☐ 4–6 days per week
- ☐ Every day

137a. Does your **multivitamin** usually contain **minerals** (such as iron, zinc, etc.)?

- ☐ NO
- ☐ YES
- ☐ Don't know

137b. For how many years have you taken **multivitamins**?

- ☐ Less than 1 year
- ☐ 1–4 years
- ☐ 5–9 years
- ☐ 10 or more years



Introduction to Question 138 appears on the next page

Over the past 12 months...

137c. Over the past 12 months, did you take any vitamins, minerals, or other herbal supplements other than your multivitamin?

☐ NO

Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- **Did not skip any pages and**
- **Crossed out the incorrect answer and circled the correct answer if you made any changes.**

☐ YES (GO TO INTRODUCTION TO QUESTION 138)

These last questions are about the vitamins, minerals, or herbal supplements you took that are NOT part of a One-a-day-, Theragran-, or Centrum-type of multivitamin.

Please include vitamins taken as part of an antioxidant supplement.

138. How often did you take **Beta-carotene** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 139)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

138a. When you took **Beta-carotene**, about how much did you take in one day?

☐ Less than 10,000 IU
☐ 10,000–14,999 IU
☐ 15,000–19,999 IU
☐ 20,000–24,999 IU
☐ 25,000 IU or more
☐ Don't know

138b. For how many years have you taken **Beta-carotene**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

Question 139 appears in the next column

139. How often did you take **Vitamin A** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 140)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

139a. When you took **Vitamin A**, about how much did you take in one day?

☐ Less than 8,000 IU
☐ 8,000–9,999 IU
☐ 10,000–14,999 IU
☐ 15,000–24,999 IU
☐ 25,000 IU or more
☐ Don't know

139b. For how many years have you taken **Vitamin A**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

140. How often did you take **Vitamin C** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 141)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

140a. When you took **Vitamin C**, about how much did you take in one day?

☐ Less than 500 mg
☐ 500–999 mg
☐ 1,000–1,499 mg
☐ 1,500–1,999 mg
☐ 2,000 mg or more
☐ Don't know

140b. For how many years have you taken **Vitamin C**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

Question 141 appears on the next page

Over the past 12 months...

141. How often did you take **Vitamin E** (**NOT** as part of a multivitamin in Question 137)?

- ☐ NEVER (GO TO QUESTION 142)
- ☐ Less than 1 day per month
- ☐ 1–3 days per month
- ☐ 1–3 days per week
- ☐ 4–6 days per week
- ☐ Every day

141a. When you took **Vitamin E**, about how much did you take in one day?

- ☐ Less than 400 IU
- ☐ 400–799 IU
- ☐ 800–999 IU
- ☐ 1,000 IU or more
- ☐ Don't know

141b. For how many years have you taken **Vitamin E**?

- ☐ Less than 1 year
- ☐ 1–4 years
- ☐ 5–9 years
- ☐ 10 or more years

142. How often did you take **Calcium** or **Calcium-containing antacids** (**NOT** as part of a multivitamin in Question 137)?

- ☐ NEVER (GO TO QUESTION 143)
- ☐ Less than 1 day per month
- ☐ 1–3 days per month
- ☐ 1–3 days per week
- ☐ 4–6 days per week
- ☐ Every day

142a. When you took **Calcium** or **Calcium-containing antacids**, about how much elemental calcium did you take in one day? *(If possible, please check the label for elemental calcium.)*

- ☐ Less than 500 mg
- ☐ 500–599 mg
- ☐ 600–999 mg
- ☐ 1,000 mg or more
- ☐ Don't know

Question 143 appears in the next column

142b. For how many years have you taken **Calcium** or **Calcium-containing antacids**?

- ☐ Less than 1 year
- ☐ 1–4 years
- ☐ 5–9 years
- ☐ 10 or more years

The last two questions ask you about other supplements you took more than once per week.

143. Please mark any of the following **single supplements** you took more than once per week (**NOT** as part of a multivitamin in Question 137):

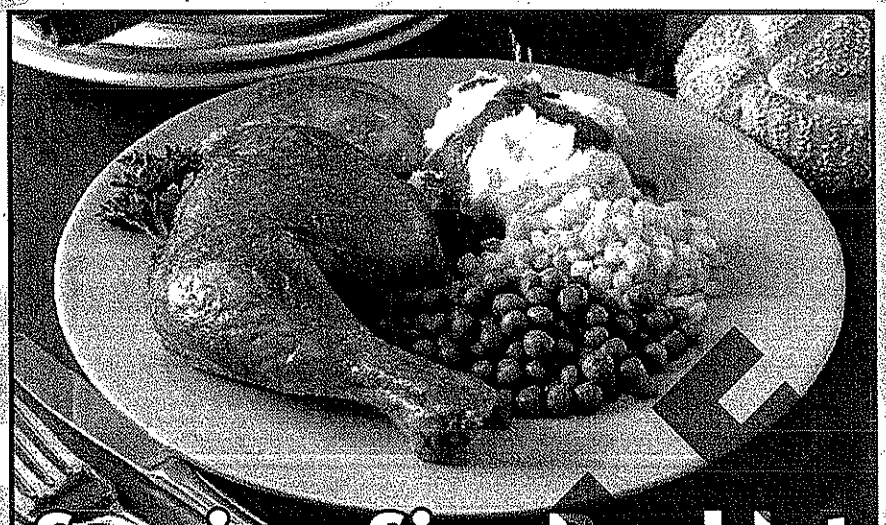
- ☐ B-6
- ☐ B-complex
- ☐ Brewer's yeast
- ☐ Cod liver oil
- ☐ Coenzyme Q
- ☐ Fish oil (Omega-3 fatty acids)
- ☐ Folic acid/folate
- ☐ Glucosamine
- ☐ Hydroxytryptophan (HTP)
- ☐ Iron
- ☐ Niacin
- ☐ Selenium
- ☐ Zinc

144. Please mark any of the following **herbal** or **botanical supplements** you took more than once per week.

- ☐ Aloe Vera
- ☐ Astragalus
- ☐ Bilberry
- ☐ Cascara sagrada
- ☐ Cat's claw
- ☐ Cayenne
- ☐ Cranberry
- ☐ Dong Kuai (Tangkwei)
- ☐ Echinacea
- ☐ Evening primrose oil
- ☐ Feverfew
- ☐ Garlic
- ☐ Ginger
- ☐ Ginkgo biloba
- ☐ Ginseng (American or Asian)
- ☐ Goldenseal
- ☐ Grapeseed extract
- ☐ Kava, kava
- ☐ Milk thistle
- ☐ Saw palmetto
- ☐ Siberian ginseng
- ☐ St. John's wort
- ☐ Valerian
- ☐ Other

Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- **Did not skip any pages and**
- **Crossed out the incorrect answer and circled the correct answer if you made any changes.**



Serving Size Booklet

SAMPLE

Thank you for participating in this important research study.

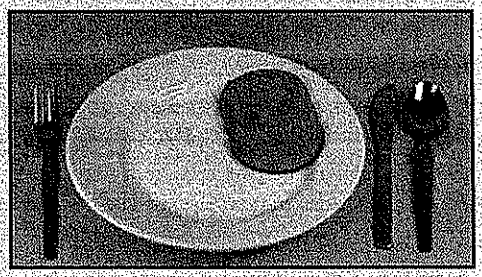
In the next few weeks, staff from the **Fred Hutchinson Cancer Research Center** in Seattle will call you to ask about the foods you ate on the previous day. You do not need to do anything special to prepare for this interview.

This booklet will help you estimate your serving sizes. Please look through it and have it available for the telephone interviews. If you told us that you preferred to be called at work, please keep this booklet at work.

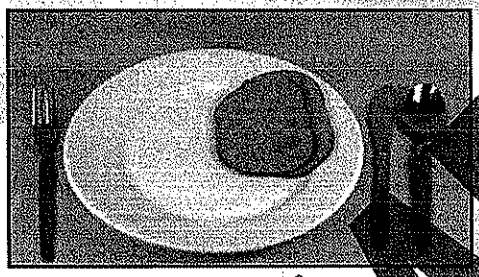
We appreciate your help with this research project.

You can estimate portion sizes **between** the picture amounts throughout this booklet.

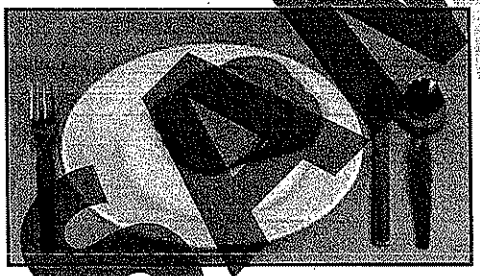
Beef, Pork, Chicken and Fish



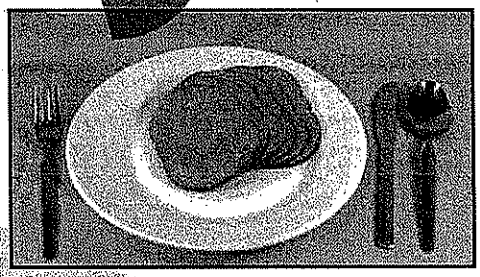
1-1/2 ounces



3 ounces



6 ounces



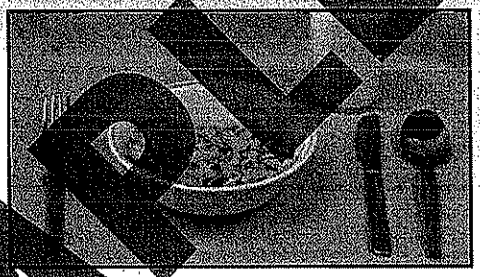
9 ounces

SAMPLE

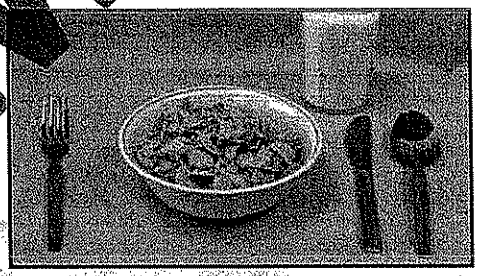
Cereal and Soup

1/2 cup

1 cup



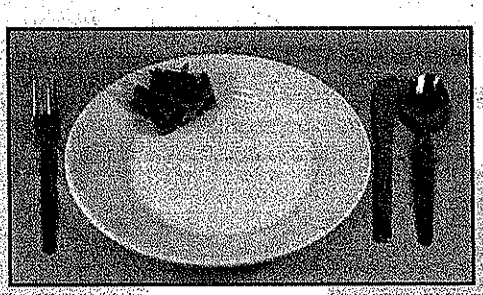
1-1/2 cups



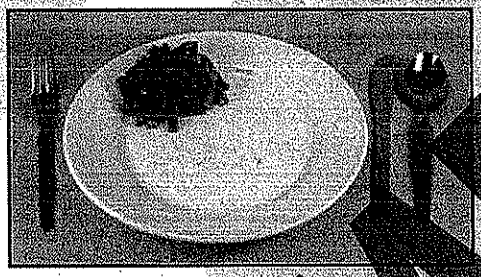
2 cups

SAMPLE

Vegetables such as Green Beans, Corn and Potatoes

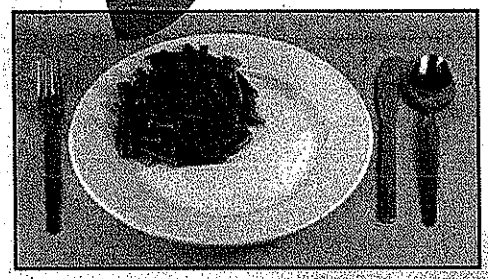


1/4 cup



1/2 cup

1 cup

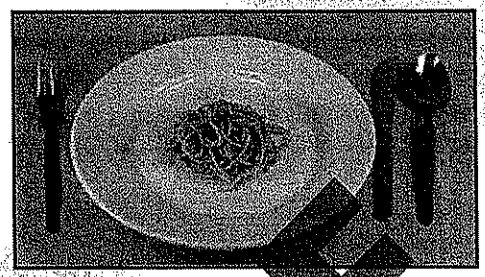


1-1/2 cups

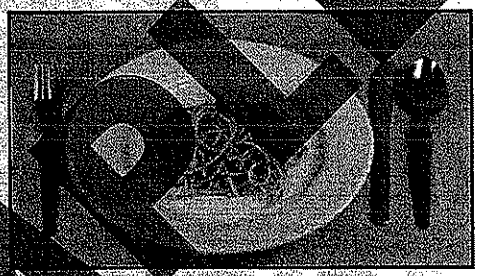
SAMPLE

Spaghetti and Casseroles

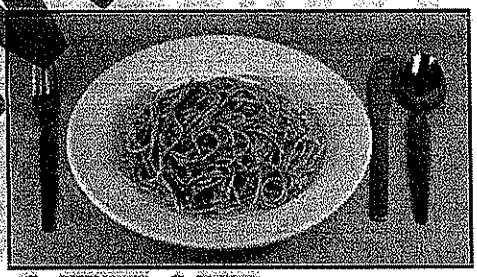
1/2 cup



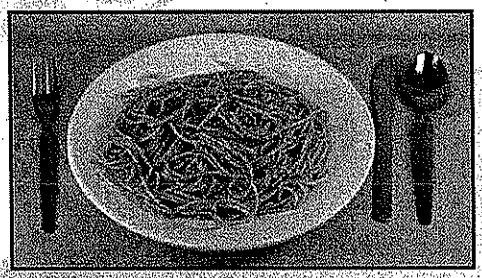
1 cup



2 cups

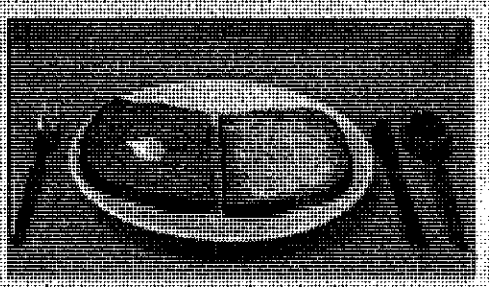


3 cups

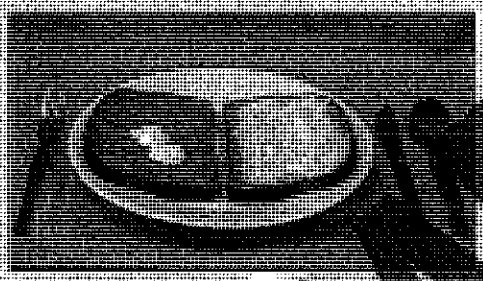


SAMPLE

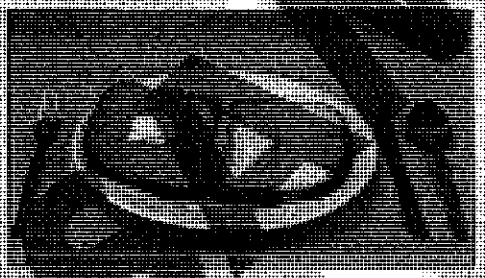
Spreads such as Butter, Margarine, Mayonnaise, or Peanut Butter



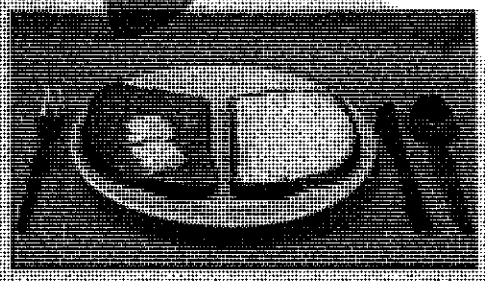
1 teaspoon



2 teaspoons



3 teaspoons
= 1 Tablespoon



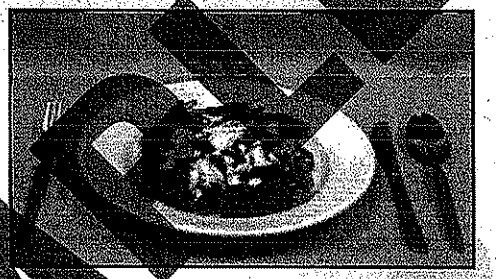
2 Tablespoons

SAMPLE

Salad Dressing

1 Tablespoon

2 Tablespoons

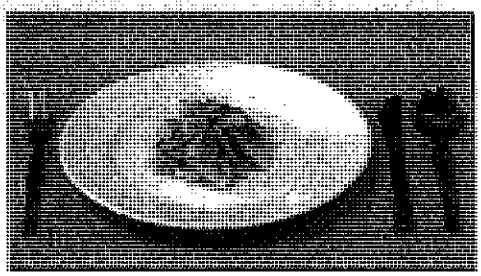


3 Tablespoons

4 Tablespoons
= 1/4 cup

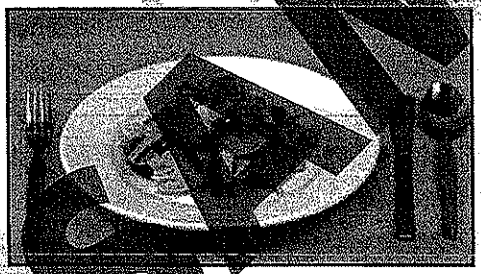
SAMPLE

Salads



1/2 cup

1 cup



1-1/2 cups

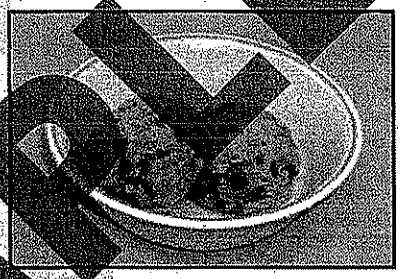
2 cups

SAMPLE

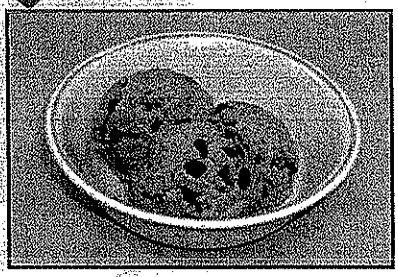
Ice Cream, Mashed Potatoes, or Cottage Cheese

1/2 cup

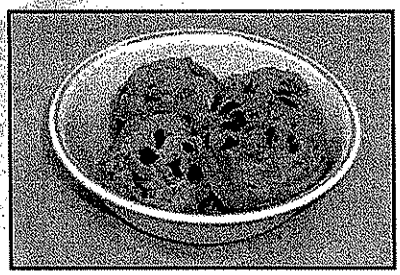
1 cup



1-1/2 cups



2 cups



SAMPLE

Seven Ways to Size Up Your Servings

1 3 ounces of meat is about the size and thickness of a deck of playing cards or an audiotape cassette.

2 A medium apple or peach is about the size of a tennis ball.

3 1 ounce of cheese is about the size of 4 stacked dice.

4 1/2 cup of ice cream is about the size of a racquetball or tennis ball.

5 1 cup of mashed potatoes or broccoli is about the size of your fist.

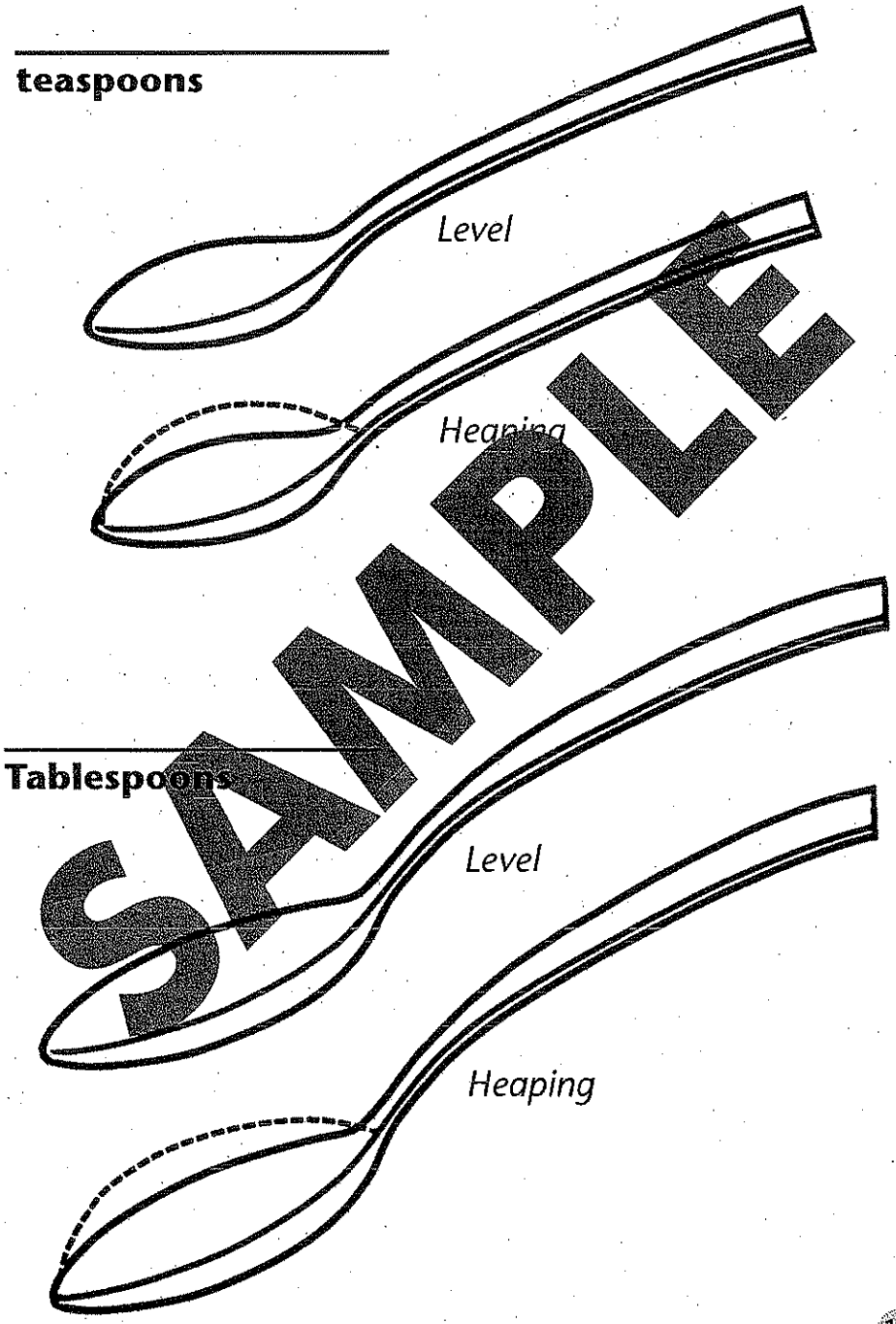
6 1 teaspoon of butter or peanut butter is about the size of the tip of your thumb.

7 1 ounce of nuts or small candies equals one handful.

SAMPLE

concept ©1996 National Dairy Council

Eating and Serving Spoons

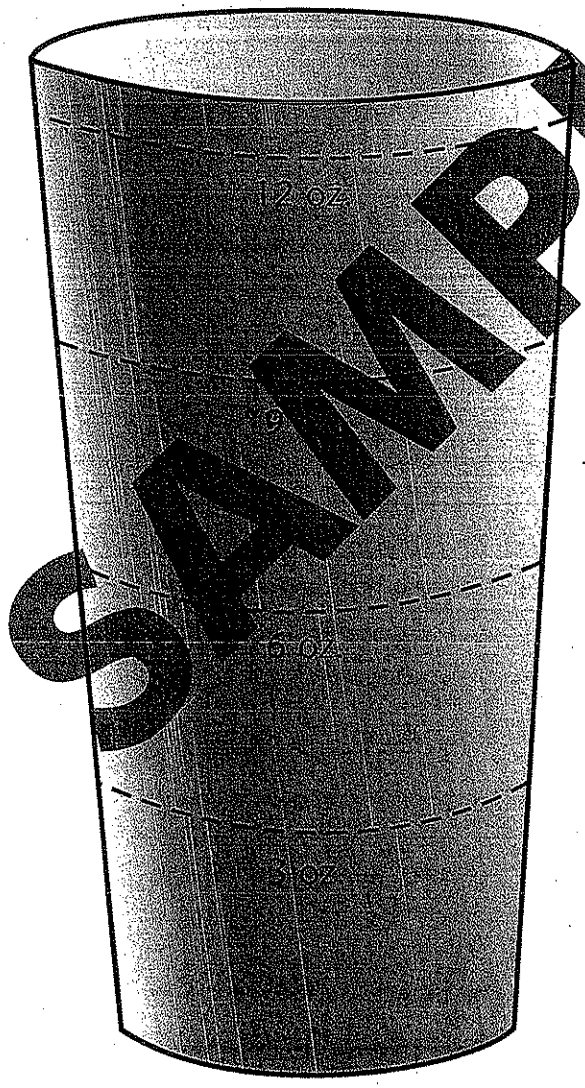


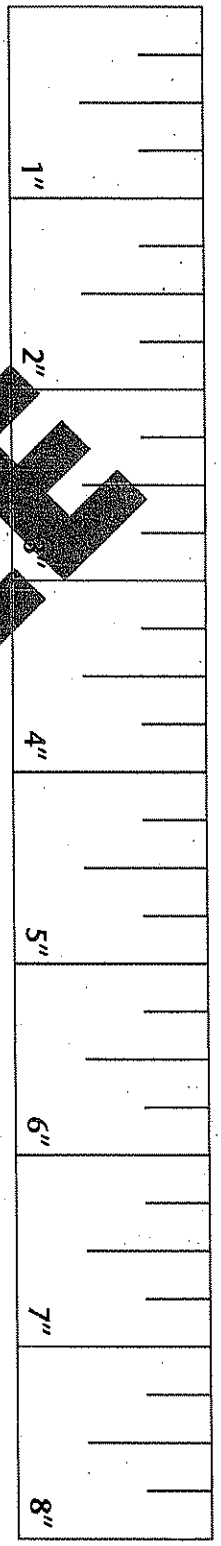
SAMPLE

Thickness

1
2
3
4
5

12 fluid ounces





SAMPLE

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Response to Minor Stipulations

Receipt Date : 1/30/2007 **Submission Type:** Initial **IRB ID :** 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong **IRB:** Biomedical **Coordinator:** Astrid Ertola

☑ Full Board Review ☐ Expedited Review

Study Description :

Submission Description :

3/16/07 Response and revised materials reviewed and approved. Ready for approval letter.

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter:
  - ☑ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date: 3/21/07 SAB)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 5 (only 1 copy of stamped c.f.)

Other attachments: ~~17~~ 18 (only 1 copy)

FINAL ACTIONS:

- ☑ Approved
- ☑ Approved with Minor Stipulations
- ☑ NHSR
- ☑ Return to Full IRB
- ☑ Return to sender
- ☑ Termination

MarieZeldin/Dr. Weber:

Attached is the following pertaining to Dr. Tong's protocol, *GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust IRB Study #: 07-0190*:

RECEIVED
MAR 1 5 2007
IRB UNC-CH

1. CRAC letter dated 2/6/07
2. PI response dated 3/2/07 - rec'd 3/6/07

Once you've looked over this and think the issues have been resolved, I'll forward the response to the IRB for their approval.

IRB:

I have looked over the attached materials provided by Dr. Tong regarding protocol *GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust IRB Study #: 07-0190* and have found his responses to be satisfactory.

Marie Zeldin 3/6/07
Marie Zeldin or David Weber
GCRC Research Subject Advocate

Paul Watkins, GCRC Director

OK per email 3/15/07
Paul Stewart, GCRC Biostatistician

N8

Full Committee Review

Receipt Date : 1/30/2007 **Submission Type:** Initial **IRB ID :** 07-0190

Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong **IRB:** Biomedical

Agenda Date 2/26/2007 Reviewer 1: ~~Kehrl, Howard~~ Bows, Cutson Reviewer 2: Weinstein, Diane

Additional Findings and/or Special Populations: ☐ Children ☐ Prisoners ☐ Pregnant Women

☐ Sensitive Info ☑ IND ☐ IDE ☐ DNA/Gene transfer ☑ Genetics ☑ GCRC ☐ ORC ☐ LAR

Meeting Notes and Required Findings :

- If excluding pregnancy, what about males & younger sexual partners? Who will pregnancy test be performed? Contraception?
- IND for Fish Oil?
- Will initial blood test for genotyping be done the same day as H&P. What is sequence?
- ~~Include~~ Endpoints for subject to proceed to next level of exposure are not clearly defined.
- CF - Language in "Purpose" section needs to be simplified.

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 2/28/07 H)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email/hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email/hard copy sent

Consent forms attached: 4

Other attachments:

FINAL ACTIONS:

- ☐ Approved
- ☒ Approved with Minor Stipulations
- ☐ Deferred
- ☐ Disapproved
- ☐ Return to sender
- ☐ Termination

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of
North Carolina
at
Chapel Hill
&
UNC
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Marie H. Zeldin, RN, BSN
Assistant Director
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Verne S. Caviness

General Clinical Research Center

RECEIVED
FEB 12 2007
IRB UNC-CH

MEMORANDUM

TO: Haiyan Tong, MD

FROM: Michael Fried, MD
Associate Director, GCRC

DATE: February 6, 2007

RE: GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

07-0190

Your protocol referenced above was reviewed at the February 6th meeting and I am pleased to inform you that it was conditionally approved for support. A few issues still remain that you will need to address before you can receive formal approval to proceed.

1. IRB Application:
   a) Clarify the exclusions (for the main study) related to drinking red wine and using olive oil as they are not stated very clearly in the application.
   b) Is there an IND number for this study? If so, please include on the IRB checklist. Confirm your use of IDS for the supplements.
2. Safety Monitoring Plan: Provide an updated SMP that includes the following:
   a) Question #1 (risks): Include the risks related to administration of nitroglycerin as part of the Brachial Artery Ultrasound and the fish oil/olive oil supplementation.
   b) Question #4 (safety tests): List the labs done at screening or on study days, including pregnancy testing, and when each is performed.
3. Biostatistics: Please see the attached comments by Dr. Paul Stewart, the GCRC Biostatistician. These include consideration of an alternative study design.
4. Pharmacy: Since your protocol requires the dispensation of clinical trial materials through the Investigational Drug Service, please contact Robert Granko, Pharm.D (rgranko@unch.unc.edu) 6-8 weeks in advance of treating your first study subject.
5. **Citation of the grant:** As you may know, our center is now being evaluated based on how many published manuscripts cite our grant number (RR000046). We may in the future be forced to withhold resources from investigators that do not appropriately cite the grant. **Please remember to cite the grant # RR000046 on any publication where this can be justified.**

Please address the above issues in a letter to me and submit 4 copies of the revised materials (highlighting the changes on 2 of the copies). In addition, if there are any IRB issues to address, send your response to those issues to this office as well.

This protocol has been established as a **Category A** study.

MWF:wcs

Attachment

GCRC# 2579

PI: Haiyan Tong, MD

STAFF REVIEW

BIOSTATISTICS

Summary

In a study of healthy adults the investigators will examine the effects of diesel exhaust, the ability of fish oil dietary supplement to mitigate these effects, and the role of the GSTM1 null or GSTM1 positive genotypes in this relationship.

Stage1: N=12 adults of ages 55-75 will be studies. The sample will be stratified as follows: n=6 with GSTM1 null genotype; n=2 with each of the 3 variants (Ile/Ile, Ile/Val, Val/Val) of GSTP1 codon 105. The goal of this stage will be used to estimate the optimum diesel exhaust (DEP) concentration which results in safe and measurable cardiovascular effects. The 12 subjects will be exposed to 100 $\mu g/m^3$ DEP for two hours during the first exposure visit. This exposure protocol will be repeated 2 and 4 weeks later at exposure levels of 200 $\mu g/m^3$, and 300 $\mu g/m^3$ DEP, respectively. The estimate of the optimal DEP concentration will be the lowest concentration which results in a statistically significant change from baseline in the primary response. Exposures at higher concentrations will be suspended once the estimate of the optimal concentration has been reached.

Stage2: N=60 adult subjects of ages 50-75 will be enrolled in a genotype-stratified, treatment-randomized, double-blind study of effects of fish oil on post-DEP cardiovascular effects.

Group I.	n=15	Fish oil	(2g/day)	and	GSTM1 pos.
Group II.	n=15	Fish oil	(2g/day)	and	GSTM1 null,
Group III.	n=15	Olive oil	(2g/day)	and	GSTM1 pos.
Group IV.	n=15	Olive oil	(2g/day)	and	GSTM1 null.

Primary Outcomes: heart rate variability (HRV), peripheral blood omega-3 fatty acid levels, peripheral blood biomarkers for specific and non-specific immune responses, peripheral blood coagulation factors, *etc.*

Secondary Outcomes: Spirometry variables, single breath diffusing capacity (DLCO) flow-mediated dilatation (endopat).

Primary hypotheses: (1) fish oil treatment reduces cardiovascular effects of DEP exposure. (2) GSTM1 pos. subjects have lower CV risk after DEP exposure.

ANOVA and a Bonferroni adjustment will be used to control for multiple comparisons where appropriate. Secondary analyses will use similar methods to explore the effect of DEP on the secondary outcomes. The investigators anticipate the Stage2 sample size will provide an 80% chance that the test (of size α=0.05) will detect a 10% treatment effect in ΔHRV.

Support

Computations for RDM will be performed by the investigators and the HSD QA manager. Statistical analysis will be performed by the investigators with the assistance of Dr. Haibo Zhou. Funding: EPA Intramural Federal Research

Concerns

1. It is not clear that Dr. Zhou has been consulted on the design of this protocol. Working closely with Dr. Zhou is encouraged.

2. There may be room for improvement in the choice of criterion in Stage I for choosing the dose. A criterion of "statistical significance" is heavily influenced by choice of sample size. Apart from safety considerations, the primary endpoint of Stage2 (HRV) should be the focus of the dose-selection during Stage1. A criterion worth considering: at each dose estimate the mean and percentiles of ΔHRV with the intent of finding the lowest dose that yields at least a 10%* change in HRV, on average. An alternative criterion: the lowest dose which causes at least 50%* of the subjects to experience a 5%* decrease in HRV. [* choose values per your expertise.]

3. A conceptual point: If the primary null hypothesis test is not statistically significant, then that test will be inconclusive. It is not true (as stated in the GCRC Addendum) that "If none of tests are statistically significant, we will know from this study that fish oil and GSTM1 genotype will not protect the cardiovascular and respirator system from diesel exhaust exposure."

4. Design of Stage2: a crossover design -or- a pre-treatment vs. post-treatment design may be more efficient than the proposed randomized parallel-arms design.

Consent Issues - #2579

Haiyan Tong

Recommendations from the RSA:

a) Clarify whether nitroglycerin will be given as part of the study procedures (endo PAT procedure) for subjects in the pilot study. If so, include in the CF the risks related to taking Nitroglycerin.

CLINICAL RESEARCH ADVISORY COMMITTEE

Scientific Review of Investigator-Initiated ("A" or "B") Studies

1. **DATE OF REVIEW** 02-02-2007

2. **PROJECT IDENTIFICATION**
   a) **Number** 2579
   b) **Title** Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
   c) **Principal Investigator(s)** Haiyan Tong, MD, PhD, James Samet, PhD, MPH

3. **GCRC PROGRAM QUESTIONS**
   a) Is this a multicenter study? No.
   b) Is this a clinical trial (i.e. assessing safety/efficacy of a treatment of device)? If yes, is this phase I, II, III or IV?Part one of this study is a phase I type trial to determine the optimum dose of diesel exhaust for testing cardiovascular effects of diesel exhaust. The main study is a trial of omega-3 fatty acids vs placebo in subjects who are exposed to the optimum dose of diesel exhaust.

4. **BRIEF SUMMARY**

This is a two part study: The first part, a pilot study, is to identify the optimum concentration of diesel exhaust to be used for testing exposure in the main study. The purpose of the main study is to 1) examine health effects, i.e., cardiac autonomic function and systemic inflammation, of diesel exhaust exposure on the cardiovascular system, 2) examine whether adverse cardiovascular effects can be attenuated by pretreatment with omega-3 fatty acid supplementation, and 3) whether healthy older individuals with genotype GSTM1 have less cardiovascular risk than the null genotype GSTM1 when exposed to diesel exhaust.

The pilot study to identify the optimum concentration (100ug/m3, 200ug/m3, 300ug/m3) of diesel exhaust will enroll six subjects, ages 50-75 males and females, healthy, 2 each of GSTM1 null and codon 105 genotypes Ile/Ile; Ile/Val and Val/Val. They will have a two hour exposure at each concentration for 3 visits 2 weeks apart. Primary endpoints are heart rate variability, pulmonary function, biomarkers including neutrophils, platelets, fibrinogen, c-reactive protein, lactate, ferritin, alpha-1 antitrypsin. An analysis will be done at the end of each level before proceeding to the next level. Testing will stop when statistically significant effects are found. 20 ml of blood will be collected during this part of the study.

The main study will enroll 60 healthy 50-75 yo male and female subjects, 30 subjects that are genotype GSTM1 positive and 30 subjects that are GSTM1 null. Study is randomized and double-blinded with four groups:

Group 1 GSTM1 positive receiving fish oil,
Group 2: GSTM1 positive receiving placebo,
Group 3: GSTM1 null receiving fish oil,
Group 4: GSTM1 null receiving placebo.

Subjects will attend a training session after being enrolled into the study, and will sign additional consent forms, complete a food frequency questionnaire, receive diet instructions regarding consumption of various dietary oils, and medication instruction sheets. Subjects will receive a 15 day course of either 2 g/day of fish oil or 2 g/day of olive oil capsules. The capsules are provided by Pharmavite, LLC. Subjects will be exposed to the optimum concentration of diesel exhaust for 2 hours following 15 days of daily oil supplementation. 50 ml of blood will be collected during this part of the study. .

Subject recruitment will be handled by the Westat Corporation. Subjects are recruited under a separate consent that has been approved by the UNC IRB. This recruitment includes a telephone screening, physical examination, medical history, cardiac arrhythmia screening, and blood sample for genotype screening.

5. **SCIENTIFIC MERIT** - What benefits or new knowledge is likely to result from study?

Omega 3 fatty acids found in fish oil are thought to have protective effects on the cardiovascular system including antiarrhythmic, antithrombotic, antiatherosclerotic, anti-inflammatory, improved endothelial function, lowering of blood pressure (see National Library of Medicine monograph attached). Likewise, substantial research has shown associations between particulate matter (PM) and adverse cardiovascular effects including all of the ones just mentioned. The effect of diesel emissions on the human respiratory system has been well studied; however the effect on the human cardiovascular system has not. This study will add new

information to the scientific literature about the health effects of diesel emissions on the human cardiovascular system.

6. **POSSIBLE HAZARDS** - Risks include the usual invasion of privacy associated with providing information for any research project, bruising associated with phlebotomy, and cardiac or respiratory distress caused by the diesel exhaust exposure. Specific procedures are described to minimize these risks including privacy procedures followed by the investigator and staff, having a physician on site during all testing, and the availability of equipment including an emergency cart with defibrillator in the event of a medical emergency. In addition, subjects are excluded from participating if any cardiac arrhythmias or abnormalities are identified during the screening procedure. There are risks associated with high doses of omega-3 fatty acids, but the doses that are being given are accepted as safe.

7. **INDUCEMENTS FOR PARTICIPATION** - Subjects will receive $1087 for participating in the main study and $1461 for participating in the pilot study. These fees are prorated based on each part of the study that they complete. The application states that if a part of the study is dropped due to changes made by the investigator, that the subject will still be compensated for that.
   - **How is informed consent obtained?**
     Informed consent is obtained by Westat during recruitment for screening procedures and by the principal investigator or their staff once the subject passes screening.
   - **Is the consent form adequate for the study?**
     The consent form appears to be adequate and there are separate consents for each part of the study.
   - **How will confidentiality be assured?**
     All subjects will be assigned a numerical identification and names will not be associated with study records.

8. **DESIGN OF STUDY POPULATION: INCLUSION OF ADEQUATE GENDER AND MINORITY REPRESENTATION.**
   This is a study of a specific high risk age group and includes men and women of all ethnic/racial backgrounds.

9. **INTERVAL AND FORM FOR PROGRESS REPORTS**
   Recommend annual report in the standard format.

10. **LIST SPECIFIC QUESTIONS AND/OR CONCERNS TO BE INCLUDED IN THE LETTER TO THE INVESTIGATOR (Please number).**
    None.

11. **RECOMMENDATIONS TO PARENT COMMITTEE**
    a) Science* : 1.5-2.0
    b) Mission*: 2.0
    c) Category@ A

Respectfully submitted,
Marjorie Busby

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097

TO: Haiyan Tong
Environmentally Protection Agency
7315

FROM: Biomedical IRB

DATE: 2/27/2007

RE: Minor Contingencies to be addressed following Full Board IRB Review on 2/26/2007
Submission Type: Initial
Study #: 07-0190
Other #: GCRC - GCRC-2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been reviewed by the above IRB at a convened meeting. This is not an IRB approval. You may not implement the research activities described in your submission until you have received a memo indicating final IRB approval. The IRB determined that this submission MAY BE APPROVED, pending stipulated changes and/or clarifications as detailed below.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the items listed below. Any additional changes (including new materials) must also be listed and discussed in the memo. You will need to return 2 copies of all revised materials to the IRB, one in which all changes are underlined and the other a "clean" copy with no underlining.

If this is a GCRC study all materials must be submitted to the GCRC before it can be reviewed by the IRB. The GCRC will send it to the IRB after they have reviewed it. If there is also a memo from the GCRC, you will need to address the GCRC issues in a separate point-by-point memo which should be included in the materials sent to the GCRC.

Details:
1. Please provide a signature for the Conflict of Interest Section.
2. Please clarify whether men should use a barrier method to avoid partner pregnancy.
3. If pregnant women will be excluded, will women be asked to use contraception? When will a pregnancy test be performed?
4. Please explain why the box for an IND has been checked on the application.
5. Please explain whether x-rays and other study related procedures will all be performed on the first day or if just the blood draw for genotyping will be done
6. Please describe what the stopping rules are for this study. The endpoints for subjects to proceed to the next level of exposure are not clearly defined..
7. Please simplify consent form language in the purpose section. Please see marked consent form for additional changes.

Call the IRB at 966-3113 if you have any questions. You can now access IRB status information at https://my.research.unc.edu/.

CC:
James Samet, Environmentally Protection Agency, , Faculty Advisor
Wanda Simmons, (GCRC), Non-IRB Review Contact

RECEIVED
MAR 1 5 2007
IRB UNC-CH

To: Biomedical IRB
UNC-Chapel Hill

From: Haiyan Tong, MD, PhD
Human Studies Division
National Health and Environmental Effects Research Laboratory

Date: 3/2/2007
Re: Responses to initial IRB full board review
IRB study #: 07-0190
GCRC #: 2579
Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Thank you for your recent memo informing us of the disposition of our above-referenced study. As instructed, we have prepared point-by-point responses to the Reviewers queries. Revised study materials are attached.

1.Please provide a signature for the Conflict of Interest Section.

We have signed the conflict of interest section page.

2. Please clarify whether men should use a barrier method to avoid partner pregnancy.

Participation in this study does not create a specific need **for men to use a barrier method to avoid partner pregnancy.**

3. If pregnant women will be excluded, will women be asked to use contraception? When will a pregnancy test be performed?

Female subjects will not be asked to use contraception in this study. However, a menstrual history will be collected on all female subjects, and a pregnancy test will be performed on the training day and the exposure day if more than 7 days since last pregnancy test it will be repeated.
These have been clarified on page 13 in the protocol and page 3 in the consent forms.

4. Please explain why the box for an IND has been checked on the application.

Thank you for pointing out this mistake. The check mark has been removed.

5. Please explain whether x-rays and other study related procedures will all be performed on the first day or if just the blood draw for genotyping will be done.

On the physical exam and genotyping day (the first day), subjects will undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG). Subjects will then have approximately 20 ml of blood collected in the pilot study and approximately 50 ml of blood collected in the main study, and a portion of these samples will be used for genetic screening (please see page 12 and 13 under the subtitle of "physical exam").

6. Please describe what the stopping rules are for this study. The endpoints for subjects to proceed to the next level of exposure are not clearly defined.

In the pilot study, subjects will be monitored for symptoms that they may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, dyspnea, pallor, ataxia, and significant cardiac arrhythmias. In addition, analyses of blood samples taken after exposure will be scrutinized for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. Symptoms and/or changes detected in the blood samples that are considered clinically significant could prompt the study physicians to stop the study.
Relevant text has been added to the protocol (page 12) and consent form (page 4).

We also deleted "statistical" from this paragraph on page 12 in the IRB protocol.

7. Please simplify consent form language in the purpose section. Please see marked consent form for additional changes.

We have simplified the language on the consent forms. Please find these changes highlighted on the main and pilot study consent forms.

8. **We have changed "form" to "from" on page 2 in the pilot study genotyping consent.**

9. **We have added examples of β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol) on page 2 in the main study and pilot study genotyping consent.**

10. **We have made changes in response to reviewer's comments, as highlighted in the main study consent form.**

11. **We have made several changes in response to reviewer's comments that are highlighted in the pilot study consent form.**

RECEIVED
MAR 1 5 2007
IRB UNC-CH

To: Michael Fried, MD
Associate Director, GCRC

From: Haiyan Tong, MD, PhD
Human Studies Division
National Health and Environmental Effects Research Laboratory

Date: 3/2/2007

Re: Responses to initial GCRC review
IRB study #: 07-0190
GCRC #: 2579

Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Thank you for your recent communications informing us of the status of our above-referenced studey application. This memorandum addresses the Reviewers' quries in poin-by-point fashion.

1. **IRB Application**

a) We have clarified the criteria of not drinking red wine and olive oil use in the IRB protocol.
b) There is no IND for this study. We have removed the mark in the IRB protocol and apologize for the confusion.

2. **Safety Monitoring Plan**

a) Question #1 (risks): We have added text addressing the risks associated with administration of sublingual nitroglycerin in the brachial artery ultrasound measurement on page 2. We have also responded to the request for and explanation of the and risks associated with fish oil/olive oil on page 3. Please note that Nitroglycerin will not be given as part of the endo PAT procedure for subjects in the pilot study.
b) Question #4 (safety tests): We have expanded the answer on question #4 on page 4.

3. **Biostatistics**

a) Dr. Zhou is a consultant on the design of this study and is working with us in the application phase. He will also assist us with data analyses.
b) The sample size of 15 in the stage 2 study was determined in order to detect a difference of 10% changes in HRV assuming a standard deviation of 9%. A 10% change in HRV is the smallest that we would consider biologically significant.
c) We agree that if the primary null hypothesis test is not statistically significant, then the test will be inconclusive. We have changed the answer on question # 4.5.4

d) The randomized study design was chosen because of the fish oil/olive oil supplementation and the need to limit the study to a single exposure. Either a crossover design or a pre/post-treatment design would require multiple exposures. In addition, the washout period for the fat soluble supplements used in this study is lengthy and would add considerable time to the participation duration for each subject.

4. **Pharmacy** We have submitted a request to the Investigational Drug Service (IDS) through a memo to Dr. Robert Granko when we submitted our initial GCRC protocol. Attached please find a memo from Dr. Robert Granko acknowledging IDS participation in this study.

5. As requested, we will cite grant number (RR00046) when we publish this study.

UNC HEALTH CARE

RECEIVED
MAR 15 2007
IRB UNC-CH

Investigational Drug Services
Department of Pharmacy
UNC Hospitals
CB 7600, Room 3001
101 Manning Drive
Chapel Hill, NC 27514
Office: 919-966-1766
Fax: 919-966-6359

FROM: Robert P. Granko, Pharm.D.
Director, Investigational Drug Services

TO: James Samet, Haiyan Tong, Robert Devlin

CC: Maryann Bassett, RN

DATE: February 8, 2007

RE: **IDS** Protocol (P07-654c) "OMEGA"

Investigational Drug Services (IDS) has been contacted to provide pharmacy services for the above protocol. A pharmacist member of the IDS staff has reviewed the protocol and the protocol has been filed in the IDS pending file until notification is received from the principal investigator that the protocol is ready to screen patients.

Once IDS receives the notification that the protocol is actively screening for patients, a meeting will be scheduled with the study coordinator to review the specifics of the protocol and set a time-line for the first dispensation of clinical trial materials.

Should you require any additional information, please do not hesitate to contact me.

Please notify IDS six weeks before you plan on randomizing the first study subject so that we may in-service all of the appropriate staff and template the study protocol orders.

In addition, the following items (if applicable) are needed to finalize the protocol with IDS for dispensation of Clinical Trial Materials:

1. A copy or access to the Investigator's Brochure.
2. A copy of the FDA 1572 form or a list of authorized prescribers if a 1572 is not required.
3. A copy of the Pharmacy Manual and/or Sponsor provided Accountability Records.
4. A 98 account or University Grant number.

IDS Protocol Budget

Please notify IDS six weeks before you plan on randomizing the first study subject so that we may in-service all of the appropriate staff and template the study protocol orders.

Investigational Drug Services
Annual Fee for Services*

☐ Industry Sponsored	$ 2000.00
☐ Investigator Initiated, Industry Sponsored	$ 1000.00
☒ NIH/Gov/Foundation Sponsored	$ 1500.00
☐ Investigator Initiated, No Sponsor	$ 500.00

*Per year, payable upon receipt of drug/study notebook completion and yearly thereafter.

Note: The section below should be filled out and signed by business administrator or person responsible for disbursement of funds, and returned to the Investigational Drug Service (mail or fax) before start of the study; if not returned, IDS is authorized to bill for its services based on this sheet as-is.

Prepared By: Robert P. Granko, Pharm.D.		*Name and address where invoices should be sent:*
Principal Investigator/ Business Administrator Signature:	Date:	
Account Number (☐University or ☐ Hospital) Departmental Internal Tracking Number if applicable:		

By signing this agreement the Investigational Drug Service agrees to provide the above services.

The Principal Investigator agrees to the following:

1. To provide a copy of or access to both the complete written protocol and the Investigator's Brochure
2. To provide full reimbursement for the above stated services
3. To provide a copy of the FDA form 1572 or a list of all authorized prescribers if a 1572 is not required
4. To provide a copy of the final IRB approval letter
5. To renegotiate the Service Agreement if (a) protocol revisions call for a change in the services needed from the Investigational Drug Service or (b) the cost of materials used in provided such services increases.

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF HUMAN SUBJECTS RESEARCH
Version 2-Nov-2006

RECEIVED
JAN 30 2007
IRB UNC-CH

For IRB Use		
Behav	Biomed	PH-Nurs
IRB Study # 07-0190		
Rec'd		
Full	Expedited	Exempt

Part A.1. Contact Information, Agreements, and Signatures

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Date: 10 January, 2007
GCRC #: 2579

original signatures

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH
Co- Principal Investigator: Robert Devlin, PhD.
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
UNC-CH PID:
Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov
For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: James M Samet, PhD, MPH
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
Phone #: (919) 966-0665 Fax #: (919) 966-6271 Email Address: samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department:
Mailing address/CB #:
Phone #:
Fax #:
Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor:
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): **Sponsor or award number**: EPA Intramural Federal Research

Include following items with your submission, where applicable.
- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. This application. One copy must have original PI signatures.	3
X	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
X	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
X	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Protocol, grant application or proposal supporting this submission; (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal).	3
X	7. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
X	10. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator — Date: Jan 25, 2007

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor — Date

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee — Date: 01/25/2007

Print Name of Department Chair or designee: Robert Devlin, PhD

Department: U.S. EPA Clinical Research Branch

Part A.2. Summary Checklist

Are the following involved?	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	X
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X	__
A.2.3. Videotaping, audiotaping, filming of subjects (newly collected or existing)?	__	X
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	X	
b. Non-English-speaking?		X
c. Decisionally impaired?		X
d. Patients?	X	
e. Prisoners, others involuntarily detained or incarcerated, or parolees?		X
f. Pregnant women?		X
g. Minors (less than 18 years)? ***If yes***, give age range: to years		X
A.2.5. a. Is this a multi-site study (sites outside UNC-CH engaged in the research)?		X
b. Is UNC-CH the sponsor or lead coordinating center?		X
If yes, include the ***Addendum for Multi-site Studies where UNC-CH is the Lead Coordinating Center***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, provide contact information for the foreign IRB.		
A.2.6. Will there be a data and safety monitoring committee (DSMB or DSMC)?	__	X
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	X
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	X
A.2.8. a. Investigational drugs? (provide **IND #** _____)	X	
b. Approved drugs for "non-FDA-approved" conditions?		X
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	X	
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #** _____)	__	X
A.2.11. Fetal tissue?	__	X
A.2.12. Genetic studies on subjects' specimens?	X	__
A.2.13. Storage of subjects' specimens for future research? ***If yes, see instructions for Consent for Stored Samples.***	X	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? ***If yes, approval by the UNC-CH Radiation Safety Committee is required.***	__	X
A.2.15. Recombinant DNA or gene transfer to human subjects? ***If yes, approval by the UNC-CH Institutional Biosafety Committee is required.***	__	X
A.2.16. Does this study involve UNC-CH cancer patients? ***If yes, submit this application directly to the Oncology Protocol Review Committee.***	__	X
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? ***If yes, obtain the GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.***	X	__

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	X no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the Sponsor of this study for the use or benefit of any member of the research team?	__ yes	X no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

OFFICE OF HUMAN RESEARCH ETHICS

Institutional Review Board

APPLICATION FOR IRB APPROVAL OF HUMAN SUBJECTS RESEARCH

Version 2-Nov-2006

RF[illegible]

MAR [illegible] 2007

IRB [illegible]

For IRB Use		
Behav	Biomed	PH-Nurs
IRB Study #_____		
Rec'd_____		
Full	Expedited	Exempt

Part A.1. Contact Information, Agreements, and Signatures

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Date: 2 March, 2007
IRB #: **07-0190**
GCRC #: 2579

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH
Co- Principal Investigator: Robert Devlin, PhD.
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
UNC-CH PID:
Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov
For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: James M Samet, PhD, MPH
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
Phone #: (919) 966-0665 Fax #: (919) 966-6271 Email Address: samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department:
Mailing address/CB #:
Phone #:
Fax #:
Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor:
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): **Sponsor or award number**: EPA Intramural Federal Research

Include following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. This application. One copy must have original PI signatures.	3
X	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
X	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
X	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Protocol, grant application or proposal supporting this submission; (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal).	3
X	7. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
X	10. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator _____ Date _____

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor _____ Date 3/5/07

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee _____ Date _____

Print Name of Department Chair or designee _____ Department _____

Part A.2. Summary Checklist

Are the following involved?	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	X
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X	__
A.2.3. Videotaping, audiotaping, filming of subjects (newly collected or existing)?	__	X
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	X	
b. Non-English-speaking?		X
c. Decisionally impaired?		X
d. Patients?	X	
e. Prisoners, others involuntarily detained or incarcerated, or parolees?		X
f. Pregnant women?		X
g. Minors (less than 18 years)? ***If yes***, give age range: to years		X
A.2.5. a. Is this a multi-site study (sites outside UNC-CH engaged in the research)?		X
b. Is UNC-CH the sponsor or lead coordinating center?		X
If yes, include the *Addendum for Multi-site Studies where UNC-CH is the Lead Coordinating Center*.		
If yes, will any of these sites be outside the United States?	__	__
If yes, provide contact information for the foreign IRB.		
A.2.6. Will there be a data and safety monitoring committee (DSMB or DSMC)?	__	X
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	X
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	X
A.2.8. a. Investigational drugs? (provide **IND #** _____)		X
b. Approved drugs for "non-FDA-approved" conditions?		X
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	X	
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #** _____)	__	X
A.2.11. Fetal tissue?	__	X
A.2.12. Genetic studies on subjects' specimens?	X	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for Consent for Stored Samples.*	X	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the UNC-CH Radiation Safety* ***Committee*** *is required.*	__	X
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the UNC-CH Institutional Biosafety* ***Committee*** *is required.*	__	X
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the Oncology Protocol Review Committee.*	__	X
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	X	__

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	X no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the Sponsor of this study for the use or benefit of any member of the research team?	__ yes	X no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ 3/5/07

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. The first part of this protocol is a pilot study to identify the optimum diesel exhaust concentration to investigate the cardiovascular effects in healthy older subjects. The main part of this proposal is to examine the health effects of diesel exhaust exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust.
Participants: Approximately 6 healthy 50-75 year-old male and female subjects with GSTM1 null and GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val; n=2 each) for the pilot study. Sixty healthy 50-75 year-old male and female subjects (30 subjects are GSTM1 positive genotype and 30 subjects are GSTM1 null) for the main study.
Procedures (methods): In the pilot study, subjects will have 3 sequential exposures to the diesel exhausts at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours with a about 2 weeks of interval between exposures. In the main study, GSTM1 positive and GSTM1 null genotype subjects will be exposed to the optimum concentration (will be determined based on the pilot study) of diesel exhaust for 2 hours after 15 days of omega-3 fatty acid fish oil or olive oil supplementations.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3).

The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13).

A recent epidemiological study involving eight European cities suggested that the primary effect of PM air pollution on cardiac admissions was likely attributable to diesel exhaust inhalation (14). Diesel exhaust is a component of air pollution in urban settings, and contributes both PM and gaseous phase compounds to the atmosphere. Diesel exhaust fumes are made up largely of fine and ultrafine carbonaceous particulates generated by incomplete combustion of fuel. The U.S. EPA and the National Research Council (NRC) PM Research Priorities currently include identification of components of PM (including PM size and chemical composition) that contribute to the reported increased morbidity and mortality (NRC, 1998; EPA, 1997). In addition, both the EPA and NRC have stressed the importance of examining gaseous co-pollutants that may also affect PM toxicity. Diesel exhaust (i.e., gas phase constituents and diesel exhaust particles (DEP)) is ideal for examining the role of these factors in inducing human health effects for the following reasons: (1) Because of the relatively high content of organic compounds on DEP (usually 10-30% by weight), DEP are ideal for examining the role of organics in inducing health effects; (2) The exhaust is a mixture of PM and gaseous pollutants including some (e.g., aldehydes, NOx, carbon monoxide) that are known to produce human toxicity in high enough concentrations that may potentially augment PM-induced effects; (3) the size of the DEP vary depending on engine type and other factors, allowing assessment of PM size on the induction of human health effects. Few controlled human exposures to diesel exhaust have been performed. One hour exposure to 300 $\mu g/m^3$ diesel exhaust with intermittent exercise in healthy human volunteers resulted in marked systemic and redox-sensitive pulmonary inflammatory responses (15, 16), and vascular dysfunction and impaired endogenous fibrinolysis that links diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (17). Despite a decade of intensive studies, much about the PM health effects problem, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that diesel exhaust exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of diesel exhaust exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (18)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death

after myocardial infarction in humans due to its anti-arrhythmic effect (19-21). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (22). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (23). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (24). Another enzyme called GSTP1 is also associated with anti-oxidants effects and we will determine the optimal concentration of diesel exhaust in the pilot study in the most vulnerable subgroup with GDTM1 null and 3 GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val). Therefore, the role of GSTs in mediating the cardiovascular effects following acute diesel exhaust exposure will also be studied in the main protocol in GSTM1 null and GSTM1 positive healthy older adults.

U.S. EPA regulations concerning diesel emissions, mandated by the Clean Air Act, have resulted in dramatic decreases in emissions from diesel engines since 1988, with the last set of significant engine modifications occurring in 1995 (EPA, 1998). The respiratory effects of diesel exhaust have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to diesel exhaust in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to diesel exhaust in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.

11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Le Tertre, A., Medina, S., Samoli, E., Forsberg, B., Michelozzi, P., Boumghar, A., Vonk, J.M., Bellini, A., Atkinson, R., Ayres, J.G., Sunyer, J., Schwartz, J. and Katsouyanni, K. (2002). Short-term effects of particulate air pollution on cardiovascular diseases in eight European cities. J Epidemiol Community Health 56:773-779.
15. Salvi, S., Blomberg, A., Rudell, B., Kelly, F., Sandstrom, T., Holgate, S.T. and Frew, A. (1999). Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. American journal of respiratory and critical care medicine 159:702-709.
16. Behndig, A.F., Mudway, I.S., Brown, J.L., Stenfors, N., Helleday, R., Duggan, S.T., Wilson, S.J., Boman, C., Cassee, F.R., Frew, A.J., Kelly, F.J., Sandstrom, T. and Blomberg, A. (2006). Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Respir J 27:359-365.
17. Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstrom, T. and Newby, D.E. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112:3930-3936.
18. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
19. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
20. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
21. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
22. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
23. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
24. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.
25. Lauer, M.S., Pothier, C.E., Chernyak, Y.B., Brunken, R., Lieber, M., Apperson-Hansen, C. and Starobin, J.M. (2006). Exercise-induced QT/R-R-interval hysteresis as a predictor of myocardial ischemia. Journal of electrocardiology 39:315-323.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal includes approximately 6 subjects with GSTM1 null and GSTP1 codon 105 (Ile/Ile, Ile/Val, and Val/Val; n=2 each) genotypes for the pilot study and 60 subjects (30 subjects GSTM1

positive and 30 subjects GSTM1 null) for the main study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria (pilot study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- No exposure will be conducted within 4 weeks of a respiratory tract infection.

Exclusion criteria (main study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin.

- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Female subjects currently taking estrogen replacement therapy.
- Because of reported cardioprotective effects of red wine, subjects will be prohibited from drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

Pilot study: Approximately 6 subjects will undergo 3 exposures to air containing approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts sequentially for 2 hours in an exposure chamber. There will be a 2-week interval between exposures. We anticipate that there will be 6 subjects at each concentration of diesel exhaust exposure during the course of this pilot study. However, circumstances beyond our control may arise (i.e. subject/subjects might drop off due to illness and/or other reasons during the study) which may prevent finishing the proposed 3 exposures, and then we will have to add new subject/subjects at that point. Each new subject will have 3 exposures at approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts.

Subjects who withdraw from the study will be compensated for all procedures and time completed to that point (See the pilot study design flow chart).

The purpose of the pilot study is to identify safe and measurable cardiovascular effects of diesel concentration. Our primary endpoints in the pilot study include HRV, pulmonary function, and biomarkers such as neutrophils, platelets, fibrinogen, c-reactive protein, lactate, ferretin, and α1-antitrypsin. We will analyze these endpoints after finishing each concentration of diesel exposure and determine whether we will continue the next higher concentration exposure. For example, if we find significant effects at approximately 100 $\mu g/m^3$ of diesel concentration then we will use that concentration for our main study. We may not need to test subjects at the next higher proposed concentrations. We will carefully monitor the symptoms that subjects may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, dyspnea, pallor, ataxia, and significant cardiac arrhythmias. In addition, analyses of blood samples taken after exposure will be scrutinized for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. Symptoms and/or changes detected in the blood samples that are considered clinically significant could prompt the study physicians to stop the study.

Main study: This will be a randomized double-blinded study. Sixty study subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) will be given, in a double-blind fashion, a container filled with enteric-coated either 2g/day of fish oil or 2g/day of olive oil capsules for 15 days. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 25-35% of eicosapentaenoic acid (EPA) and 30-65% of docosahexaenoic acid (DHA). Each 1000 mg of these olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. There will be four groups of subjects in this study with 15 subjects in each group: the first group is fish oil supplement in GSTM1 positive subjects, the second group is fish oil supplement in GSTM1 null subjects, the third group is olive oil supplement in GSTM1 positive subjects, and the fourth group is olive oil supplement in GSTM1 null subjects. In the exposure study, each subject will be exposed to air containing approximately optimum diesel exhaust for 2 hours in an exposure chamber. (See the main study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the Westat recruitment office in the USEPA Human Studies Facility (HSF). At that time the study protocol will be outlined, a medical screening informed consent form will be signed, and a medical history form completed that contains information on general personal and family medical history.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical and the medical history form completed that contains information on general personal and family medical history. At that time a genotype screening informed consent form and a consent form for storing blood with identifying information will also be signed. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead

electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. Subjects will then have approximately 20 ml of blood collected in the pilot study and approximately 50 ml of blood collected in the main study and a portion of the blood will be used for genetic screening of GSTM1 and/or GSTP1 Ile105 Val polymorphism. A menstrual history will be collected on all female subjects.

- ***GSTM1/GSTP1 genotype screening***: A portion of the blood sample (about 10 ml) will be used for genotyping of GSTM1 and GSTP1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene and GSTP1 Ile105Val polymorphism. Since the pilot study only seeks people that are GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotypes, and since the joint GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotype is present in about 8%, 17%, and 14% respectively of the population, it is likely that about 80-90 % of the subjects will be excluded in this pilot study but they will be informed to participate in our future study which will enroll both GSTM1 positive and null genotypes. Those subjects with GSTM1 positive will not be asked to continue with the study and will be compensated for their time. Since the main study seeks to enroll equal numbers of people that are GSTM1 positive and null, it is likely that as the study nears completion, we will still be seeking people with the null allele but not with the sufficient allele. If that is the case, those subjects will not be asked to continue with the study and will be compensated for their time.

Food frequency questionnaire in the main study: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the university of south carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Training session: Those subjects who are not excluded on the basis of the physical exam and genetic screening will undergo a training session to familiarize them with the study protocol, obtain informed consent to complete the study and sign a consent form for storing blood with identifying information, and will ask any questions they might have regarding their participation in the study. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the main study:*** Subjects will be given an approximately 15 days supply of either fish oil or olive oil supplements randomly assigned. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of energy and nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase.

Subjects will be asked to keep two 3-day food records (one 3-day record per week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 15 day of supplementation period. We will give the subjects another 15 days supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

- ***HRV measurement before exposure***: In the pilot study, subjects will come to the human study facilities (HSF) at EPA in the morning of the exposure to have baseline heart rate viability (HRV) measured by a Holter monitor. Electrodes for telemetry and HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. Subjects will be released while wearing the Holter monitor and return to the HSF the next morning to get the Holter monitor removed. In the main study, subjects will come to the HSF in the morning of the last supplement to get baseline heart rate viability (HRV) by Holter monitor before exposure and the monitor will be remained for approximately 24 hours.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.

- Not to eat or drink anything for 2 hours prior to the training day visit.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (blood pressure, HRV, endothelial cell function by brachial artery ultrasound (BAU) and possibly by Endo-PAT, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement in both pilot and main study*** will be done by a Holter monitor.
- ***Brachial artery ultrasound in the main study:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.
- ***Peripheral endothelial function by Endo-PAT in both pilot and main study***: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be induced by sudden cuff deflation. The PWA recordings will be measured continuously during suprasystolic occlusion and deflation reactive hyperemia. PAT measurement will be displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).
- ***Pulmonary function in both pilot and main study*** will be measured by spirometry.
- ***Blood sampling in both pilot and main study***: approximately 50 ml of blood samples will then be collected in the pilot study and approximately 80 ml of blood samples will then be collected in the main study.

In the pilot study: Subjects will then enter the diesel exposure chamber for 3 sequentially exposures at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours each with a about 2-week interval between exposures. In the main study: Subjects will have only one exposure at an optimum concentration of diesel exhaust that will be identified from the pilot study.

Exposure: All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. The diesel exhaust will be generated from a 6-cylinder, 205 hp, 5.9 L displacement diesel engine (Cummins, Columbus IN) mounted on a vehicle located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean filtered and humidified air by approximately 1/30th to give a chamber concentration of approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$. If a real-time PM concentration exceeds 300 $\mu g/m^3$ for 5 min, then the exposure will be terminated. We do not believe a short exposure to about 300 $\mu g/m^3$ will be harmful to the subjects, as volunteers have not shown adverse effects in response to exposures to 300 $\mu g/m^3$ in other studies, while workers are regularly exposed to 1-2 mg/m^3 in some mining operations. It is expected that 75 to 95% of the diesel exhaust PM from this type of engine will be centered around approximately 0.1-0.2 μm. Preliminary testing has shown the mean particle size to be approximately 0.14 μm. Levels of carbon monoxide, and oxides of nitrogen (NOx; mainly NO, and some NO2), will be kept under 10 ppm CO, 15 ppm NO, and 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) for these substances are: carbon monoxide=50 ppm; NO2=5 ppm; NO=25 ppm; The American Conference of Governmental Industrial Hygienists (ACGIH) 8 hr threshold limit values (TLVs) are: NO=25 ppm, NO2=3 ppm, and CO=25 ppm]. Diesel fuel used for the study will be purchased as a certified fuel (Chevron Phillips Chemical Co., Borger TX, 0.05 LS Certification Fuel, type II), and the same lot utilized throughout the study. This certified fuel represents diesel fuel composition that is typically available at gas stations for most regions of the country except for California. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured with a thermal element oscillating microbalance (TEOM) and/or a Data-RAM, particle number per cm^3 by a Condensation Particle Counter (CPC), and chamber PM concentrations during exposure further validated by weighing filters obtained from versatile air pollution samplers (VAPS) impacters. Particle size distribution will be determined by scanning mobility particle size (SMPS) not during the subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles.

Post-exposure: Subjects will be released while wearing the Holter monitor. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 50 ml of blood will be collected in the pilot study and approximately 80 ml of blood will be collected in the main study.

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement

will be obtained, and approximately 50 ml of blood will be collected in the pilot study and approximately 80 ml of blood will be taken in the main. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound and finger arteries Endo-PAT). Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood (about 50 ml in the pilot study about 80 ml in the main study) will be drawn by standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In the main study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to a diesel exhaust and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of diesel exhaust on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.
- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound and finger arteries Endo-PAT:** There are no known risks associated with imaging of the brachial artery and index finger arteries. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left

ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.

- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Diesel exposure** The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban areas. Diesel particulate concentrations proposed for the pilot study will be 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m3$ and the optimum concentration will be used for the main study. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. During exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have EKG leads attached during the exposure to monitor cardiac function during exercise. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for their participation up to that point. The investigators or duty physician will end the exposure if the subject is found to be suffering from any significant adverse effect. The small pilot study will allow us to monitor the adverse effects of diesel exhaust closely and we will determine whether we will go to the next higher concentration based on the analysis. We will be able to identify a safe concentration of diesel exhaust that induces a measurable change in our study endpoints. No major adverse health effects were observed in a recently completed study that exposed subjects

(18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

- **Confidentiality** Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test our hypothesis that diesel exhaust causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust. We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to diesel will also be measured (e.g., blood pressure, HRV, brachial arterial blood flow rate, change in blood coagulation factors, vasoconstrictor endothelin, NOx, platelets, fibrinogen, c-reactive protein, lactate, ferretin, and α1-antitrypsin). In this study, our primary endpoints will be HRV measurement and peripheral

venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 was derived using a power of 0.8 and an α of 0.05.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to diesel exhaust compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial blood flow rate, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial blood flow rate. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of diesel exhaust on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen and platelets, changes in IL 6, IL 8 and TNFα, comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. X Names
b. X Telephone numbers
c. X Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. X Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. X Electronic mail addresses
g. __ Social security numbers
h. X Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, characteristic or code, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.11. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

X No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:

__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.12. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

X Secure network X Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question 7 above)
__ Locked suite or office
X Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.13. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects.** If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent.** *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true:

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study involves sensitive data that could be damaging if disclosed). __ yes __ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no
Explain.

If you checked "yes" to either, will consent be oral? Will you give out a fact sheet? Use an online consent form, or include information as part of the survey itself, etc?

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**

If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ *If this does not apply to your study, do not submit this section.*

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with your IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Westat recruitment office in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Will the information collected be limited only to that necessary to contact the subjects to ask if they are interested in participating in the study?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the pilot study will be approximately 3 months and the main study will take approximately 18 months. Participant recruitment and screening is expected to be continuous throughout the study until the intended number of participants is reached.

If the subject is eligible for the pilot study they will have 8 visits to the HSF over approximately 6-8 weeks. Subjects will have about 2 weeks break between 2 exposures. If the subject is eligible for the main study of diesel exhaust exposure the individual will have 6 visits to the research facility over approximately 5-6 weeks. The pre-enrollment genotyping and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after genotyping of the blood sample. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment for the main study) will require approximately 3 hours. Exposure day will last approximately 6 hours for the pilot study and 8 hours for the main study. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours for the pilot study and 4 hours for the main study.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Include food or refreshments that may be provided.

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in

effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be paid for the entire study, excluding completion bonus.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled, and will be paid 50% up to a total maximum of $100 for canceled procedures. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study.

1. If following genotyping of the blood samples on screening day, it is determined that the individual's genotype is not needed for completion of the study, then that subject may be informed that his/her participation in this study is no longer necessary. The subject will be notified by phone and the total compensation for this study will be approximately $60.
2. If the subject is qualified and finished the entire pilot study, the total compensation for completion of this study will be approximate $1461.
3. If the subject is qualified and finished the entire main study, the total compensation for completion of this study will be approximate $1087.

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30

Pre-study qualification total = $60

Subjects based on their genotype assessment may not be asked to further participate in the study.

Training day (3 hours)

Time (3h @$12/h)	$36

Pilot study:

Exposure (6 hours)

Venipuncture (~50ml, pre; 3@$30 each)	$90
Holter monitor (3 @$100 each)	$300
Chamber exposure (3 @$72 each)	$216
Venipuncture (~50ml, post; 3@$30 each)	$90
Time (3X6h @$12/h)	$216
Finger artery Endo-PAT (6 @$10 each)	$60

Lunch (3@$5 each)	$15

Total for completion of exposure = $987

Eighteen hours after exposure (3 hour)

Venipuncture (~50ml; 3@$30 each)	$90
Time (3X3h @$12/h)	$108
Finger artery Endo-PAT (3 @$10 each)	$30

Total for completion of post-exposure = $228

Protocol Completion Bonus $150

Approximate TOTAL for completion of pilot study = $1461

Main study:

Exposure (8 hours)

Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Finger artery Endo-PAT (2 @$10 each)	$20
Lunch:	$5
Food records (2 times)	$300

Total for completion of exposure = $753

Eighteen hours after exposure (4 hour)

Venipuncture (~80ml)	$30
Time (4h @$12/h)	$48
Brachial artery ultrasound	$50
Finger artery Endo-PAT	$10

Total for completion of post-exposure = $138

Protocol Completion Bonus $100

Approximate TOTAL for completion of main study = $1087

Subjects will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Data, Records or Human Biological Specimens without Direct Contact with Subjects

→ *If this does not apply to your study, do not submit this section.*

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do all of these data records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

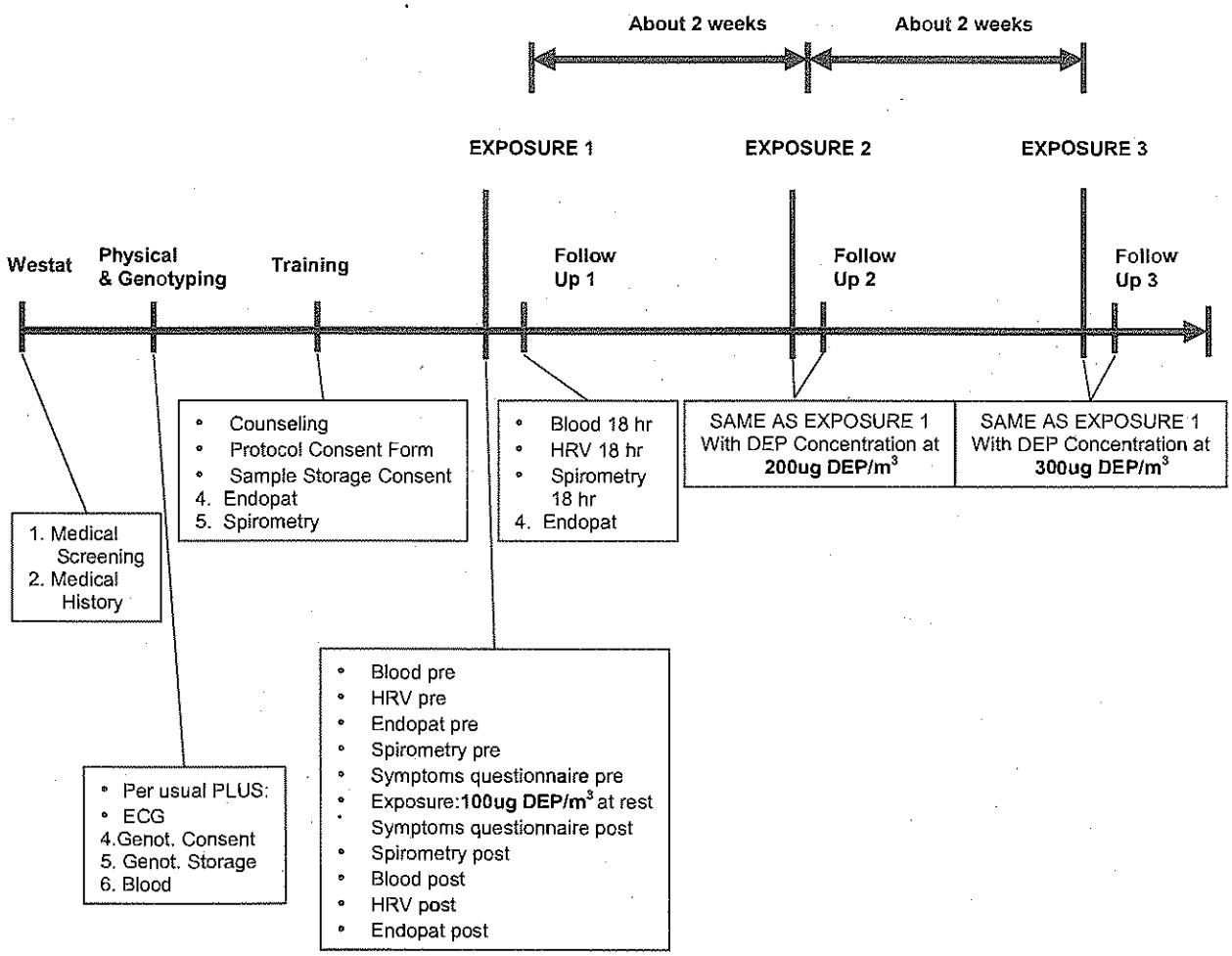
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

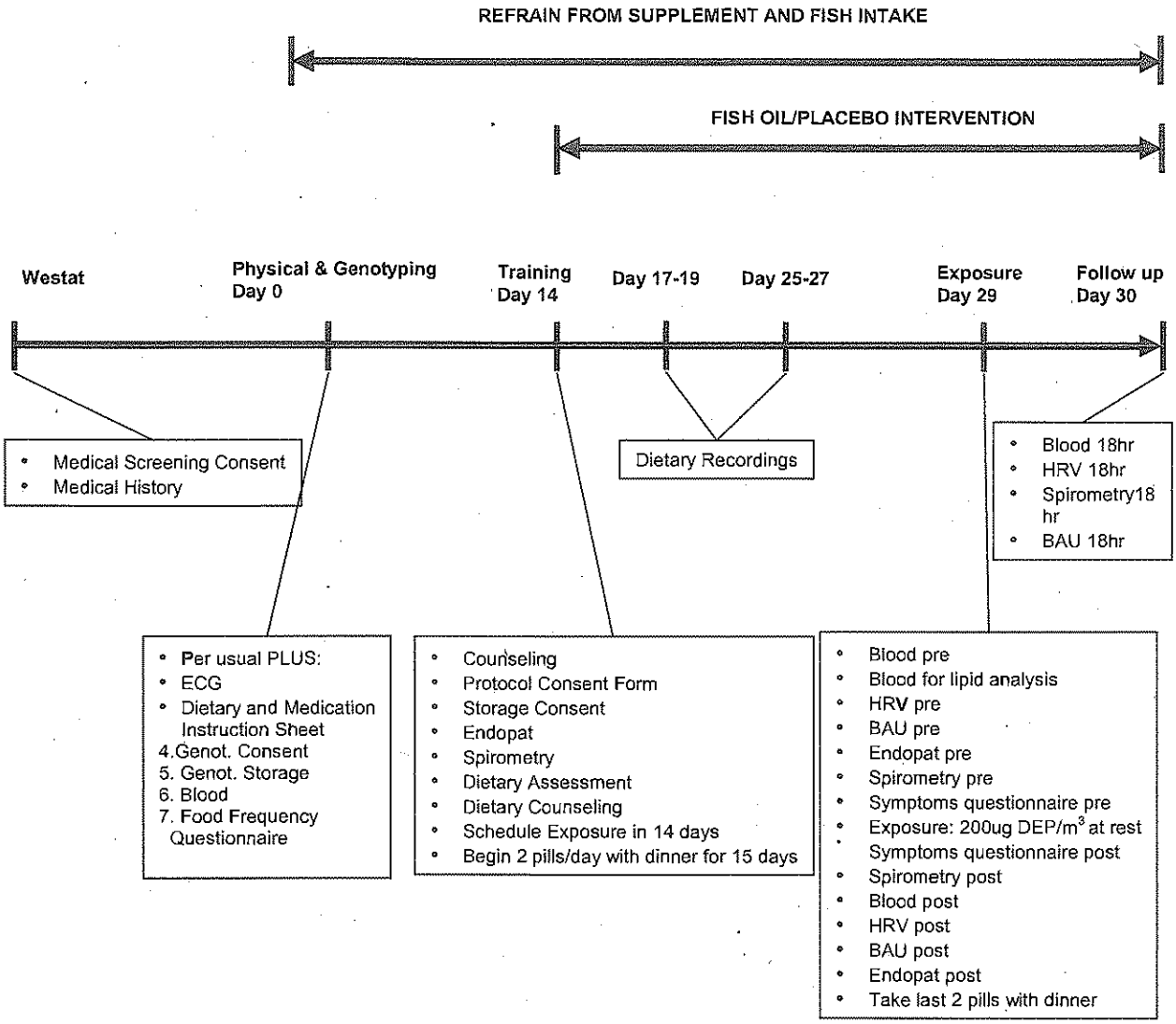
INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



PILOT PHASE FLOW DIAGRAM



MAIN STUDY FLOW DIAGRAM

GCRC ADDENDUM
to
IRB APPLICATION

(5 paper copies and one electronic copy (Word or PDF) of IRB application and this addendum are required for new submissions)

GCRC #: 2579

Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Date: 2 March 2007

If you have had previous GCRC protocols, as PI or Co-PI, please list publications related to these protocols, from the last 3 years, and state whether or not they cited GCRC grant (RR00046).

Publications	Cited GCRC Yes	Cited GCRC No
	☐	☐
	☐	☐
	☐	☐
	☐	☐
	☐	☐
	☐	☐

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USE OF THE GENERAL CLINICAL RESEARCH CENTER:

SERVICE (check all that apply)	YES	NO
Clinical Trial If so, what phase?	☐	☒
Outpatient rooms	☐	☒
Inpatient rooms	☐	☒
Blood drawing	☐	☒
Subjects seen at locations other than the GCRC	☒	☐
Subjects seen at the Dental Research GO Health Center	☐	☒
Use of GCRC investigator lab space for specimen processing	☐	☒
Temporary storage of samples in GCRC freezers	☐	☒
Bioanalytical Core (BAC) Lab Assays	☐	☒
Medication administration	☒	☐
Meals (Research diets, regular meals/nourishments for inpatients or for extended outpatient visits)	☐	☒
Research Nutritionist services (nutrition assessment, dietary intake data, diet instruction)	☐	☒
Body composition lab (DXA, Indirect calorimetry, pQCT, Erythromycin Breath Test, Activity Monitors, BIA, Physical Activity Questionnaires)	☐	☒
Database creation or other data management help	☒	☐
Other requests- specify:	☐	☒

Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH; and Robert Devlin, PhD.	**Phone:** 966-0665 (James Samet) **Pager:**	**Email:** Samet.james@epa.gov
Coordinator: Maryann Bassett	**Phone:** 966-6234 **Pager:**	**Email:** Bassett.maryann@epa.gov

A. SAFETY-MONITORING PLAN

All GCRC supported studies must have a written Safety Monitoring Plan that addresses the questions below. This is an NIH requirement and this Safety Monitoring Plan must be available for review by the NIH. For more information, contact David Weber, **dweber@unch.unc.edu**, phone 843-0725 OR Marie Zeldin, **mzeldin@med.unc.edu**, phone 966-6844. (*note: you can copy the email addresses in the preceding text boxes and paste into your email program.)*

If your study is not a clinical trial and does not involve a research intervention (administering a substance, exposure to experimental conditions or doing a procedure for research purposes) you may answer "N.A." to questions that do not apply.

1. **What potential risks are inherent in the study?** This should correspond with the risks that are described in the protocol and consent form.

Subjects in this study will be in GCRC only for brachial artery ultrasound measurements (flow-mediated dilatation, or FMD). This is a non-invasive test which involves minimal risk. A highly skilled investigator or technician will obtain the measurements. Occluding blood flow to the forearm may cause mild discomfort and unusual sensations such as tingling and numbness. There is a small risk (<1%) of developing petechiae in the arm which is examined. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.

General risks that may be encountered outside the GCRC are described below:

Diesel exposure is not expected to produce any permanent adverse health effects in this study. Dyspnea is the only predictable effect that may be encountered in the short term. All endpoints measurements, except for venipuncture, are non-invasive and involve only minimal risk.

The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy

intersections in large urban areas. Diesel particulate concentrations proposed for the pilot study will be 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 μg/m3 and an optimum concentration will be used for the main study. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had DEP levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City.

Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.

There is minimal risk associated with non-invasive endo-PAT measurement, which is similar to the risk associated with brachial artery ultrasound measurement, except that there is no nitroglycerin administration in the endo-PAT measurement

2. Identify and justify the level of risk to subjects for this protocol. Refer to "GCRC Protocol Risk Assessment and Monitoring Guidelines click here at **http://gcrc.med.unc.edu/forms/Risk Assessment Guidelines.doc**. (*note: double click on the URL in the preceding text box and paste into your browser.)*

2. Select Risk Level Justify Risk: Brachial artery ultrasound measurement at GCRC, Endo-PAT, ECG, and pulmonary function test will be rated at risk level 1. Exposure sessions in the pilot study will be imposed a moderate risk and we will minimize the risk by continuously monitoring (see #3 below). The exposure session in the main study will be rated at risk level 2.

3. How will risks be minimized? Describe the procedures that will be used to minimize risk to the subject.

3. Subjects will undergo a physical exam and 12-lead ECG to determine whether they can participate in this study. A physician will be onsite during the exposure. Heart rate, ECG, and pulse goniometry will be monitored continuously during the exposure. The subjects will be monitored for significant respiratory distress, chest pain, significant cardiac arrhythmias, pallor, and ataxia. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect which could be attributed to the exposure. The human exposure laboratory is also equipped with an emergency "crash cart" with standard emergency medications, IV fluids and a defibrillator in the unlikely event of a medical emergency during any exposure study. All subjects will return for assessment approximately 18 hours after the exposure as part of the study design. As standard procedure for all EPA studies, nursing personnel will make follow-up calls to the subjects in the days following the exposure to ensure that subjects are well and all subjects will be provided with a contact telephone number for access to a study physician who is on call 24 hours/day.

4. What tests, evaluations, or observations will be performed to monitor subject safety? List the specific tests, evaluations, examinations, observations, questionnaires, that will be used, and their frequency, to assess subject safety, to monitor for toxicity, or to detect adverse events. If there is a table or schedule of events, it should be reproduced here or referenced clearly.

1) On the physical exam day, subjects will undergo an ECG to screen for baseline cardiac arrhythmias. Subjects will have blood CBC, chemistries, and lipid tests to screen for hyperlipidemia, abnormal blood cell counts and differentials, and abnormal electrolytes.
2) A menstrual history will be collected on all female subjects on the physical exam day. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and the exposure day if it is more than 7 days since last pregnancy test it will be repeated.
3) Heart rate, heart rate viability (HRV), blood pressure, pulmonary function test, brachial artery ultrasound or endopat, blood sampling will be measured before exposure on the exposure day.
4) Heart rate, ECG, and pulse oximetry will be monitored continuously during the exposure. In addition, the subjects will be continuously monitored by a study investigator, duty physician, or nursing personnel.
5) Immediately after exposure, subjects will undergo blood pressure, heart rate, lung function, brachial artery ultrasound measurements and blood sampling.
6) Eighteen hours after exposure, subjects will undergo blood pressure, heart rate, lung function, brachial artery ultrasound measurements and blood sampling. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat.

5. Identify the classification scale that will be used to grade adverse events. Identify a scale that will be used to grade the severity of adverse events. If a protocol does not already have a specific grading scale, it is recommended that the Common Toxicity Criteria (CTC scale) be utilized. It can be found at **http://ctep.cancer.gov/**. For simple studies, a mild, moderate, severe scale may be utilized. (*note: double click on the URL in the preceding text box and paste into your browser.)*

5. A subjective adverse event classification will not be used in this study. If a subject experiences any event that he or she, or the investigators feel is sufficient to warrant stopping the exposure or other study procedure, that subjects' participation will immediately be stopped. Objective criteria which will lead the investigators to stop a study procedure will include:
1. Oxygen saturation < 91%.
2. Arrhythmia other than sinus tachycardia or sinus bradycardia.

6. List specific stopping rules that would lead to discontinuing a subject from the study or a change in the research intervention. If there is a specific number and grade of adverse events that will lead to subject discontinuation from the research intervention or if there are rules for dose escalation, please describe.

6. See #5 above. Additionally, if any subject experiences an event that requires significant medical attention the study will be stopped until a through review is completed by the investigators, the EPA National Health and Environmental Effects Research Laboratory (NHEERL) Human Research Official, and potentially the UNC IRB. The study will only be resumed after this review if all of these parties deem it appropriate.

7. For multicenter or high risk protocols, describe the Data Safety Monitoring Board or Safety Monitoring Committee that will monitor subject safety across all sites and describe study wide stopping rules. Provide details of the frequency of meetings, the membership, study wide stopping rules, and a copy of the DSMB charter. If a DSMB or SMC will not be utilized, explain why not. Confirm that the IRB will receive all reports of DSMB meetings and other safety reports.

7. N/A.

8. For single center studies or studies of moderate or lower risk, describe who is responsible for receiving and reviewing adverse events in aggregate across the entire study population. If this study is Phase I or II with no DSMB, please summarize how and at what intervals the safety data will be aggregated and reviewed, and who will be involved in these decisions.

8. There is no DSMB involved with this study, although adverse events will be monitored and noted by the investigators continually throughout the duration of the study. The UNC IRB will receive a report form the investigators regarding any adverse event that would cause a subject to be excluded from further participation in the study.

9. To whom and with what frequency will adverse events be reported? Discuss reporting to the IRB and GCRC as well as to sponsor, FDA and NIH if applicable, of expected and unexpected events.

9. See #8 above.

10. Describe who at UNC will monitor the safety of research subjects. Identify the individuals (Investigators, MDs, RNs, Research Associates, etc.) responsible *at this site* for monitoring the safety of research subjects. Describe the reporting mechanisms of the study staff to the P.I. and the frequency with which the P.I. reviews protocol data. Specify whether an outside monitor will review study charts and data.

10. All investigators having contact with the subjects during the course of the study will monitor the safety of the subjects. In addition, the health and well-being of the subjects during and for a time after the study will also be monitored by the EPA Human Studies Division nursing staff and physicians. As standard protocol for all EPA studies, the EPA nurses and physicians report any potential adverse events to the study PI.

B. Investigational Drug Service

The Investigational Drug Service is **not** a core service of the GCRC, however, all administration / dispensing of any agents (commercial or investigational) **must** go through the IDS for GCRC protocols. The GCRC addendum requires completion of the "Request For Investigational Drug Services" at **http://intranet.unchealthcare.org/site/w3/pharmacy** which is reviewed at the time of the GCRC protocol review. The IDS is located down the hall from the GCRC in Room 3001 of the main hospital. Please direct any questions to IDS by calling 966-3469. (*note: double click on the URL or email address in the preceding text boxes and paste into your browser or email application.)*

C. Biostatistics

All of the following questions should be answered in sufficient detail to allow useful biostatistical review. If you have already completely and adequately addressed these questions in previous sections of the IRB-GCRC application, then you may simply reference those sections rather than repeat the details in the boxes below. Alternatively, if you prefer to answer all the questions in the form of an attached word document, feel free to do that. (The protocol that was written for your study should have a complete statistical considerations section.) If your study is a multi-center trial with a master protocol that sufficently addresses all of these questions, you may paste all appropriate text in the first box below, OR reference the applicable page numbers of the master protocol. The master protocol must be submitted with this addendum.

For more information, contact Paul Stewart, **paul_stewart@unc.edu**, phone 966-7276.

Paste in page references to IRB Application or Master Protocol here:

1. What are the scientific aims and practical objectives of this study? (e.g., publication, get data for a grant proposal, pilot-test procedures/feasibility, estimate prevalence, test hypotheses, etc.)

This proposal is to examine the health effects of diesel exhaust exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust.
The proposed study is designed to test the null hypothesis that there is no protective effect of omega-3 fatty acid supplements and presence of a functional GSTM1 gene on the effects of diesel exhaust exposure.

2. What are the important features of the study design ?

In the pilot phase, three exposures to incremental increased levels of diesel exhaust with 2 weeks of interval to identify a safe dose of diesel exhaust that induces an alteration in heart rate viability (HRV) in healthy older subjects with GSTM1 null and GSTP1 Ile105Val polymorphism (Ile/Ile, Ile/Val, and Val/Val).
The main study will follow a randomized, double-blinded design to test the effects of dietary supplementation and GSTM1 status on the effect of optimal level of diesel exhaust identified from the pilot study. The study coordinator will provide subjects randomization assignments for dietary supplementation. The study investigators and subjects will be blinded for the dietary supplements and GSTM1 genotype.

3. Data used in statistical analyses

3.1 For what variables will measurements be recorded? (e.g., age, blood pressures, treatment.)

Heart rate viability (HRV), flow-mediated dilation of the brachial artery, blood pressure, lung function, and blood biomarkers in responses to fish oil/olive oil, GSTM1 and GSTP1 genotypes, and different levels of diesel exhaust exposure will be recorded.
Safety monitoring will include continuous observation of heart rate, electrocardiogram, pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmia, pallor, and ataxia.

3.2. How often and on what occasions will these measurements be made?

Heart rate viability, flow-mediated dilation of the brachial artery, blood pressure, lung function, and blood biomarkers will be obtained at baseline before diesel exposure (0 min), immediately after exposure (2 hours), and 18 hours after exposure (20 hours).

3.3. As used in the analyses, what will be the units and scales of these variables?

HRV will be expressed as the standard deviation of normal-to-normal RR intervals (SDNN) and the square root of the mean of squared differences between adjacent RR intervals (r-MSSD), the unit of HRV is second. Flow-mediated dilation of the brachial artery will be expressed as % change to the baseline level. Blood pressure will be in mmHg. Blood markers will be expressed as ug/ml or units/ml. Lung function will be expressed as liter and liter/first second.

3.4. What is the role of each of these variables? (e.g., primary outcome of the study, secondary outcome, explanatory covariate, etc.)

The primary endpoint will be HRV and peripheral blood samples. Secondary endpoints will include flow-mediated dilation (FMD) of brachial artery and lung function will be collected. Explanatory variable will be "period", "exposure level" in the pilot study, and "treatment" and "GSTM1 and GSTP1 genotypes" in the main study.

4. Statistical analysis of the data

4.1 Who will perform the statistical computations and who will provide interpretation of the results? (The Biostatistics Core does not provide these efforts. Referrals may be available.)

Study investigators will perform the statistical analysis and interpret the results with the

assistance of biostatistician.

4.2. What statistical methods will be used?

We will compare the different responses to diesel exposure between fish oil and olive oil groups and GSTM1 positive and GSTM1 null genotypes using ANOVA, and the differences between pre-exposure and post-exposure measurement using Student's paired t-test.

4.3. What are the details of the statistical models (if any) that will be fitted to the data? (e.g. ANOVA, linear regression, nonlinear PK models.)

ANOVA will be used for the main study to examine the differences between fish oil and olive oil treatment, GSTM1 null and positive genotypes; paired t-test will be used to examine the differences between pre- and post-exposures.

4.4. For what parameters (e.g., means, differences, etc.) will statistical estimates be obtained ?

Means will be obtained.

4.4.1. Are adequate levels of precision anticipated for these estimates? (e.g., results are not informative if confidence intervals are too wide.)

Yes.

4.4.2. What are your plans for computing confidence intervals for these estimates?

Supposed we will have a normal shape of viable from subjects and we will use 95% confidence intervals values for one-tailed test.

4.5. What hypothesis tests (if any) will be performed ?

Our hypothesis is that diesel exhaust causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment will attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower HRV responses cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust.

4.5.1. What statistical testing procedures will be used for these tests?

ANOVA will be used for the main study to examine the differences between fish oil and olive oil treatment, GSTM1 null and positive genotypes; paired t-test will be used to examine the differences between pre- and post-exposures.

4.5.2. What is your strategy for controlling the probability of type I errors (false discoveries) ?

We control the probability of type I errors at 0.05. If we only have one comparison, then it means a p-value of 0.05 or less will be considered significant. If we have multiple comparisons, since the number of comparisons is not big, we will use Bonferroni adjustment. That is, we compare the p-value of each of the comparison to 0.05/number of comparisons. Then we can decide whether each individual comparison is significant or not.

4.5.3. What levels of power are anticipated for these tests ?

We anticipate power will be 0.8.

4.5.4. Will this study be a success even if none of the tests are statistically significant?

If the primary null hypothesis test is not statistically significant, then the test will be inconclusive.

4.6. What are the plans for coping with incomplete data (if any) ?

Every effort will be made to obtain complete data on subjects according to protocol—including those subjects who do not complete the protocol. It is preferable to include the data from subjects with incomplete response in the primary statistical analysis computations. All available data values will be used whenever possible.

Since we only have 15 subjects and we do not expect missing data problem would be an issue. Furthermore, when deciding the sample size of 15, we have already taken into account the retention. Even if we lose a couple of subjects in some endpoints, we can still get the targeted results.

Several plausible mechanisms have the potential to cause unexpected missing data in this study. It will be necessary to carefully examine the apparent cases of unexpected missing data and subject dropout. The occurrences of unexpectedly missing our mis-timed data values will be carefully examined and explanations will be sought for the causes. If indicated, auxiliary analyses designed for non-ignorable missing data will be performed to gauge implications.

5. What were the primary considerations in choosing the proposed sample size?

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 was derived using a power of 0.8 and an α of 0.05.

The proposed sample size of 15 subjects was chosen based on considerations of feasibility, previous experience with levels of precision in similar studies, statistical methods to be used, and formal computations of statistical power for the proposed hypothesis tests. Toward achieving acceptable levels of *precision* and *power*, we have considered information available from previous studies about the standard deviations and correlations that can be anticipated in the proposed study. By making reasonable conjectures about the underlying distributions of key variables, and about enrollment and retention, power calculations useful for sample size selection have been made and have been presented in the discussion of the primary analysis plans. These considerations collectively indicate that the proposed sample sizes and study design are appropriate.

6. What are the details of the randomization procedure (if any) and who will perform the computations?

In the main study, each subject will be randomized to receive either fish oil or olive oil supplementation; however, we will try to balance the assignment by genotypes so that we have 15 subjects in each of the four study group (fish oil/GSTM1 positive, fish oil/GSTM1 null, olive oil/GSTM1 positive, and olive oil/GSTM1 null). The randomization procedure will be implemented in two stages. In stage 1, the study coordinator will use a computer program to generate a sequence of ID numbers and assignment profiles. The study coordinator will securely store the assignment key in a blinded manner, but with provision of ready access in case of medical emergency. Stage 1 will be completed before enrollment begins. Stage 2, each of the eligible subjects will be assigned to one of the randomization ID numbers. The method of generating the assignment sequence in stage 1 will use a *permuted block* method.

Stage 2 will use the simplest approach—an "envelope system"—implemented as described below. This implementation can be a literal one using paper envelope. Upon completing stage

1, a set of sealed envelopes, each bearing a "randomization ID number" printed on the outside. These envelopes will be available for use one-by-one according to numbered sequence. The study coordinator will open the next envelop whenever she is notified that another eligible subject has be enrolled and needs to be randomized.

Other information / comments:

D. DATABASE MANAGEMENT AND CONFIDENTIALITY

If this is a multi-center trial with a master protocol that sufficently covers all the required details, you may paste all appropriate text in the first box below, OR reference the applicable page numbers of the master protocol. The master protocol must be submitted with this addendum. For more information, contact Clarence Potter, **cpotter@med.unc.edu**, phone 966-4802

1. How will you secure the data from unauthorized access or inadvertent alteration?

All electronic records generated at the EPA Human Studies Facility (HSF) will be kept on a computer-database that is password protected. Subject paper files will be maintained and kept in the EPA HSF nursing station. The EPA HSF building can only be entered by persons with EPA identification or accompanied by persons with EPA identification. Flow-mediated dilatation data generated in the GCRC will be stored on a stand-alone CPU and access to this data will be limited to the investigators and the necessary GCRC personnel. Other data or patient information generated by the GCRC will be maintained either on the UNC CIS system or medical records system.

2. How is confidentiality maintained of the subject's protected health information (PHI)?

All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files which may contain the subject's name or screening number are secure in the EPA building which has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

3. What are the plans for research database management (RDM)? (i.e., system development, data entry, quality assurance, data querying and editing, data retrieval, data archival, etc.)

The study investigators will be responsible for ensuring data management quality. While the study protocol defined the scientific objectives and methods of the study, additional written guidelines and detailed procedure manuals will be needed for uniform adherence to the detailed intent of the protocol. In the end a written record of how the study was performed is essential. Preferably, this documentation is written prior to the execution of the study and is updated during the course of the study. The study investigators will have overall responsibility for the definition and production of the documents necessary to describe all aspects of the study in sufficient detail to insure the study can be conducted in a scientifically sound, standardized manner. The study investigators will be assisted by their data management staff in monitoring adherence to

protocol.

4. How will you verify that the data has been entered into the database correctly from data collection forms or other sources?

Data collection will adhere to precise written instructions included in manual of operations. All data collected will be double-key verified on entry into the data management system. Data collected will be stored in the EPA HSF. This system is password protected and protected by daily archival to magnetic tape.
The error rate should be exceed one erroneous data value per 100,000 data values. For estimation purposes, a 5% random sample of data collection forms, stratified by type, will be compared against the database at six months and then annually during the years of data collection.
This study will involve a variety of data sources. The study investigators will be responsible for managing the data streams, reviewing data-monitoring reports, and investigating questions raised about remarkable data values.

5. Who will perform the computations for RDM?

Study investigators or the HSD QA manger will perform the computations for RDM.

6. What software will be used for database management ?

Database analysis will be carried out using lotus 123, Microsoft excel, and Prism 3 from GraphPad, Inc.

7. How will you validate formulas, functions, or macros to ensure that they will perform data manipulations correctly?

One study investigator will enter the formulas and the other investigator will confirm the entry is correct. And this will be validated by HSD QA manager.

8. What protections are there from lost data?

All paper files will be maintained and kept in the EPA HSF nursing station. All computer-database will be backed up regularly.

Other information / comments:

We do not anticipate using the Informatics Core for database management for out study.

E. NURSING INVOLVEMENT

For more information, contact Nancy Cozart, **ncozart@med.unc.edu**, phone 843-0560, pager 216-6991

Nursing Requirements	Yes	No
1. Do you require vital sign measurement? **If yes,** Indicate frequency: One measurement before diesel exposure and 1 immediately after and 1 at 18 hrs after diesel exposure (these measurements will be done by nurses at EPA).	☒	☐
2. Does your protocol require blood sampling? Hospital labs and/ or study kit: Lab?	☒	☐

Nursing Requirements	Yes	No
Blood sampling will occur at EPA medical station.		
3. Will you be using the GCRC investigator lab for **specimen processing?**	☐	☒
4. Will you be using the GCRC investigator lab for **temporary (< 90 days) sample** storage?	☐	☒
5. Are you requesting GCRC staff to **process any samples**? GCRC can only be responsible for sample processing after 1pm each day. If yes, provide processing and storage instructions.	☐	☒
6. Does your protocol require IV placement, IVF administration, and monitoring? If yes, please provide details	☐	☒
7. Does the protocol involve **administration of chemotherapy, drugs** or study medication **infusions**? Length of infusion: Repeated (frequency):	☐	☒
8. Does the protocol require **other tests or procedures**, such as : EKG, PFT, etc?	☒	☐

Procedure	Description / Who will perform procedure and where?
Brachial artery ultrasound (BAU)	BAU will be obtained by Dr. Alan Hillderliter or a research technician instructed by him at NCMH using a 12.5 MHz image probe interfaced with an ATL HDI 5000 ultrasound machine.
ECG	ECG will be obtained by physicians and nurses at EPA.
PFT	PFT will be performed by investigators in this study.
Endo-PAT	Endo-PAT will be performed by investigators in this study.

9. Are you requesting any assistance to **perform study interventions?** i.e., exercise testing, patient education.	☐	☒
10. Will you have any **special patient care** needs, such as telemetry, chest PT, isolation, indwelling catheters? Please describe:	☐	☒
11. Does the protocol require an **inpatient stay**? (over midnight admission) Will you be able to do advanced scheduling (at least one week before)?	☐ ☐	☒ ☐
12. Does the protocol require **outpatient visits**? Please estimate length of outpatient visits (30 minutes, 1 hour, etc.)	☒	☐

Intervention	Description

Nursing Requirements	Yes	No
1 hr for 3 times.		
13. Will your protocol require unscheduled (< 48 hours notice) visits?	☐	☒
14. Are you requesting that the GCRC provide any supplies or special equipment necessary to conduct the protocol? If yes, describe here and add to Cost Assignment Sheet. BAU will be measured at NCMH using a 12.5 MHz image probe interfaced with an ATL HDI 5000 ultrasound machine.	☒	☐
15. Describe any other requests of GCRC nursing services: None.		
16. Are there personnel working on the protocol that have not been oriented by GCRC staff? If so, please contact Nancy Cozart prior to study initiation to complete this orientation.	☐	☒
Other information / comments: The GCRC nursing stuff will only be asked to check the subjects in and out as outpatients; there are no requests for data to be collected during this process. The endpoint to be measured is flow-mediated dilation as measured by brachial artery ultrasound which will be obtained by study investigator Dr. Alan Hillderliter or a research technician instructed by him.		

For extensive outpatient visits or inpatient admissions, a Time and Event schedule is required to outline the requirements of your protocol. Use the sample format found at: **http://gcrc.med.unc.edu/ftp/forms/time_event.dot** *(note: double click on the URL in the preceding text box and paste into your browser.)*

F. Nutrition, Body Composition, and Research Kitchen

For more information, contact Marjorie Busby, **mgbusb@med.unc.edu**, phone 966-4746, pager: 216-3469

Please define **ALL** of the specific procedures that you request the GCRC Nutrition Service to carry out during the course of the study:

Nutrition Requirements	Yes	No
Inpatient All inpatients require meal description. Describe meal below.	☐	☐
Outpatient	☐	☒
If yes to either inpatient or outpatient meals, please describe the type of diet, e.g., research, regular; or nourishment required:		
Measurements by Research Nutritionist	Yes	No
Subject Screening for Research Diet Adherence	☒	☐
Nutrition Assessment / Diet History	☒	☐
Food Frequency Questionnaire	☒	☐

Food Records / Dietary Intake (# of days per patient)	☒	☐
Diet Instruction	☒	☐
Nutrient Intake Analysis of Research Diet	☒	☐
Other: These measurements will be obtained by Dr. Susan Steck, a nutritionist at the University of South Carolina.		
Body Composition Measurements	Yes	No
DXA (bone density, body composition) Which measurements? If so, fill out the DXA Radiation Safety form (**http://gcrc.med.unc.edu/forms/diet/radcommitteeapp-dexa.doc**). Send the completed Radiation Safety Form to the Radiation Safety Office. The address is on the form. .	☐	☒
peripheral QCT (bone structure) If so, fill out the pQCT Radiation Safety form (**http://gcrc.med.unc.edu/forms/diet/radcommitteeapp-pQCT.doc**). Send the completed Radiation Safety Form to the Radiation Safety Office. The address is on the form.	☐	☒
Indirect Calorimetry (Resting Energy Expenditure, Respiratory Quotient)	☐	☒
Bioelectric Impedance Analysis	☐	☒
Anthropometric Measurements	☐	☒
Physical Activity Monitors	☐	☒
Physical Activity Questionnaires	☐	☒
Other information / comments:		

G. GCRC BIOANALYTICAL CORE (BAC) LABORATORY

For more information, contact David Barrow, **david_barrow@dentistry.unc.edu**, 962-0633

Here's the URL to review BAC Laboratory Policies is
(http://gcrc.med.unc.edu/investigators/corelab/BACLabPolicies.doc).

The URL for assay planning document is (**http://gcrc.med.unc.edu/forms/BACAssay.xls**). Identify the labs and quantities in the planning document, then email the document to David Barrow prior to submitting this addendum to the GCRC. After coordinating with David, attach the final version of the assay planning document, to this addendum. (*note: double click on the URL in the preceding text box and paste into your browser.)*

	Yes	No
Will the BAC lab store samples for the investigator? If so, for how long?	☐	☒

H. GCRC Specimen Processing Facilities

	Yes	No
1. If so, have the staff that will use these facilities completed the GCRC lab online training? If not please go to **http://gcrc.med.unc.edu/investigators/labtraining**	☐	☒
3. Do you need temporary (< 90 days) storage of samples in the GCRC freezers?	☐	☒

I. Cost Assignment Sheet

For more information, contact Mary Beth Lister, 966-4767, **marybeth_lister@med.unc.edu**

Cost Sheet.xls must be attached to this addendum before submitting to the GCRC. It was included in the zip file with this document. It can be downloaded from **http://gcrc.med.unc.edu/forms/cost%20sheet.xls**. *(note: double click on the URL in the preceding text box and paste into your browser.)*

What are the funding sources for this protocol? Check all that apply

☐ Industry-Funded
Must choose one: ☐ Industry-Initiated
☒ Investigator-Initiated

If Industry-Initiated, check if applicable: ☐ Rare Disease
☐ Therapeutic Disease Network

If you marked industry-funded and investigator-initiated, please provide a **justification** for the GCRC Clinical Research Advisory Committee to consider this a Category A protocol. Use the criteria found at http://gcrc.med.unc.edu/investigators/admin/category.htm :

Justification goes here

Check any other sources of funding:

☐ Department Funds

☐ NIH (Please provide Grant #) ___ ___

☒ Other (Please specify) _X__EPA intramural federal research ___

☐ No funding source outside GCRC resources

J. Data Sharing Plan

As of October 1, 2003, the NIH requires all investigators requesting $500,000 or more in direct costs in any single year to include a plan for data sharing or to state why data sharing is not possible. For more information on the NIH policy on data sharing, click on http://grants.nih.gov/grants/policy/data_sharing/. The General Clinical Research Center supports the concept of data sharing and agrees that data sharing is essential for expedited translation of research results into knowledge, products and procedures to improve human health.

	Yes	No
Do you have an NIH grant totaling $500,000 or more in direct cost funding per year?	☐	☒

If you checked yes, please select one of the following options:

☐ I agree to share data collected for this research study in accordance with NIH regulations upon publication of the main findings from the final data set in a timely manner (provide below a description of your data sharing plan).
☐ Research data will not be made available for sharing as explained below (state reason why data sharing is not possible).
Description of data sharing / Reason for not sharing:

Checklist for submission to the GCRC

Required sections for all applications:

- ☒ A. Safety Monitoring Plan
- ☒ C. Biostatistical considerations
- ☒ D. Database Management and Confidentiality of Research Data
- ☒ I. Cost Assignment Sheet
- ☒ J. Data Sharing Plan

Must be submitted if you are requesting use of these services:

- ☒ B. Investigational Drug Service
- ☐ E. Nursing
- ☐ F. Dietary/Nutrition Services
- ☐ G. GCRC Bioanalytical Core Laboratory

We continuously strive to improve this process, please send suggestions for improvements or corrections to this form to **gcrc-sys-mgr@med.unc.edu**.

07-0190

Verne S. Caviness

General Clinical Research Center

School of Medicine
The University
of
North Carolina
at
Chapel Hill
&
UNC
Hospitals

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Research Subject Advocacy**
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(919) 966-6844

RECEIVED
JAN 30 2007
IRB OFFICE

MEMORANDUM

TO: Stephen Bernard, MD
Biomedical IRB

FROM: Wanda Simmons
General Clinical Research Center

DATE: January 26, 2007

RE: **New Submission - GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust**

Principal Investigator: **Dr. Haiyan Tong**

The above named protocol has now been submitted to the GCRC and has been assigned a GCRC number. Enclosed are three copies of the protocol for the committee's review. Please forward any pertinent correspondence regarding this protocol to the GCRC office.

This protocol will be on the February 6, 2007 agenda for the Clinical Research Advisory Committee.

Thank you.

Verne S. Caviness

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Assistant Director
(919) 966-6844

MEMORANDUM

TO: Dr. Haiyan Tong

FROM: Dr. Paul B. Watkins, Director

DATE: March 22, 2007

SUBJECT: **GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust**
IRB Study #: 07-0190

Both the Clinical Research Advisory Committee and the Committee on the Protection of the Rights of Human Subjects have reviewed this proposal and have approved it until **February 25, 2008.** Approximately two months prior to its expiration date, you will receive a renewal form.

REMEMBER:
This approval is contingent on your citing the GCRC grant number (RR00046) in any publications that might ensue from the work conducted on this NIH funded center.

Inpatient admissions should be scheduled with Ms. Nancy Cozart (843-0560) and outpatient visits with Mrs. Annie Johnson (966-1437). The approved consent form must be signed by each patient before any studies are done. A copy of this signed consent form must be left at the Nursing Station, and one should be placed in the UNC Hospitals record.

Should any significant side effects occur in any volunteer participating in this study, you are expected to inform the Director of the General Clinical Research Center and the Chairman of the Human Rights Committee immediately.

Paul B. Watkins, MD, Director

Enclosure

cc: Stephen Bernard, MD
IRB Chair

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097

TO: Haiyan Tong
Environmentally Protection Agency, CB# 7315

FROM: Biomedical IRB

Stephen Bernard / SKA
Authorized signature on behalf of IRB

APPROVAL DATE: 6/08/2007

EXPIRATION DATE OF APPROVAL: 2/25/2008

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Approved Research
Study #: 07-0190
Other #: GCRC-2579
Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this research is no more than minimal.

Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Should any adverse event or unanticipated problem involving risks to subjects or others occur it must be reported immediately to the IRB using the adverse event form at the same web site.

Submission Description:
This modification dated 5 June 2007 submits the following changes:

1) Adds use of ACE inhibitors and latex allergies to exclusion criteria

2) Includes multiple changes and additions to clarify study procedures

3) Rearranges the sequence of study procedures in order to reduce the number of office visits and reduce study costs

4) Outlines conditions under which exposures will be terminated prematurely

5) Changes to exploratory endpoints

6) Changes the duration of various study visits to adjust for changes in and timing of procedures

7) Changes number of study visits and exposure periods during the study

8) Increases the total payment for completion of the Pilot study to $1893 and the Main study to $1095

9) Removes Richard Hermann form study personnel

10) Changes, "What will happen if you participate in this study" section of Pilot study genotype screening and Main study genotype screening consent form to include additional procedures

11) Combine genetic screening and physical exam blood collection in the Pilot study genotype screening study and Main study genotype screening

12) Changes the benefits section of the Pilot study genotype screening consent form to include blood cholesterol levels

13) Adds Brachial Arterial Ultrasound measurement to the Pilot study

14) Lunch is provided during Main study to control fat intake

15) Newly submitted documents include: Recruitment letter, to be mailed to prior study subjects; Appointment Reminder/Parking display ticket; OMEGA Phone Screen Report; and Symptom Questionnaire for Chamber Exposure Studies.

The IRB application, consent forms (version date: 1 June 2007) and previously- approved recruitment materials and study flow diagrams have been updated appropriately.

Details: Call the IRB at 966-3113 if you have any questions. You can now access IRB status information at https://my.research.unc.edu/.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), and 21 CFR 50 & 56 (FDA), where applicable.

The University of North Carolina at Chapel Hill holds a Federal Wide Assurance approved by the Office for Human Research Protections, Department of Health and Human Services (FWA # 4801).

CC:
James Samet, Environmentally Protection Agency, , Faculty Advisor; Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 6/8/07 AND 2/25/08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information--PILOT STUDY **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: 1 June, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Maryann Bassett, RN; Andrew Ghio MD; Debbie Levin, BSN; Tracey Montilla, RN; Michael Schmitt MS
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 50 ml of blood for genotyping and blood analysis. If you are re-contacted from the previous study, you will not need genotype screening but you will need to get blood drawn for other blood analysis. At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office.

How will the blood sample be collected?
You will have about 50 ml (about 5 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers and lipid lowering statins. You need to

Initial/Date _____

be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

Initial/Date _____

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Initial/Date _____

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 6/8/07 AND 2/25/08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Storing Biological Specimens With Identifying Information **2**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: 29 May 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Maryann Bassett, RN; Andrew Ghio MD; Debbie Levin, BSN; Tracey Montilla, RN; Michael Schmitt MS
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

Initial/Date _____

Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to the effect of air pollutants such as ozone and diesel exhaust. The purpose of this research study is to examine the role of the GSTM1 gene in mediating the cardiovascular effects following acute diesel exhaust exposure in GSTM1 null and GSTM1 sufficient healthy older adults.

Diesel exhaust exposure has been found to cause a range of lung and systemic changes in normal subjects. We will be collecting blood samples which will help us to further study this condition. In order to do so, we will need to collect and store blood. Blood samples will be collected in order to look for but not limited to indicators of inflammation due to the diesel exhaust exposure. We may further isolate RNA from blood for genetic analysis. RNA may be isolated for genetic analysis to see if there is a difference between pre- and post diesel exhaust exposures.

All samples will be stored where only project members will have access to the samples. There is a need to store samples in such a repository because this will be an ongoing study where samples from subjects will be collected over an extended period of time. Storing of samples allows for all samples to be processed at the same time and also allows our scientist the opportunity to further study these samples with as yet unknown questions and techniques.

How will the specimen be collected?
The description of the samples to be collected and the manner in which this will be done have been described in the main study consent.

What will happen to the specimen?
Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the diesel exhaust exposure. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens (or data) for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens (or data) will be provided without identifiers to these other researchers by employing a data use agreement.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding the cardiovascular effects of diesel exhaust exposure in older population.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:

Initial/Date _____

1. Blood sampling will be performed by medical personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you for storage of the specimens?
There will be no cost to you for the storage and use of the specimens for research purposes.

Will you receive anything for being in this study?
You will not receive anything for taking part in this study with regard to storage of excess specimens. Reimbursement for participation in the main study is addressed in the detailed consent for that study.

Who owns the specimens?
Any blood, body fluids, or tissue specimens obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the

Initial/Date _____

privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the specimens from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not

Initial/Date _____

waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 6/8/07 AND 2/25/08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY

3

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 1 June, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

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Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if…

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal

Initial/Date _____

anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately

Initial/Date _____

200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed.

Initial/Date _____

These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck

Initial/Date _____

driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.

- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

Initial/Date _____

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Initial/Date _____

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical

Initial/Date _____

Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $1893.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you

Initial/Date _____

are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
Lunch (3@$5 each) (delete)	$15
On-time bonus (3@$25 each)	$75
Total for completion of exposure = $1419	
18 hours after exposure (3 hour)	
Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Initial/Date _____

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $1893

Subjects will be asked to bring (delete) provided a lunch by GCRC for the exposure day. and a refrigerator and microwave will be available. (delete) If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect

Initial/Date _____

your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

______________________________ ____________

Signature of Research Subject Date

____

Printed Name of Research Subject

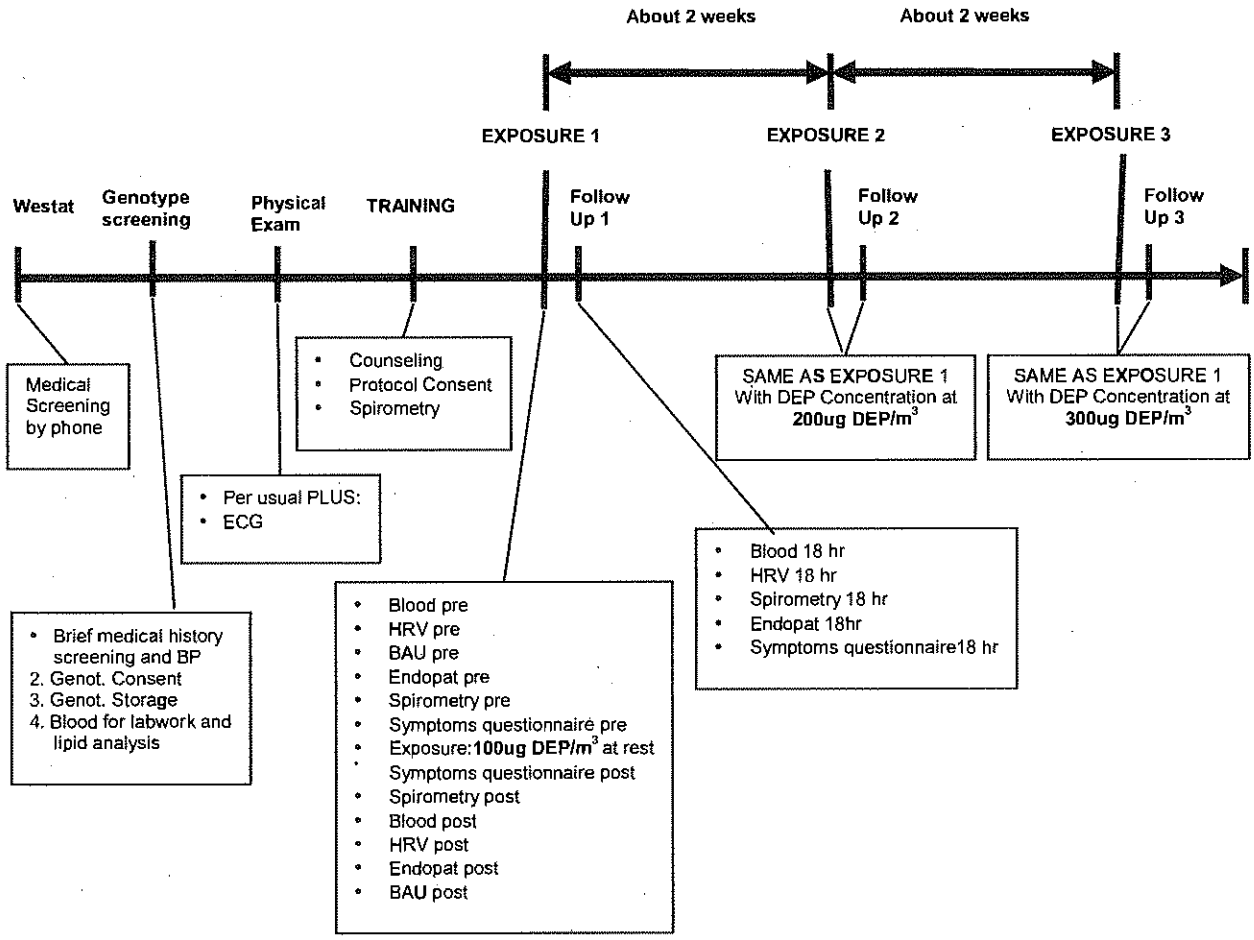
______________________________ ____________

Signature of Person Obtaining Consent Date

____

Printed Name of Person Obtaining Consent

Initial/Date ______________



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 6/8/07 AND 2/25/08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information—MAIN STUDY 4

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: 1 June 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Maryann Bassett, RN; Andrew Ghio MD; Debbie Levin, BSN; Tracey Montilla, RN; Michael Schmitt MS
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to diesel exhaust. We will have equal numbers of subjects with GSTM1 sufficient gene or GSTM1 null gene enrolled in this study. The purpose of this screening session is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 60 participants in this study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and you will be instructed to mail the FFQ. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 8 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should

Initial/Date _____

not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol) and lipid lowering statins. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Initial/Date _____

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Initial/Date _____

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 6/8/07 AND 2/25/08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—MAIN STUDY **5**

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: 29 May, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, and chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.

Initial/Date _____

- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Use olive oil for cooking, dressings, and sauces during the study.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 60 people in this research study.

How long will your participation in this study last?

You will have up to 3 visits to the research facility over approximately 5-6 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) two-week of fish oil/olive oil treatments part, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

Initial/Date _____

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a 15-day fish oil or olive oil supplementation and the exposure session. You will be given a 15-day supply of either fish oil or olive oil tablets. Please take 2 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next two weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this 15-day of supplementation period. We will give you another 15-day supply of either fish oil or olive oil tablets and you will need to restart the pills again.

In the morning of your last pill of supplements, you will come to the HSF for the baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel

Initial/Date _____

exhaust causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF in the next morning for the exposure and the monitor will be removed.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat before midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we will perform on your last pill day).
- Have about 80 ml blood drawn (about 8 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- We will bring you back to the HSF where we may also measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT) at HSF and we will compare the findings with ultrasound measurement of the artery in your arm. We will place two small PAT probes to both of your index fingers. The procedure is similar to that of ultrasound measurement and we will only place a blood pressure cuff on one of your arm. We will obtain recordings

Initial/Date _____

for 5 minutes at rest, 5 minutes during the blood pressure cuff inflated, and 5 minutes after the blood pressure cuff released.

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust (the concentration will be determined based on the pilot study) for 2 hours in a small exposure chamber. This particle concentration is representative of diesel exhaust particle (DEP) levels which you would inhale if you were occupationally exposed to DEP, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over a period at a busy intersection in a polluted city (such as Los Angeles) where DEP concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will take you back to the GCRC at the NCMH for another ultrasound of the artery in your arm. (delete)
- We will obtain another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 cc or about 8 tablespoons).
- Fill out a symptom score questionnaire.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)

Initial/Date _____

You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will take you back to the GCRC at the NCMH for another ultrasound of the artery in your arm. (delete)
- You will have another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 cc or about 8 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: The concentration of diesel exhaust in this study has been chosen based on a pilot study in susceptible older subjects. Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that

Initial/Date _____

exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from healthy food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication,

Initial/Date _____

nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Finger Endo-PAT: Similar to the measurement by brachial artery ultrasound, there are no significant risks associated with index fingers arteries measurement.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If

Initial/Date _____

such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1095.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Initial/Date _____

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml) (genotyping)	$30
Pre-study qualification total =	$60
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Exposure sessions (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Brachial artery ultrasound (2@ $50 each)	$100
Finger Endo-PAT	$20
Time (8h @$12/h)	$96
Lunch (delete)	$5
Food record (2 times)	$300
FFQ	$50
On-time bonus	$25
Total for completion of exposure =	$823
18 hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3 hrs @$12/h)	$36
Brachial artery ultrasound (delete)	$50
Finger Endo-PAT	$10
Day 3 total for completion of post-exposure =	$76
Protocol Completion Bonus	$100
Approximate TOTAL for completion of the study =	$1095

Subjects will be asked to bring (delete) provided a lunch by GCRC for the exposure day. and a refrigerator and microwave will be available.(delete) If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a

Initial/Date _____

scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division

Initial/Date _____

Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

____________________________________ _______________

Signature of Research Subject Date

____

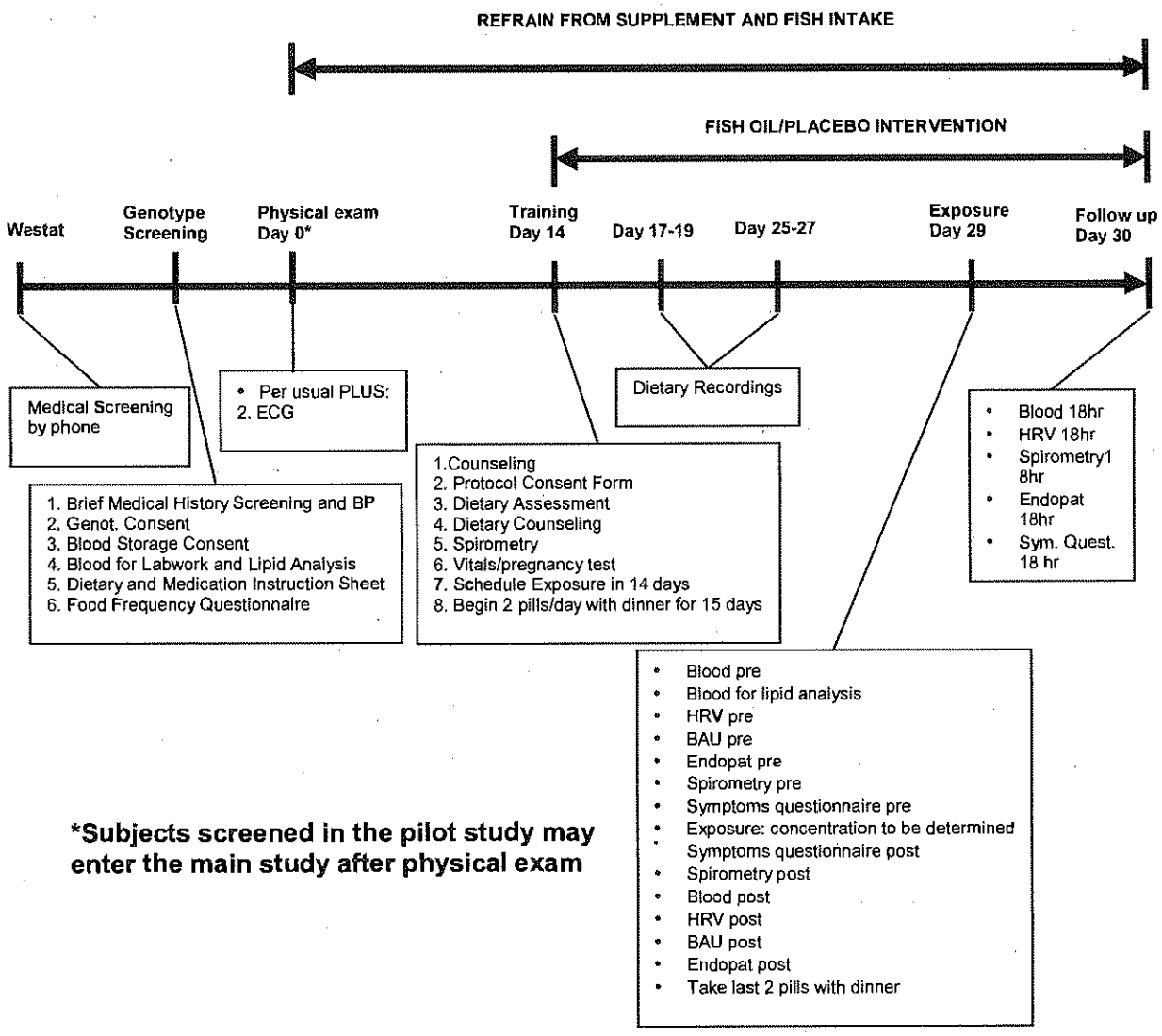
Printed Name of Research Subject

____________________________________ _______________

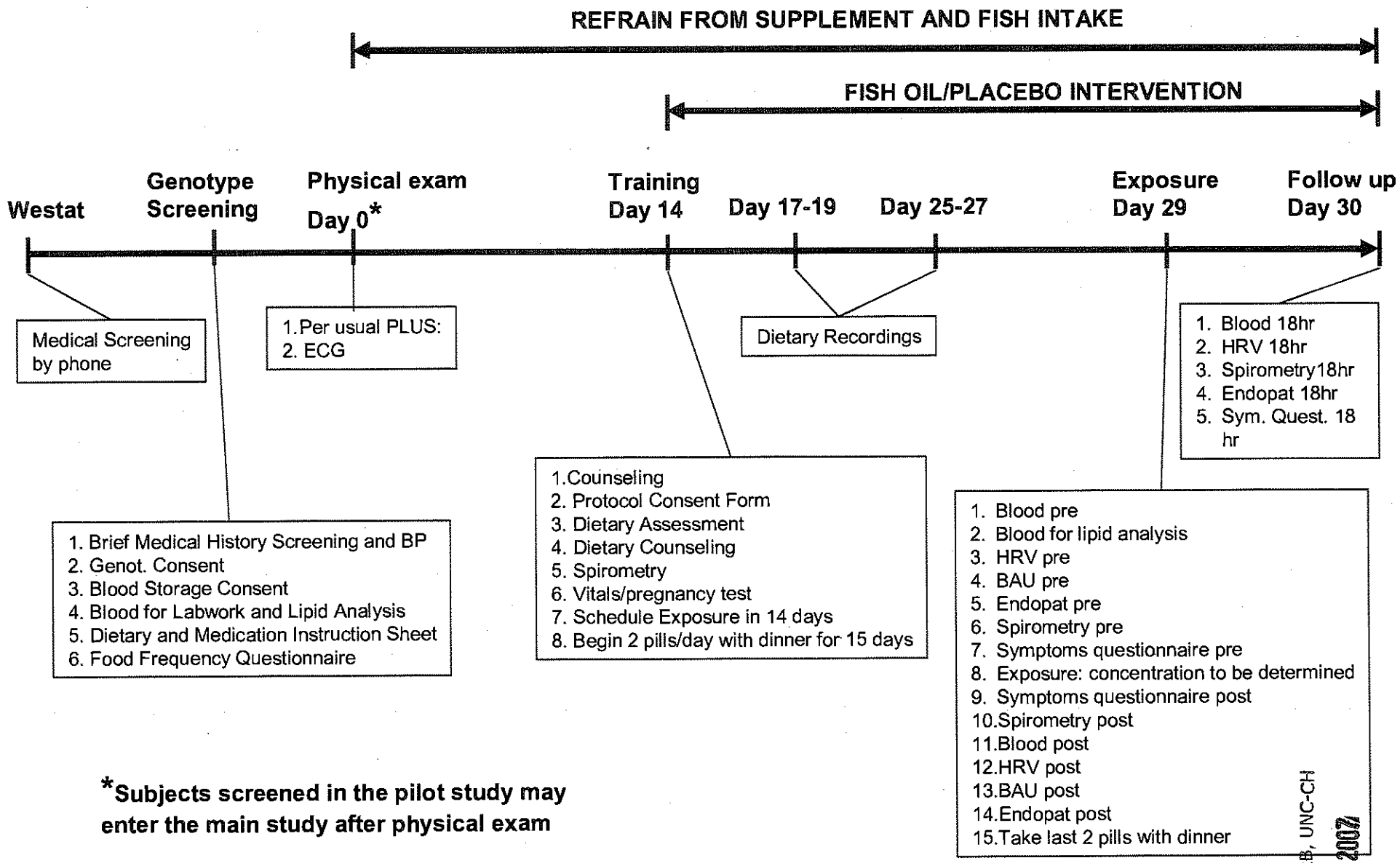
Signature of Person Obtaining Consent Date

____

Printed Name of Person Obtaining Consent



Initial/Date _____



***Subjects screened in the pilot study may enter the main study after physical exam**

MAIN STUDY FLOW DIAGRAM

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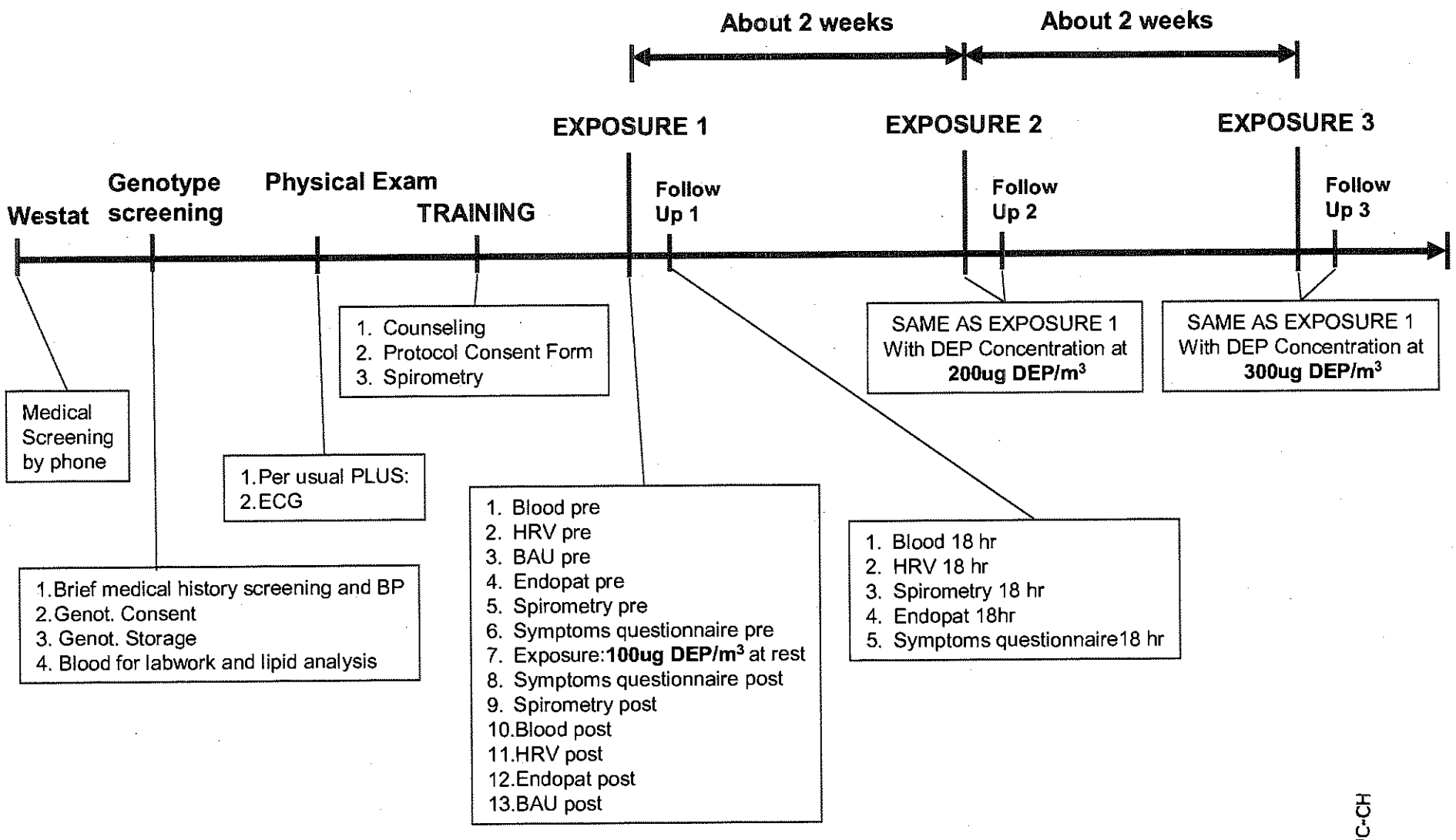
JUN 08 2007



PILOT PHASE FLOW DIAGRAM

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JUN 0 8 2007

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JUN 0 8 2007

SUBJ #_____ DATE_____

SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4

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JUN 08 2007

OMEGA Phone Screen Report

6/1/2007

Name: Age: Phone Phone

Phone Screen Date: _____ Gender: _____

Current smoker? Past smoker? Smoking history _____

Date quit _____ Total no of years: _____ Packs per day: _____

Are you presently in any other studies or ever in EPA studies?

Do you have asthma?

Do you have chronic respiratory disease?

Do you have seasonal allergies or hay fever?

Major medical conditions? accidents? operations?

Description:

Are you taking any medications?

Medication type:

Medication:

Add comments:

Have you donated blood in the past 30 days?

Do you have diabetes?

Do you have kidney insufficiency or failure?

Do you use supplemental oxygen?

Have you been hospitalized for breathing problems in the past 3 months?

Have you ever had kidney dialysis?

Do you have heart rhythm problems or heart disease?

Have you ever had a heart attack? Most recent heart attack:

Have you had coronary artery bypass grafting? Most recent bypass:

Do you have an internal cardiac defibrillator?

Do you have a pacemaker?

Name: Age: Phone Phone

Have you ever had a stroke?

Have you been diagnosed with mitral valve prolapse?

Is your b/p greater than 160/90?

Do you have glaucoma treated with medications?

Have you fainted or passed out in the past 12 months?

Have you had cancer requring radiation or chemotherapy?

Are you unable to tolerate having EKG leads applied to your skin?

Additional Comments:

Westat Interviewer: _____

Date: _____

Medical Station

Approved: _____ **Not Approved:** _____

Signed: _____ **Date:** _____

Reason(s) for exclusion: _____

_____

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JUN 08 2007

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA STUDY – GENETIC SCREENING VISIT**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA STUDY SCREENING GUIDELINES
For Genetic Screening Visit

Illness. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores.

No active allergies. If you have questions about allergies, please call the EPA medical station at 966-6232.

If you are unable to keep your appointment, please call Recruitment. Please be on time!

Thank You!
Westat EPA Support Services

Recruitment Letter Prior Subjects: **PILOT and MAIN study**

APPROVED - IRB, UNC-CH

JUN 08 2007

[Date]

Dear [Name]:

During 2005 and 2006 the EPA conducted a research study for older adults during which blood and urine samples were collected. Our records indicate that you completed that study (called CARHOP), and consented to allow a sample of your blood to be stored for possible future studies. The EPA has initiated two new studies about diesel exhaust and genetics and has determined that your specific genotype may make you eligible for one or both of theses studies.

The main purpose of the first study is to identify an effective level of diesel exhaust which can be used to study cardiac changes in healthy older adults. Nearly everyone is exposed to diesel exhaust because it is a component of ambient air pollution, but there is little research to indicate at what levels mild inflammatory changes may occur, particularly in older people. The second study will use information gathered from the first study to test whether fish oil supplements lessen the risk of cardiac changes caused by diesel exhaust.

Both of these studies will require a genotyping visit to the EPA Human Studies Facility plus medical screening, which includes completing medical history forms and a physical exam. For those who qualify by genotype, the pilot study then requires a training visit followed by up to three diesel exposure sessions. Exposure sessions will include blood draws, heart rate monitoring, blood vessel imaging and breathing tests, as well as two-hour exposures to diesel exhaust. Each exposure session will include a follow up session the day after. Diesel exposure levels will never exceed the levels recommended by OSHA (Occupational Safety and Health Administration) or experienced at busy intersections in large urban areas.

Volunteers who qualify for the second study, that of dietary supplementation and diesel exhaust exposure, will also complete genotyping and a training session similar to that described above, but in addition will undergo a dietary assessment and counseling, learn to keep a diet record, and will begin either fish oil or olive oil supplementation. Fifteen days after the training session, volunteers will undergo a single exposure session, followed a day later with additional testing.

Volunteers will be paid for all portions of this research. Genotyping and medical screening pays a total of $60 and training pays $36. Individuals who qualify for the pilot study will be paid $549 for each exposure session and follow-up day plus a bonus of $150 if all three sessions are required and completed. Individuals who qualify for the dietary supplementation study will be paid for screening and training and $999 for completing the single exposure and follow-up.

If you are interested in learning more about these studies or to see if you qualify for screening, please call the Westat EPA Recruiting Office at (919) 966-0604 or 1 (888) 279-9353.

Sincerely,

Andrea Ware, MPH, MEd, RN
Project Manager
Westat EPA Support Services

APPROVED - IRB, UNC-CH
JUN 0 8 2007

OMEGA MAIN STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1095.

Procedures:

Volunteers initially undergo a genetic screening visit and blood work, followed by routine medical screening at the EPA Human Studies Facility, which includes ~~one visit to complete~~ completing medical history forms and ~~another for~~ a physical exam ~~and blood draw for genotyping~~. You will be paid $30 for the genetic testing visit and $30 for the additional medical screening ~~$60 for completing these two screening appointments.~~ If it is determined that your genotype is not needed for this study, no further visits will be scheduled. If you are eligible for the remainder of the study you will have up to 3 visits over approximately 5 to 6 weeks.

Participation in this study will include two-weeks of fish oil or olive oil treatments (taken as capsules) which will begin on the day of your physical exam. A training visit, lasting about 3 hours, will be scheduled 14 days later, and a diesel exposure visit and follow up day will occur two weeks after that. The exposure day lasts 8 hours and the follow up day, about 3. You will also be asked to record your food intake twice for 3 days each between the training session and exposure.

Procedures involved in this study include breathing tests, heart rate monitoring, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a does of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of diesel exhaust, no more than you might encounter if you visited a large city such as Las Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be available for training 14 days after the physical exam and for the exposure 15 days after that. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Must not eat or drink anything for 2 hours prior to the training day visit.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Genotyping Visit, Dietary Instructions, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (1 hour)

Physical Exam, ~~Genotyping, Dietary Instructions~~: days and times TBD (Day 1, 2 hours)

Training: 14 days after the Physical Exam Day (3 hours):

Exposure Day: 8:00 AM on Day 29 after Physical Exam (8 hours)

Follow-up Day: 18 hours after Exposure Day (3 hours)

APPROVED - IRB, UNC-CH
JUN 08 2007

OMEGA PILOT STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this pilot study is to identify ~~a safe~~ an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will be used to determine the type of particular genes you carry. The genes, called glutathione-S-transferase, are responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying an inactive form of one of these genes and a variance of another may be more susceptible to the effects of air pollutants. Therefore, in this pilot research we will study the responses to diesel exhaust in healthy older subjects with very specific genetic characteristics.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1893 if all study visits are completed.

Procedures:

Volunteers initially undergo a genetic screening visit and blood work, followed by routine medical screening at the EPA Human Studies Facility, which includes ~~one visit to complete~~ completing medical history forms and ~~a another for~~ a physical exam. ~~and blood draw for genotyping~~. You will be paid $30 for the genetic testing visit and $30 for the additional medical screening ~~$60 for completing these two screening appointments~~. If it is determined that your genotype is not needed for this study, no further visits will be scheduled for the pilot study, but you may be eligible for the full study at a later time.

If you are eligible for the remainder of the pilot study you will have up to 7 visits over approximately ~~5 to 6~~ 18 to 20 weeks. The goal of this pilot study is to find ~~a safe~~ an optimal concentration of diesel exhaust to be used in the future study. Results will be analyzed after finishing each proposed exposure to decide whether to continue on to the next level of proposed exposure. You may not have all these 3 exposures if this pilot study is terminated at some point based on interim results.

Your participation in this pilot study will include one training session for about 3 hours, up to 3 exposure sessions, each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours. Exposures are separated by about 2 weeks.

Study procedures will include breathing tests, heart rate monitoring, blood draws, and exposures to controlled amounts of diesel exhaust. Even at the highest level of exposure, you will not be exposed to more particles than you might encounter if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure session.

Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In order to participate you must be willing to observe several important restrictions. They are:

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Do not eat or drink anything for 2 hours prior to the training day visit.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposures and follow-ups.

Would you be able to comply with these restrictions? If so, we can schedule you for ~~an office~~ a visit to complete the initial part of the screening.

Study Schedule

Genotyping visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (about 1 hour)

Physical Exam: days and times TBD (2 hours)

Training: days/times TBD (3 hours)

Exposure Day 1: 9:00 AM days TBD (8 hours)
Follow-up 1: 18 hours after Exposure 1 (3 hours)

Exposure Day 2: 9:00 AM days TBD, about 2 weeks after Exposure 1 (8 hours)
Follow-up 2: 18 hours after Exposure 2 (3 hours)

Exposure Day 3: 9:00 AM days TBD, about 2 weeks after Exposure 2 (8 hours)
Follow-up 3: 18 hours after Exposure 3 (3 hours)

Web Site Advertising: www.epastudies.org

OMEGA: Elder Diesel Main Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

Participation in this study will include two-weeks of fish oil or olive oil treatments and one exposure to a controlled amount of diesel exhaust, no more than you might encounter if you visited a large city such as Las Angeles, New York, or Mexico City on a smoggy day. Study procedures include breathing tests, heart rate monitoring, blood tests, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a does of nitroglycerine, which is a short-acting vasodilator. Total time commitment after screening is 3 visits after eligibility screening, about 15 hours, over 5-6 weeks. Compensation is provided.

APPROVED - IRB, UNC-CH
JUN 08 2007

Web Site Advertising: www.epastudies.org

OMEGA PILOT: Elder Diesel Pilot Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this pilot study is to identify an optimal ~~safe~~ concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of particular genes you carry. The genes, glutathione-S-transferase (GSTM1 and GSTP1) are genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying an inactive form of GSTM1 and a variance of GSTP1 may be more susceptible to the effects of air pollutants. Therefore, in this pilot study, we will study the responses to diesel exhaust in healthy older subjects with very specific genetic characteristics.

If you are eligible for the remainder of the study after screening you will have up to 7 visits over approximately 18 to 20 ~~5 to 6~~ weeks. Total time commitment after eligibility screening is about 36 hours. Procedures involved include breathing tests, heart rate monitoring, blood tests, and exposures to controlled amounts of diesel exhaust. Even at the highest level of exposure, you will not be exposed to more particles than you might encounter if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day. Compensation is provided.

APPROVED - IRB, UNC-CH
JUN 0 8 2007

OMEGA MAIN STUDY
E-mail Announcement

SUBJECT: INFORMATIONAL: Research study for Healthy Older Adults

The main purpose of this research study is to determine if a component of diesel exhaust elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Volunteers ages 50 to 75 will be tested to determine the type of a particular gene they carry, one of several genes responsible for protecting the body against oxidants such as diesel exhaust. Participation will include two-weeks of fish oil or olive oil treatments and one exposure to a controlled amount of diesel exhaust. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Committee on the Protection of the Rights of Human Subjects Biomedical Institutional Review Board. IRB # 07-0190 (GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

APPROVED - IRB, UNC-CH
JUN 08 2007

OMEGA PILOT STUDY
E-mail Announcement

SUBJECT: INFORMATIONAL: Pilot Research study for Healthy Older Adults

The main purpose of this pilot study is to identify an optimal ~~safe~~ concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This pilot research will study the responses to diesel exhaust in healthy older subjects with the specific GSTM1 and GSTP1 genotype. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Committee on the Protection of the Rights of Human Subjects Biomedical Institutional Review Board. IRB # 07-0190 (GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

APPROVED - IRB, UNC-CH
JUN 08 2007

"Main" study

The US Environmental Protection Agency is seeking

ADULT VOLUNTEERS

Ages 50 to 75 for Research

APPROVED - IRB, UNC-CH
JUN 08 2007

This is a research study about genetics, diet supplementation and exposure to diesel exhaust.

We are looking for healthy older adults to study diet supplementation and the effects of diesel exhaust exposure on heart and lung function.

Study participants will complete screening and 3 visits over about 5-6 weeks. Total time commitment after screening is about 15 hours. You will receive payment for screening, the study, and out of town travel. Parking is provided.

1-888-279-9353 or
919-966-0604
www.epastudies.org

WESTAT • EPA Recruiting • In care of the environment

The Human Studies Facility is located on the UNC-CH campus

APPROVED - IRB, UNC-CH

JUN 08 2007

The US Environmental Protection Agency is seeking

ADULT VOLUNTEERS

Ages 50 to 75 for Research

This is a pilot study about genetics and exposure to diesel exhaust.

We are looking for healthy older adults with a very specific genotype to study the effects of diesel exhaust exposure on heart and lung function.

If you qualify for this study, you will complete screening and up to 7 visits over about 18-20 weeks. Total time commitment after screening is about 36 hours. You will receive payment for screening, the study, and out of town travel. Parking is provided.

1-888-279-9353 or
919-966-0604
www.epastudies.org

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The Human Studies Facility is located on the UNC-CH campus

First Review of IRB Submission

Receipt Date : 6/07/2007 **Submission Type:** Modification **IRB ID :** 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong **IRB:** Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☑ Expedited (Category: 110(b)(2)) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Submitted by: Beverly Fields

Study Description:

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):
- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter:
  - ☑ Draft letter prepared DT 6-08-07
  - ☑ Approved by chair as attached (Initials/Date: 6/11/07 MB)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 5
Other attachments: 14

FINAL ACTIONS:
- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Jan-2007

RECEIVED
JUN 07 2007
IRB UNC-CH

For IRB Use		
Behav	Biomed	PH-N
IRB Study #_____		
Rec'd_____		
Expiration Date_____		
Full		Expedited

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instrnctions are not followed.**

Check	Item	Total No. of Copies
☐	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
☐	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
☐	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining.	2
☐	4. The sponsor's document describing the amendment, if any.	1
☐	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

IRB study #: 07-0190 **Date: 5 June, 2007**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James M Samet, PhD, MPH (if applicable)

Haiyan Tong — 6/5/2007
Signature of Principal Investigator or designee — Date

June 5, 2007
Signature of Faculty Advisor (if applicable) — Date

List and describe each proposed change:

I. Modifications to IRB Study Protocol.

1). page 6 and 9, add "both" before GSTM1 null.
2). page 10 and 11, add "Currently taking ACE inhibitors." and "Have an allergy to latex." in the exclusion criteria of both main and pilot studies. ACE inhibitors will affect the measurements of BAU and EndoPat. EndoPat device is made of latex, thus we will exclude the subjects who have allergy to latex.
3). page 12, add "plasminogen activator inhibitor" in the biomarker analysis; add "We will also assess the endothelial cell function by brachial artery ultrasound and finger artery Endo-Pat" in the pilot study. And add "Therefore, we will select the diesel concentration based solely on safety considerations" in the last sentence to clarify the purpose of the pilot study.
clarif.
4). page 12 and 13, in the section of "subject qualification", arrange the sequence of genetic screening, physical exam, and training session. We move the paragraph of "GSTM1/GSTP1 genotype screening" and "food frequency questionnaire in the main study" to the "screening session" and make wording accordingly. These changes will reduce the number of subject's office visits and also reduce the cost of physical exam on unqualified subjects.
5). page 13, change the amount of blood for genetic screening from "10 ml" to "5 ml".
6). page 15, add "the both pilot and" in the BAU measurement of pre-exposure section. Reports suggested that BAU measurement is a non-invasive and very sensitive method to detect the vascular effect of air pollutants.
7). page 16 and 17, add "***Symptoms questionnaire*** before exposure will be collected" in the pre- and post-exposure sections.
clarif.
8). page 16, change from "thermal" to "tapered" for definition of TEOM.
9). page 16, delete "and the same lot utilized throughout the study" from the exposure paragraph. It is impossible to use the same lot of diesel for more that 60 exposures.
10). page 16, change the exposure limit of diesel exhaust. We delete a paragraph of "If a real-time PM concentration exceeds 300 $\mu g/m^3$ for 5 min, then the exposure will be terminated. We do not believe a short exposure to about 300 $\mu g/m^3$ will be harmful to the subjects, as volunteers have not shown adverse effects in response to exposures to 300 $\mu g/m^3$ in other studies, while workers are regularly exposed to 1-2 mg/m^3 in some mining operations". We now set the exposure limit as the following:

Exposure limit controls for pilot and main study exposures to DEP. This text describes the criteria that will be used to limit exposure of study subjects to DEP. An exposure will be terminated prematurely if one of the two following conditions is met.

1) The monitoring analyzer registers a concentration greater than 100 ug/m^3 above the target concentration. Specifically, exposures will be terminated at values $\geq$200, 300 or 400 during runs in which the exposure targets are 100, 200 and 300 ug/m^3, respectively.

2) The running sum of the 2-minute average concentrations displayed by the monitoring analyzer during the 2 hr exposure period exceeds 18,000 ug/m^3. This safety limit is specifically designed to address exposures for which the target concentration is 300

ug/m^3. It will prevent an inhalational exposure greater than 600 ug, which assumes that the resting subject will inhale 2 m^3 during the 120 min exposure.
11). page 17, add "The minute ventilation during exposure will be measured." in the exposure section.
12). Page 21, delete "vasoconstrictor endothelin, NOx" and add "plasminogen activator inhibitor" in the biomarkers measurements. Also delete "and TNFα" in the exploratory endpoints.
13). Page 26, in the section of recruiting, change from "Westat recruitment office" to "Medical station" for the genetic screening, and add "and the completed one will be mailed to Westat as per 95-EPA-66. " for the medical history form.
14). page 27, in the paragraph of duration of the study, change the number of visits and duration of the pilot study and the 18-hr post-exposure in the main study from "4 hr" to "3 hr" because we re-arrange the sequence of the office visits and add BAU measurement which will take more time to finish the study.
15). page 28 and 29, change the payment detail and amount of the payment. See the explanation of **4. 11)** and **2. 3).**
16). page 33 and 34, change study flow charts accordingly based on the changes of study protocol.

II. Modifications to Consent Form.

1. Remove Dr. Richard Hermann's name from all the consent forms. He left the office of the EPA NHEERL Human Research Protocol in March 2007.

2. Pilot study genotype screening consent: all the changes are highlighted in yellow.
1). page 2, change the duration of the screening from "15 min" to "30 min" because more time will be needed to briefly review the medical history.
2). page 2, rewrite the paragraph under the subtitle "what will happen if you participate in this study" in order to briefly review the medical history and collect more blood sample.
3). page 2, change the amount of blood drawn from "20 ml" to "50 ml". We will combine all blood draw (genetic screening and physical exam blood) into one sampling and thus increase the amount of blood needed at this time, and it will eliminate the blood sampling on the physical exam day.
4). page 3, add "you will know your blood cholesterol levels and" in the "what are the possible benefits to you?".
5). page 3, change the payment of "$60" to "$30". We split the $60 payment into recruiting ($15), physical exam ($15), and genetic screening ($30). For genetic screening only the payment is $30. The subjects will get another $30 payment on the physical exam.

3. Main study genotype screening consent: all the changes are highlighted in yellow.
1). page 2, change the duration of the study from "15 min" to "30 min". Explanation is the same as **2. 1).**
2). page 2, rewrite the paragraph under the subtitle "what will happen if you participate in this study" in order to get brief medical history and collect more blood sample. And administer the Medical History Form and Food Frequency Questionnaire (FFQ) that is moved from the training day to genotyping day.

3). page 2, change the amount of blood drawn from "50 ml" to "80 ml". Explanation is the same as **2. 3).**
4). page 2, add "(about 5 ml)" after blood sample and "and another portion of the blood will be used for cholesterol and lipid analysis" after GSTM1 gene.
5). page 3, add "you will know your blood cholesterol levels and" in the "what are the possible benefits to you?".
6). page 3, change the payment of "$60" to "$30". Explanation is the same as **2. 5).**

4. Pilot study consent: all the changes are highlighted in yellow.
1). page 2, change "a safe" to "an optimal" under subtitle "what is the purpose of the study?" to clarify the purpose of the pilot study.
2). page 2, add "You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril)" and "You have an allergy to latex." ACE inhibitors will affect the measurements of BAU and EndoPat. EndoPat device is made of latex, thus we will exclude the subjects who have allergy to latex.
3). page 3, change from "5-6 weeks" to "18-20 weeks" in the duration of the study and from "6 hours" to "8 hours" for the exposure session, because we will add BAU measurement (1 hr/measurement) on the exposure day which will increase the duration of the exposure day and will also limit the number of exposures we can do per week.
4). page 4, add "your participation from this study" after "You may terminate".
5). page 4, change "a safe" to "an optimal" after "Our goal of this pilot study is to find" to clarify the purpose of the pilot study.
6). page 4, change from "9 am" to "8 am" for the subject arrival time since we will add BAU measurement.
7). page 5, add a paragraph of the brachial arterial ultrasound (BAU) method to the prior exposure section. We add BAU measurement in the pilot study in order to obtain more information.
8). page 8, add the BAU risk in the "possible risk…" section.
9). page 8, change from "no-call" to "on-call" physician.
10). page 9, change the total amount of payment from "$1461" to "$1893".
11). page 10 and 11, change the detail payments of the study because BAU will be add on the exposure day and lunch will be provided.
12). change flow chart accordingly.

5. Main study consent: all the changes are highlighted in yellow.
1). page 2 and 3, add "You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril)" and "You have an allergy to latex." ACE inhibitors will affect the measurements of BAU and EndoPat. EndoPat device is made of latex, thus we will exclude the subjects who have allergy to latex.
2). page 3, reduce the number of visits from "4 visit" to "3 visit" and the duration of post-exposure from "4 hours" to "3 hours", because we re-arrange the sequence of office visits and drop off BAU of the post-exposure.
3). page 6, change from "4 hours" to "3 hours" for the 18 hrs follow up visit, because we drop off the BAU measurement.
4). page 7, delete "We will take you back to the GCRC at the NCMH for another ultrasound of the artery in your arm." from the 18 hr post-exposure section.
5). page 9, change from "no-call" to "on-call" physician.

6). page 10, change the total amount of payment from "$1087" to "$1095". Take out $5 lunch payment because lunch will be provided to control the fat intake.
7). page 11, change the detail payments of the study and change the screening blood collecting amount from "50 ml" to "80 ml". Explanation is the same as **2. 3).**
8). Change flow chart accordingly.

III. Revised Recruitment Materials.

Recruitment materials are changed accordingly with the changes of protocol.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

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GCRC

OFFICE OF
RESEARCH AND DEVELOPMENT

To: Michael Fried, MD
Associate Director, GCRC

From: Haiyan Tong, MD, PhD
Human Studies Division
National Health and Environmental Effects Research Laboratory

Date: 6/5/2007

Re: Amendment of GCRC
IRB study #: 07-0190
GCRC #: 2579

Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

All the changes are highlighted in yellow in this amendment.

1. We check the box of Meals (research diets for outpatient visits) on the cover page of the GCRC ADDENDUM.
2. On page 13, we check the box of outpatient nutrition requirements. We will send subjects to GCRC for the brachial artery ultrasound (BAU) measurements on the exposure day. Since BAU measurement requires subjects to have low-fat diet, we will request nutritional support from GCRC to provide subjects with a low-fat lunch on the exposure day for better control of their fat intake.

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Institutional Review Board

APPLICATION FOR IRB APPROVAL OF HUMAN SUBJECTS RESEARCH
Version 2-Nov-2006

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For IRB Use		
Behav	Biomed	PH-Nurs
IRB Study #_____		
Rec'd_____		
Full	Expedited	Exempt

Part A.1. Contact Information, Agreements, and Signatures

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Date: 1 June, 2007
IRB #: **07-0190**
GCRC #: 2579

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH
Co- Principal Investigator: Robert Devlin, PhD.
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315

UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov
For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: James M Samet, PhD, MPH
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
Phone #: (919) 966-0665 Fax #: (919) 966-6271 Email Address: samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor:
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): **Sponsor or award number**: EPA Intramural Federal Research

Include following items with your submission, where applicable.
- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.

- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. This application. One copy must have original PI signatures.	3
X	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
X	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
X	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
□	6. Protocol, grant application or proposal supporting this submission; (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal).	3
X	7. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
X	10. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist

Are the following involved?	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	X
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X	__
A.2.3. Videotaping, audiotaping, filming of subjects (newly collected or existing)?	__	X
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	X	
b. Non-English-speaking?		X
c. Decisionally impaired?		X
d. Patients?	X	
e. Prisoners, others involuntarily detained or incarcerated, or parolees?		X
f. Pregnant women?		X
g. Minors (less than 18 years)? ***If yes***, give age range: to years		X
A.2.5. a. Is this a multi-site study (sites outside UNC-CH engaged in the research)?		X
b. Is UNC-CH the sponsor or lead coordinating center?		X
If yes, include the ***Addendum for Multi-site Studies where UNC-CH is the Lead Coordinating Center***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, provide contact information for the foreign IRB.		
A.2.6. Will there be a data and safety monitoring committee (DSMB or DSMC)?	__	X
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	X
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	X
A.2.8. a. Investigational drugs? (provide **IND #** _____)		X
b. Approved drugs for "non-FDA-approved" conditions?		X
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	X	
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #** _____)	__	X
A.2.11. Fetal tissue?	__	X
A.2.12. Genetic studies on subjects' specimens?	X	__
A.2.13. Storage of subjects' specimens for future research? ***If yes***, *see instructions for* ***Consent for Stored Samples***.	X	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? ***If yes***, *approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	X
A.2.15. Recombinant DNA or gene transfer to human subjects? ***If yes***, *approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	X
A.2.16. Does this study involve UNC-CH cancer patients? ***If yes***, *submit this application directly to the* ***Oncology Protocol Review Committee***.	__	X
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? ***If yes***, *obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	X	__

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	X no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the Sponsor of this study for the use or benefit of any member of the research team?	__ yes	X no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. The first part of this protocol is a pilot study to identify the optimum diesel exhaust concentration to investigate the cardiovascular effects in healthy older subjects. The main part of this proposal is to examine the health effects of diesel exhaust exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust.
Participants: Approximately 6 healthy 50-75 year-old male and female subjects with both GSTM1 null and GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val; n=2 each) for the pilot study. Sixty healthy 50-75 year-old male and female subjects (30 subjects are GSTM1 positive genotype and 30 subjects are GSTM1 null) for the main study.
Procedures (methods): In the pilot study, subjects will have 3 sequential exposures to the diesel exhausts at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours with a about 2 weeks of interval between exposures. In the main study, GSTM1 positive and GSTM1 null genotype subjects will be exposed to the optimum concentration (will be determined based on the pilot study) of diesel exhaust for 2 hours after 15 days of omega-3 fatty acid fish oil or olive oil supplementations.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from

cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13).

A recent epidemiological study involving eight European cities suggested that the primary effect of PM air pollution on cardiac admissions was likely attributable to diesel exhaust inhalation (14). Diesel exhaust is a component of air pollution in urban settings, and contributes both PM and gaseous phase compounds to the atmosphere. Diesel exhaust fumes are made up largely of fine and ultrafine carbonaceous particulates generated by incomplete combustion of fuel. The U.S. EPA and the National Research Council (NRC) PM Research Priorities currently include identification of components of PM (including PM size and chemical composition) that contribute to the reported increased morbidity and mortality (NRC, 1998; EPA, 1997). In addition, both the EPA and NRC have stressed the importance of examining gaseous co-pollutants that may also affect PM toxicity. Diesel exhaust (i.e., gas phase constituents and diesel exhaust particles (DEP)) is ideal for examining the role of these factors in inducing human health effects for the following reasons: (1) Because of the relatively high content of organic compounds on DEP (usually 10-30% by weight), DEP are ideal for examining the role of organics in inducing health effects; (2) The exhaust is a mixture of PM and gaseous pollutants including some (e.g., aldehydes, NOx, carbon monoxide) that are known to produce human toxicity in high enough concentrations that may potentially augment PM-induced effects; (3) the size of the DEP vary depending on engine type and other factors, allowing assessment of PM size on the induction of human health effects. Few controlled human exposures to diesel exhaust have been performed. One hour exposure to 300 $\mu g/m^3$ diesel exhaust with intermittent exercise in healthy human volunteers resulted in marked systemic and redox-sensitive pulmonary inflammatory responses (15, 16), and vascular dysfunction and impaired endogenous fibrinolysis that links diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (17). Despite a decade of intensive studies, much about the PM health effects problem, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that diesel exhaust exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of diesel exhaust exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatherosclerotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (18)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (19-21). Fish oil

supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (22). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (23). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (24). Another enzyme called GSTP1 is also associated with anti-oxidants effects and we will determine the optimal concentration of diesel exhaust in the pilot study in the most vulnerable subgroup with GDTM1 null and 3 GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val). Therefore, the role of GSTs in mediating the cardiovascular effects following acute diesel exhaust exposure will also be studied in the main protocol in GSTM1 null and GSTM1 positive healthy older adults.

U.S. EPA regulations concerning diesel emissions, mandated by the Clean Air Act, have resulted in dramatic decreases in emissions from diesel engines since 1988, with the last set of significant engine modifications occurring in 1995 (EPA, 1998). The respiratory effects of diesel exhaust have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to diesel exhaust in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to diesel exhaust in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.

12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Le Tertre, A., Medina, S., Samoli, E., Forsberg, B., Michelozzi, P., Boumghar, A., Vonk, J.M., Bellini, A., Atkinson, R., Ayres, J.G., Sunyer, J., Schwartz, J. and Katsouyanni, K. (2002). Short-term effects of particulate air pollution on cardiovascular diseases in eight European cities. J Epidemiol Community Health 56:773-779.
15. Salvi, S., Blomberg, A., Rudell, B., Kelly, F., Sandstrom, T., Holgate, S.T. and Frew, A. (1999). Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. American journal of respiratory and critical care medicine 159:702-709.
16. Behndig, A.F., Mudway, I.S., Brown, J.L., Stenfors, N., Helleday, R., Duggan, S.T., Wilson, S.J., Boman, C., Cassee, F.R., Frew, A.J., Kelly, F.J., Sandstrom, T. and Blomberg, A. (2006). Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Respir J 27:359-365.
17. Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstrom, T. and Newby, D.E. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112:3930-3936.
18. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
19. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
20. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
21. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
22. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
23. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
24. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.
25. Lauer, M.S., Pothier, C.E., Chernyak, Y.B., Brunken, R., Lieber, M., Apperson-Hansen, C. and Starobin, J.M. (2006). Exercise-induced QT/R-R-interval hysteresis as a predictor of myocardial ischemia. Journal of electrocardiology 39:315-323.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal includes approximately 6 subjects with both GSTM1 null and GSTP1 codon 105 (Ile/Ile, Ile/Val, and Val/Val; n=2 each) genotypes for the pilot study and 60 subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) for the main study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria (pilot study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Currently taking ACE inhibitors.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Have an allergy to latex.

Exclusion criteria (main study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin.

- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Currently taking ACE inhibitors.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Female subjects currently taking estrogen replacement therapy.
- Because of reported cardioprotective effects of red wine, subjects will be prohibited from drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Have an allergy to latex.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

Pilot study: Approximately 6 subjects will undergo 3 exposures to air containing approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts sequentially for 2 hours in an exposure chamber. There will be a 2-week interval between exposures. We anticipate that there will be 6 subjects at each concentration of diesel exhaust exposure during the course of this pilot study. However, circumstances beyond our control may arise (i.e. subject/subjects might drop off due to illness and/or other reasons during the study) which may prevent finishing the proposed 3

exposures, and then we will have to add new subject/subjects at that point. Each new subject will have 3 exposures at approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts. Subjects who withdraw from the study will be compensated for all procedures and time completed to that point (See the pilot study design flow chart).

The purpose of the pilot study is to identify safe and measurable cardiovascular effects of diesel concentration. Our primary endpoints in the pilot study include HRV, pulmonary function, and biomarkers such as neutrophils, platelets, fibrinogen, c-reactive protein, lactate, ferretin, plasminogen activator inhibitor, and α1-antitrypsin. We will also assess the endothelial cell function by brachial artery ultrasound and finger artery Endo-Pat. We will analyze these endpoints after finishing each concentration of diesel exposure and determine whether we will continue the next higher concentration exposure. For example, if we find effects at approximately 100 $\mu g/m^3$ of diesel concentration then we will use that concentration for our main study. We may not need to test subjects at the next higher proposed concentrations. We will carefully monitor the symptoms that subjects may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, dyspnea, pallor, ataxia, and significant cardiac arrhythmias. In addition, analyses of blood samples taken after exposure will be scrutinized for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. Symptoms and/or changes detected in the blood samples that are considered clinically significant could prompt the study physicians to stop the study. Therefore, we will select the diesel concentration based solely on safety considerations.

Main study: This will be a randomized double-blinded study. Sixty study subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) will be given, in a double-blind fashion, a container filled with enteric-coated either 2g/day of fish oil or 2g/day of olive oil capsules for 15 days. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 25-35% of eicosapentaenoic acid (EPA) and 30-65% of docosahexaenoic acid (DHA). Each 1000 mg of these olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. There will be four groups of subjects in this study with 15 subjects in each group: the first group is fish oil supplement in GSTM1 positive subjects, the second group is fish oil supplement in GSTM1 null subjects, the third group is olive oil supplement in GSTM1 positive subjects, and the fourth group is olive oil supplement in GSTM1 null subjects. In the exposure study, each subject will be exposed to air containing approximately optimum diesel exhaust for 2 hours in an exposure chamber. (See the main study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for a genetic screening appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotype screening informed consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 26, methods of recruiting) will not need genotype screening. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 50 ml of blood collected in the pilot study and approximately 80 ml of blood collected in the main study. A portion of the blood

will be used for genetic screening of GSTM1 and/or GSTP1 Ile105 Val polymorphism and another portion of the blood will be used for the cholesterol levels, fatty acid analysis, and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back after they are selected on the genotype.

- ***GSTM1/GSTP1 genotype screening***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1 and GSTP1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene and GSTP1 Ile105Val polymorphism. Since the pilot study only seeks people that are GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotypes, and since the joint GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotype is present in about 8%, 17%, and 14% respectively of the population, it is likely that about 80-90 % of the subjects will be excluded in this pilot study but they will be informed to participate in our future study which will enroll both GSTM1 positive and null genotypes. Those subjects with GSTM1 positive will not be asked to continue with the study and will be compensated for their time. Since the main study seeks to enroll equal numbers of people that are GSTM1 positive and null, it is likely that as the study nears completion, we will still be seeking people with the null allele but not with the sufficient allele. If that is the case, those subjects will not be asked to continue with the study and will be compensated for their time.

- ***Food frequency questionnaire in the main study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the university of south carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the genetic screening will be scheduled for a physical examination in the HSF. Those subjects who are genetically screened but are not eligible for the pilot study, may be included in the main study if they are interested in participating. During this visit, subjects will sign an informed consent for a physical and the medical history form completed that contains information on general personal and family medical history. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam and genetic screening will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the main study:*** Subjects will be given an approximately 15 days supply of either fish oil or olive oil supplements randomly assigned. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of energy and nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record per week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 15 day of supplementation period. We will give the subjects another 15 days supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

- ***HRV measurement before exposure***: In the pilot study, subjects will come to the human study facilities (HSF) at EPA in the morning of the exposure to have baseline heart rate viability (HRV) measured by a Holter monitor. Electrodes for telemetry and HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. Subjects will be released while wearing the Holter monitor and return to the HSF the next morning to get the Holter monitor removed. In the main study, subjects will come to the HSF in the morning of the last supplement to get baseline heart rate viability (HRV) by

Holter monitor before exposure and the monitor will be remained for approximately 24 hours.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat or drink anything for 2 hours prior to the training day visit.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (blood pressure, HRV, endothelial cell function by brachial artery ultrasound (BAU) and possibly by Endo-PAT, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement in both pilot and main studies*** will be done by a Holter monitor.
- ***Brachial artery ultrasound in both the pilot and main studies:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.
- ***Peripheral endothelial function by Endo-PAT in both pilot and main studies***: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be induced by sudden cuff deflation. The PWA recordings will be measured continuously during suprasystolic occlusion and deflation reactive hyperemia. PAT measurement will be

displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).

- ***Pulmonary function in both pilot and main studies*** will be measured by spirometry.
- ***Blood sampling in both pilot and main studies***: approximately 50 ml of blood samples will then be collected in the pilot study and approximately 80 ml of blood samples will then be collected in the main study.
- ***Symptoms questionnaire*** before exposure will be collected.

In the pilot study: Subjects will then enter the diesel exposure chamber for 3 sequentially exposures at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours each with a about 2-week interval between exposures. In the main study: Subjects will have only one exposure at an optimum concentration of diesel exhaust that will be identified from the pilot study.

Exposure: All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. The diesel exhaust will be generated from a 6-cylinder, 205 hp, 5.9 L displacement diesel engine (Cummins, Columbus IN) mounted on a vehicle located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean filtered and humidified air by approximately 1/30th to give a chamber concentration of approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$. If a real-time PM concentration exceeds 300 $\mu g/m^3$ for 5 min, then the exposure will be terminated. We do not believe a short exposure to about 300 $\mu g/m^3$ will be harmful to the subjects, as volunteers have not shown adverse effects in response to exposures to 300 $\mu g/m^3$ in other studies, while workers are regularly exposed to 1-2 mg/m^3 in some mining operations (delete). The monitoring analyzer registers a concentration greater than 100 ug/m^3 above the target concentration as the exposure limit. Specifically, exposures will be terminated at values $\geq$200, 300 or 400 during runs in which the exposure targets are 100, 200 and 300 ug/m^3, respectively. The running sum of the 2-minute average concentrations displayed by the monitoring analyzer during the 2 hr exposure period exceeds 18,000 ug/m^3. This safety limit is specifically designed to address exposures for which the target concentration is 300 ug/m^3. It will prevent an inhalational exposure greater than 600 ug, which assumes that the resting subject will inhale 2 m^3 during the 120 min exposure. It is expected that 75 to 95% of the diesel exhaust PM from this type of engine will be centered around approximately 0.1-0.2 μm. Preliminary testing has shown the mean particle size to be approximately 0.14 μm. Levels of carbon monoxide, and oxides of nitrogen (NOx; mainly NO, and some NO2), will be kept under 10 ppm CO, 15 ppm NO, and 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) for these substances are: carbon monoxide=50 ppm; NO2=5 ppm; NO=25 ppm; The American Conference of Governmental Industrial Hygienists (ACGIH) 8 hr threshold limit values (TLVs) are: NO=25 ppm, NO2=3 ppm, and CO=25 ppm]. Diesel fuel used for the study will be purchased as a certified fuel (Chevron Phillips Chemical Co., Borger TX, 0.05 LS Certification Fuel, type II), and the same lot utilized throughout the study (delete). This certified fuel represents diesel fuel composition that is typically available at gas stations for most regions of the country except for California. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured with a tapered element oscillating microbalance (TEOM) and/or a Data-RAM, particle number per cm^3 by a Condensation Particle Counter (CPC), and

chamber PM concentrations during exposure further validated by weighing filters obtained from versatile air pollution samplers (VAPS) impacters. Particle size distribution will be determined by scanning mobility particle size (SMPS) not during the subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. The minute ventilation during exposure will be measured.

Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 50 ml of blood will be collected in the pilot study and approximately 80 ml of blood will be collected in the main study.

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 50 ml of blood will be collected in the pilot study and approximately 80 ml of blood will be taken in the main. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound and finger arteries Endo-PAT). Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood (about 50 ml in the pilot study about 80 ml in the main study) will be drawn by standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In the main study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to a diesel exhaust and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of diesel exhaust on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound and finger arteries Endo-PAT:** There are no known risks associated with imaging of the brachial artery and index finger arteries. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Diesel exposure** The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban areas. Diesel particulate concentrations proposed for the pilot study will be 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m3$ and the optimum concentration will be used for the main study. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it

exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. During exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have EKG leads attached during the exposure to monitor cardiac function during exercise. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for their participation up to that point. The investigators or duty physician will end the exposure if the subject is found to be suffering from any significant adverse effect. The small pilot study will allow us to monitor the adverse effects of diesel exhaust closely and we will determine whether we will go to the next higher concentration based on the analysis. We will be able to identify a safe concentration of diesel exhaust that induces a measurable change in our study endpoints. No major adverse health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

- **Confidentiality** Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test our hypothesis that diesel exhaust causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust. We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to diesel will also be measured (e.g., blood pressure, HRV, brachial arterial blood flow rate, change in blood coagulation factors, vasoconstrictor endothelin, NOx (delete), platelets, fibrinogen, c-reactive protein, lactate, plasminogen activator inhibitor, ferretin, and α1-antitrypsin). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 was derived using a power of 0.8 and an α of 0.05.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to diesel exhaust compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial blood flow rate, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial blood flow rate. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of diesel exhaust on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen and platelets, changes in IL 6, IL 8 and TNFα, (delete) comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. X Names
b. X Telephone numbers
c. X Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. X Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. X Electronic mail addresses
g. __ Social security numbers
h. X Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, characteristic or code, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.11. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

X No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:

__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.12. **Data security for storage and transmission**. Please check all that apply.

For electronic data:

X Secure network X Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question 7 above)
__ Locked suite or office
X Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.13. **Post-study disposition of identifiable data or human biological materials**. Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true:

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study involves sensitive data that could be damaging if disclosed). __ yes __ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no
Explain.

If you checked "yes" to either, will consent be oral? Will you give out a fact sheet? Use an online consent form, or include information as part of the survey itself, etc?

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**

If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ *If this does not apply to your study, do not submit this section.*

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with your IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Will the information collected be limited only to that necessary to contact the subjects to ask if they are interested in participating in the study?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the pilot study will be approximately 3 months and the main study will take approximately 18 months. Participant recruitment and screening is expected to be continuous throughout the study until the intended number of participants is reached.

If the subject is eligible for the pilot study they will have 9 visits to the HSF over approximately 18-20 weeks. Subjects will have about 2 weeks break between 2 exposures. If the subject is eligible for the main study of diesel exhaust exposure the individual will have 5 visits to the research facility over approximately 5-6 weeks. The pre-enrollment genotyping and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after genotyping of the blood sample. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment for the main study) will require approximately 3 hours. Exposure day will last approximately 8 hours for the pilot and main studies. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours for the pilot and the main study. study and 4 hours for the main study (delete).

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Include food or refreshments that may be provided.

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be paid for the entire study, excluding completion bonus.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled, and will be paid 50% up to a total maximum of $100 for canceled procedures. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study.

1. If following genotyping of the blood samples on screening day, it is determined that the individual's genotype is not needed for completion of the study, then that subject may be informed that his/her participation in this study is no longer necessary. The subject will be notified by phone and the total compensation for this study will be approximately $60.
2. If the subject is qualified and finished the entire pilot study, the total compensation for completion of this study will be approximate $1893.
3. If the subject is qualified and finished the entire main study, the total compensation for completion of this study will be approximate $1095.

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml of pilot and ~80ml of main study; genotyping)	$30
Pre-study qualification total =	$60

Subjects based on their genotype assessment may not be asked to further participate in the study.

Training day (3 hours)

Time (3h @$12/h)	$36

Pilot study:

Exposure (8 hours)

Venipuncture (~50ml, pre; 3@$30 each)	$90
Holter monitor (3 @$100 each)	$300
Chamber exposure (3 @$72 each)	$216
Venipuncture (~50ml, post; 3@$30 each)	$90
Time (3X8h @$12/h)	$228
Finger artery Endo-PAT (6 @$10 each)	$60
Brachial artery ultrasound (6@$50 each)	$300
Lunch (3@$5 each) (delete)	$15
On-time bonus (3@$25 each)	$75

Total for completion of exposure = $1419

Eighteen hours after exposure (3 hour)

Venipuncture (~50ml; 3@$30 each)	$90
Time (3X3h @$12/h)	$108
Finger artery Endo-PAT (3 @$10 each)	$30

Total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each)	$150

Approximate TOTAL for completion of pilot study = $1893

Main study:

Exposure (8 hours)

Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Finger artery Endo-PAT (2 @$10 each)	$20
Lunch: (delete)	$5
Food records (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $823

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound (delete)	$50
Finger artery Endo-PAT	$10

Total for completion of post-exposure = $76

Protocol Completion Bonus	$100

Approximate TOTAL for completion of main study = $1095

Subjects will be asked to bring (delete) provided a lunch by GCRC for the exposure day. and a refrigerator and microwave will be available. (delete) If a subject is terminated from the study or

chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Data, Records or Human Biological Specimens without Direct Contact with Subjects

→ *If this does not apply to your study, do not submit this section.*

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

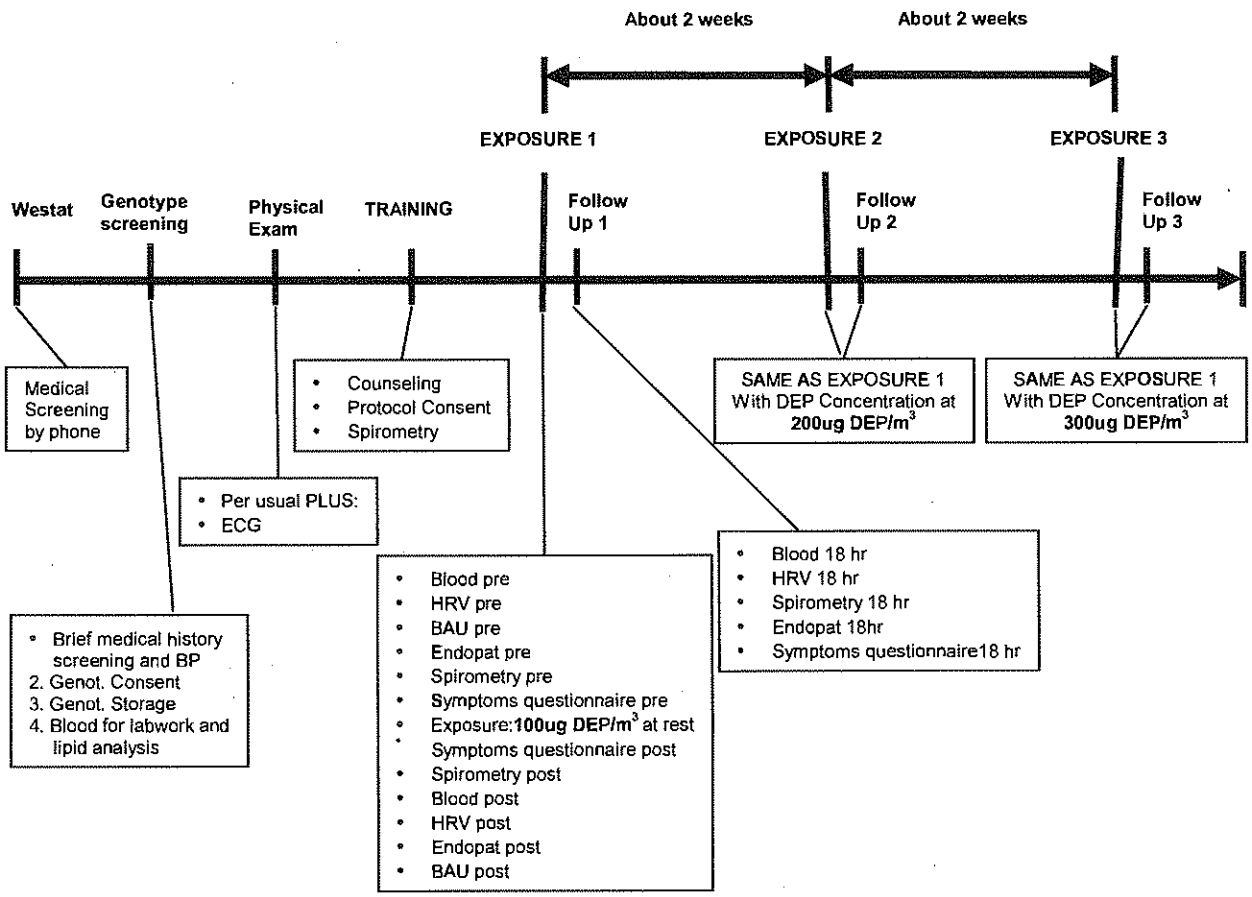
C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

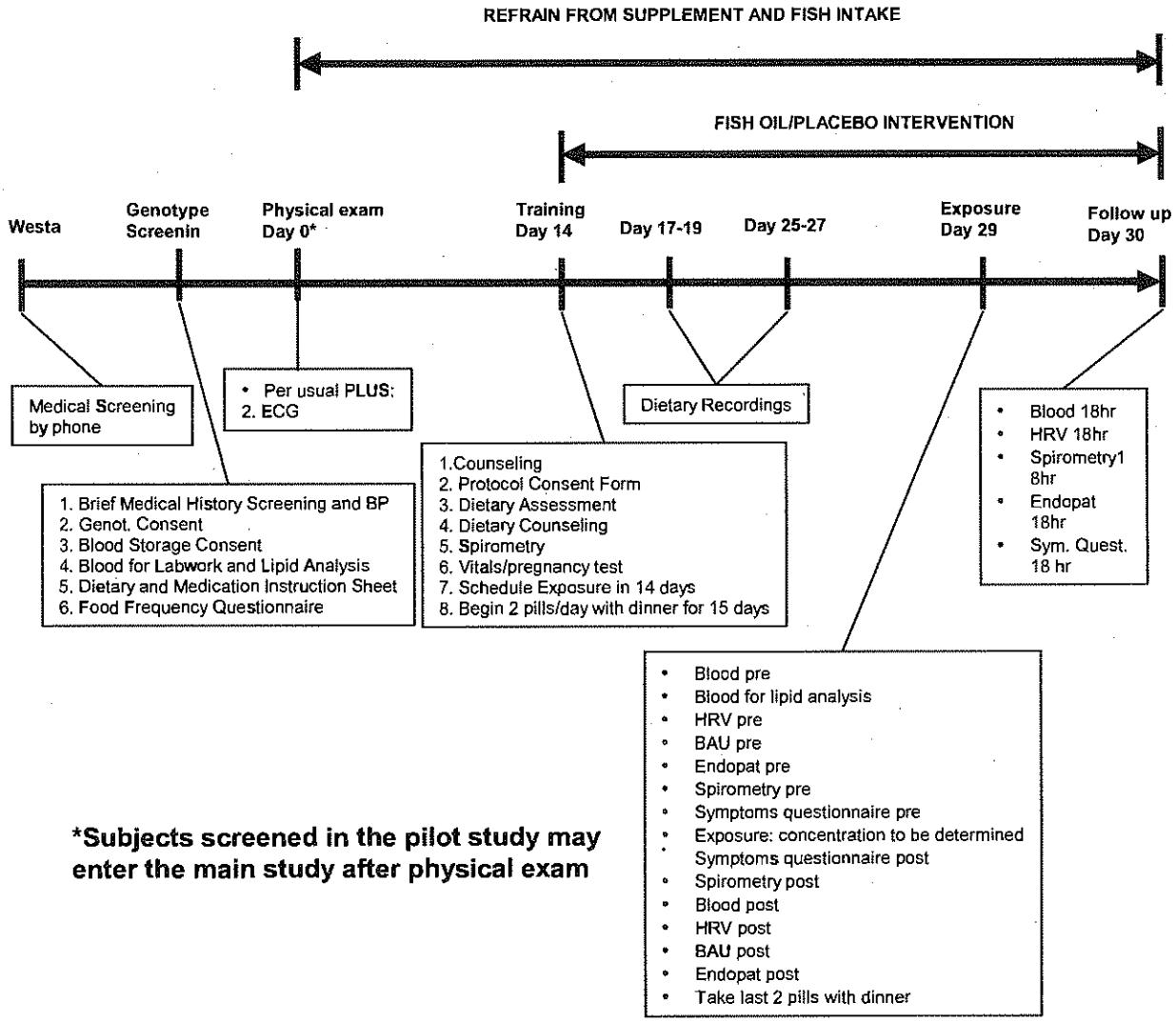
__ yes __ no __ not applicable (explain)

C.6. Do all of these data records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain



PILOT PHASE FLOW DIAGRAM



MAIN STUDY FLOW DIAGRAM

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097

TO: Haiyan Tong
Environmentally Protection Agency, CB-7315

FROM: Biomedical IRB

Stephen Bernard/JK
Authorized signature on behalf of IRB

APPROVAL DATE: 6/22/2007

EXPIRATION DATE OF APPROVAL: 2/25/2008

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Approved Research
Study #: 07-0190
Other #: GCRC-2579
Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this research is no more than minimal.

Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Should any adverse event or unanticipated problem involving risks to subjects or others occur it must be reported immediately to the IRB using the adverse event form at the same web site.

Submission Description:
This modification dated June 21, 2007 submits the following changes: **1)** Pre and post-exposure urine samples will be collected to look at a carcinogen biomarker in the urine in response to diesel exhaust exposure. **2)** Consent form, dated June 21, 2007, has been revised to reflect this change. **3)** Recruitment materials have been changed accordingly.

Details: Call the IRB at 966-3113 if you have any questions. You can now access IRB status information at https://my.research.unc.edu/.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), and 21 CFR 50 & 56 (FDA), where applicable.

The University of North Carolina at Chapel Hill holds a Federal Wide Assurance approved by the Office for Human Research Protections, Department of Health and Human Services (FWA # 4801).

CC: James Samet, Environmentally Protection Agency, Faculty Advisor, Wanda Simmons, (GCRC), Non-IRB Review Contact

APPROVED - IRB, UNC-CH
JUN 22 2007

OMEGA PILOT STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will be used to determine the type of particular genes you carry. The genes, called glutathione-S-transferase, are responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying an inactive form of one of these genes and a variance of another may be more susceptible to the effects of air pollutants. Therefore, in this pilot research we will study the responses to diesel exhaust in healthy older subjects with very specific genetic characteristics.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1953 if all study visits are completed.

Procedures:

Volunteers initially undergo a genetic screening visit and blood work, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genetic testing visit and $30 for the additional medical screening. If it is determined that your genotype is not needed for this study, no further visits will be scheduled for the pilot study, but you may be eligible for the full study at a later time.

If you are eligible for the remainder of the pilot study you will have up to 7 visits over approximately 18 to 20 weeks. The goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in the future study. Results will be analyzed after finishing each proposed exposure to decide whether to continue on to the next level of proposed exposure. You may not have all these 3 exposures if this pilot study is terminated at some point based on interim results.

Your participation in this pilot study will include one training session for about 3 hours, up to 3 exposure sessions, each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours. Exposures are separated by about 2 weeks.

Study procedures will include breathing tests, heart rate monitoring, blood draws, and exposures to controlled amounts of diesel exhaust. Even at the highest level of exposure, you will not be exposed to more particles than you might encounter if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure session.

Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In order to participate you must be willing to observe several important restrictions. They are:

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Do not eat or drink anything for 2 hours prior to the training day visit.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposures and follow-ups.

Would you be able to comply with these restrictions? If so, we can schedule you for a visit to complete the initial part of the screening.

Study Schedule

Genotyping visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (about 1 hour)

Physical Exam: days and times TBD (2 hours)

Training: days/times TBD (3 hours)

Exposure Day 1: 9:00 AM days TBD (8 hours)
Follow-up 1: 18 hours after Exposure 1 (3 hours)

Exposure Day 2: 9:00 AM days TBD, about 2 weeks after Exposure 1 (8 hours)
Follow-up 2: 18 hours after Exposure 2 (3 hours)

Exposure Day 3: 9:00 AM days TBD, about 2 weeks after Exposure 2 (8 hours)
Follow-up 3: 18 hours after Exposure 3 (3 hours)

APPROVED - IRB, UNC-CH
JUN 22 2007

Recruitment Letter Prior Subjects: **PILOT and MAIN study**

[Date]

Dear [Name]:

During 2005 and 2006 the EPA conducted a research study for older adults during which blood and urine samples were collected. Our records indicate that you completed that study (called CARHOP), and consented to allow a sample of your blood to be stored for possible future studies. The EPA has initiated two new studies about diesel exhaust and genetics and has determined that your specific genotype may make you eligible for one or both of theses studies.

The main purpose of the first study is to identify an effective level of diesel exhaust which can be used to study cardiac changes in healthy older adults. Nearly everyone is exposed to diesel exhaust because it is a component of ambient air pollution, but there is little research to indicate at what levels mild inflammatory changes may occur, particularly in older people. The second study will use information gathered from the first study to test whether fish oil supplements lessen the risk of cardiac changes caused by diesel exhaust.

Both of these studies will require a genotyping visit to the EPA Human Studies Facility plus medical screening, which includes completing medical history forms and a physical exam. For those who qualify by genotype, the pilot study then requires a training visit followed by up to three diesel exposure sessions. Exposure sessions will include blood draws, heart rate monitoring, blood vessel imaging and breathing tests, as well as two-hour exposures to diesel exhaust. Each exposure session will include a follow up session the day after. Diesel exposure levels will never exceed the levels recommended by OSHA (Occupational Safety and Health Administration) or experienced at busy intersections in large urban areas.

Volunteers who qualify for the second study, that of dietary supplementation and diesel exhaust exposure, will also complete genotyping and a training session similar to that described above, but in addition will undergo a dietary assessment and counseling, learn to keep a diet record, and will begin either fish oil or olive oil supplementation. Fifteen days after the training session, volunteers will undergo a single exposure session, followed a day later with additional testing.

Volunteers will be paid for all portions of this research. Genotyping and medical screening pays a total of $60 and training pays $36. Individuals who qualify for the pilot study will be paid $569 for each exposure session and follow-up day plus a bonus of $150 if all three sessions are required and completed. Individuals who qualify for the dietary supplementation study will be paid for screening and training and $999 for completing the single exposure and follow-up.

If you are interested in learning more about these studies or to see if you qualify for screening, please call the Westat EPA Recruiting Office at (919) 966-0604 or 1 (888) 279-9353.

Sincerely,

Andrea Ware, MPH, MEd, RN
Project Manager
Westat EPA Support Services

Westat is a contract Research Organization providing research support to the U.S. Environmental Protection Agency Human Studies Division in Chapel Hill, NC.

First Review of IRB Submission

Receipt Date : 6/21/2007 **Submission Type:** Modification **IRB ID :** 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong **IRB:** Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☒ Expedited (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Submitted by: Sonya H Bateman

Study Description:

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter: DT 6.22.07
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 6/25/07 SHB)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☒ Hard copy sent

Consent forms attached: 1

Other attachments: 2

FINAL ACTIONS:

- ☐ Approved
- ☒ Approved with Minor Stipulations
- ☒ NHSR
- ☒ Return to sender
- ☒ Termination

For IRB Use		
Behav	Biomed	PH-N
IRB Study #_____		
Rec'd_____		
Expiration Date_____		
Full	Expedited	

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

version 29-Jan-2007

RECEIVED
JUN 21 2007
IRB UNC-CH

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

IRB study #: 07-0190 **Date: 21 June, 2007**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James M Samet, PhD, MPH (if applicable)

Signature of Principal Investigator or designee — Date: June 21, 2007

Signature of Faculty Advisor (if applicable) — Date

List and describe each proposed change:

I. Modifications to Pilot Consent Form.

All the changes are highlighted in yellow.

1). page 5 and 6, add "Have about 30 ml of urine sample" in the pre- and immediately post-exposure. The urine sampling is added because an investigator at EPA in RTP will look at a carcinogen biomarker in the urine in response to diesel exhaust exposure.

2). page 9-11, change the payments to cover the $10 for each urine sampling.

3). page 13, add urine sampling in the study flow chart.

II. Revised Recruitment Materials.

Recruitment materials are changed accordingly with the changes of consent.

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097

TO: Haiyan Tong
Environmentally Protection Agency
7315

FROM: Biomedical IRB

Authorized signature on behalf of IRB

APPROVAL DATE: 7/06/2007

EXPIRATION DATE OF APPROVAL: 2/25/2008

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Study #: 07-0190
Other #: GCRC - GCRC-2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this research is no more than minimal.

Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Should any adverse event or unanticipated problem involving risks to subjects or others occur it must be reported immediately to the IRB using the adverse event form at the same web site.

Submission Description:
This amendment, dated June 29, 2007, addresses the following modifications to the Pilot Consent Form: 1) On page 4, under the heading "Exposure day" the phrase "and bring your lunch" is deleted.

The GCRC will provide lunch for subjects. 2) On page 6, under the heading "Immediately following the exposure, you will": "Have another arm artery ultrasound at GCRC" is added to the list of procedures. This measurement is already in the IRB protocol but it was left out of the consent form. The payment for this measurement was already included in the payment section. This amendment also provides and explanation of the method to transport subjects to the GCRC for the BAU measurement. An EPA government vehicle will be used to transport subjects from the EPA building to the GCRC in order to reduce the uncontrolled exposure to air pollutants. If the government vehicle is not available, a personal vehicle will be used as an alternate.

Details:
Call the IRB at 966-3113 if you have any questions. You can now access IRB status information at https://my.research.unc.edu/.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164(HIPAA), and 21 CFR 50 & 56 (FDA), where applicable.

The University of North Carolina at Chapel Hill holds a Federal Wide Assurance approved by the Office for Human Research Protections, Department of Health and Human Services (FWA # 4801).

CC: James Samet, Environmentally Protection Agency, , Faculty Advisor
Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 7-6-07 AND 2-25-08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY

3

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 29 June, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
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Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal

Initial/Date _____

anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately

Initial/Date _____

200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed.

Initial/Date _____

These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you

Initial/Date _____

would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.

- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).

Initial/Date _____

- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?
You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an

Initial/Date _____

intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your

Initial/Date _____

information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $1953.

Initial/Date _____

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90
Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75
Total for completion of exposure = $1479	
18 hours after exposure (3 hour)	

Initial/Date _____

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $1953

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

Initial/Date _____

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

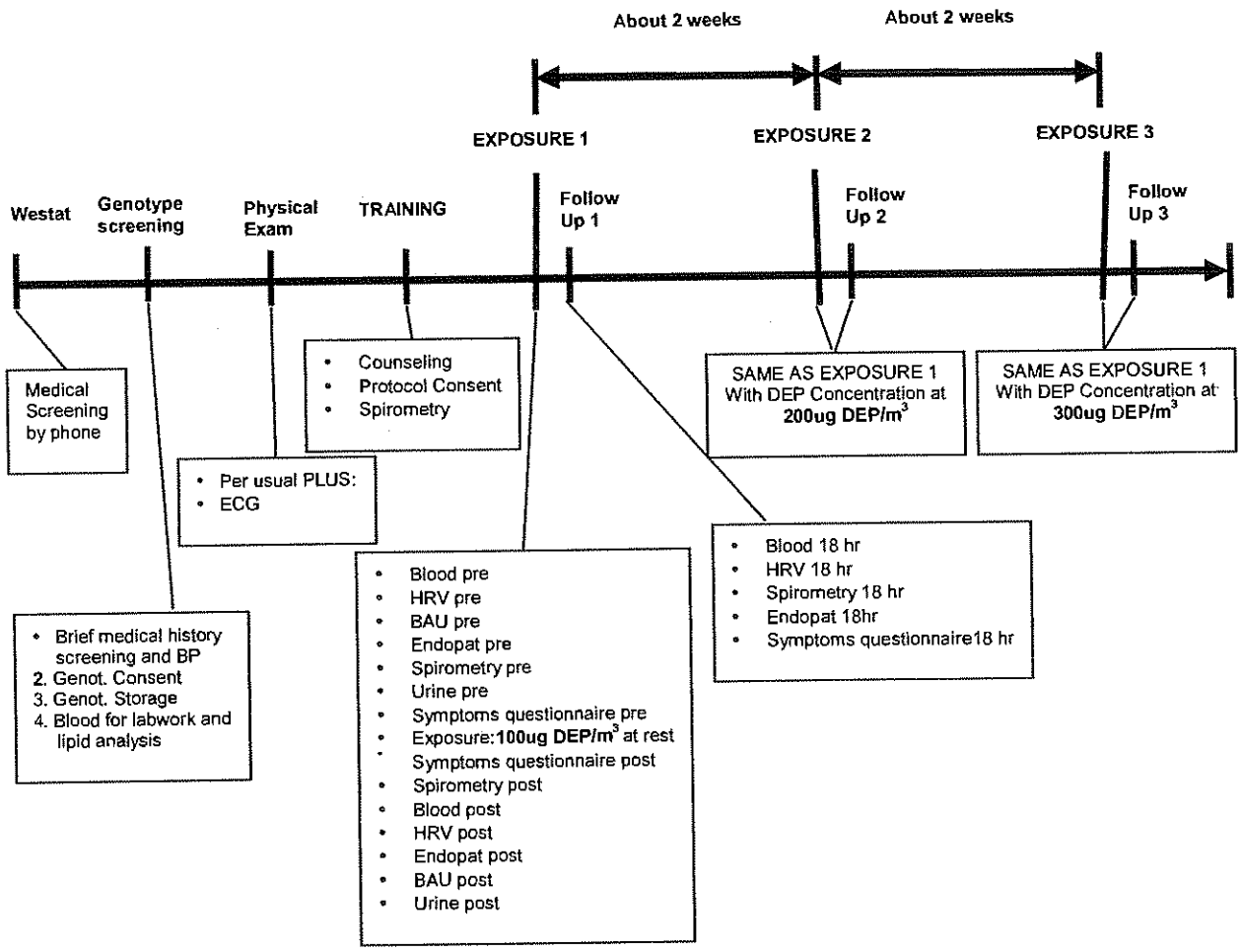
____________________________________ ________________
Signature of Research Subject Date

____
Printed Name of Research Subject

____________________________________ ________________
Signature of Person Obtaining Consent Date

____
Printed Name of Person Obtaining Consent

Initial/Date ________________



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

First Review of IRB Submission

Receipt Date : 6/29/2007 **Submission Type:** Modification **IRB ID :** 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong **IRB:** Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☒ Expedited (Category: 7) ☐ Full IRB

Agenda Date Reviewer 1: CPW Reviewer 2: Minor change to ~~existing~~ prior approved research

Submitted by: Sonya H Bateman

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults ages 50 to 75 will complete 7 visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter:
  - ☑ Draft letter prepared JH 7-6-07
  - ☑ Approved by chair as attached (Initials/Date: 7-06-07 ENK)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 1

Other attachments: Ø

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Jan-2007

RECEIVED
JUN 29 2007
UNC-(IRB)

For IRB Use
Behav Biomed PH-N
IRB Study # 07-1090
Rec'd
Expiration Date
Full Expedited

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

IRB study #: 07-0190 **Date: 29 June, 2007**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James M Samet, PhD, MPH (if applicable)

Haiyan Tong — 6/29/07
Signature of Principal Investigator or designee — Date

6/29/07
Signature of Faculty Advisor (if applicable) — Date

List and describe each proposed change:

I. Modifications to the Pilot Consent Form.

All the changes are highlighted in yellow.

1) Page 4, delete "and bring a lunch". GCRC will provide the subjects with a bagged lunch.

2) Page 6, add "Have another arm artery ultrasound at GCRC" in the immediately post-exposure to assess the effect of diesel exhaust on the peripheral artery. This measurement is already in the IRB protocol but it was left out in the consent form and the payment for this measurement was already included in the payment section.

II. Explanation of the method to transport subjects to the GCRC for the BAU measurement.

1. We will use EPA government vehicle to transport subjects from our EPA building to the GCRC in order to reduce the uncontrolled exposure to air pollutant.
2. We will use a personal vehicle for alternate if the government vehicle is not available.

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY **3**

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 29 June, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
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Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if…

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal

Initial/Date _____

anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately

Initial/Date _____

200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch (delete).

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed.

Initial/Date _____

These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you

Initial/Date _____

would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.

- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).

Initial/Date _____

- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?
You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an

Initial/Date _____

intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?

You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your

Initial/Date _____

information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $1953.

Initial/Date _____

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90
Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75
Total for completion of exposure = $1479	

18 hours after exposure (3 hour)

Initial/Date _____

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $1953

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

Initial/Date _____

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Subject's Agreement:

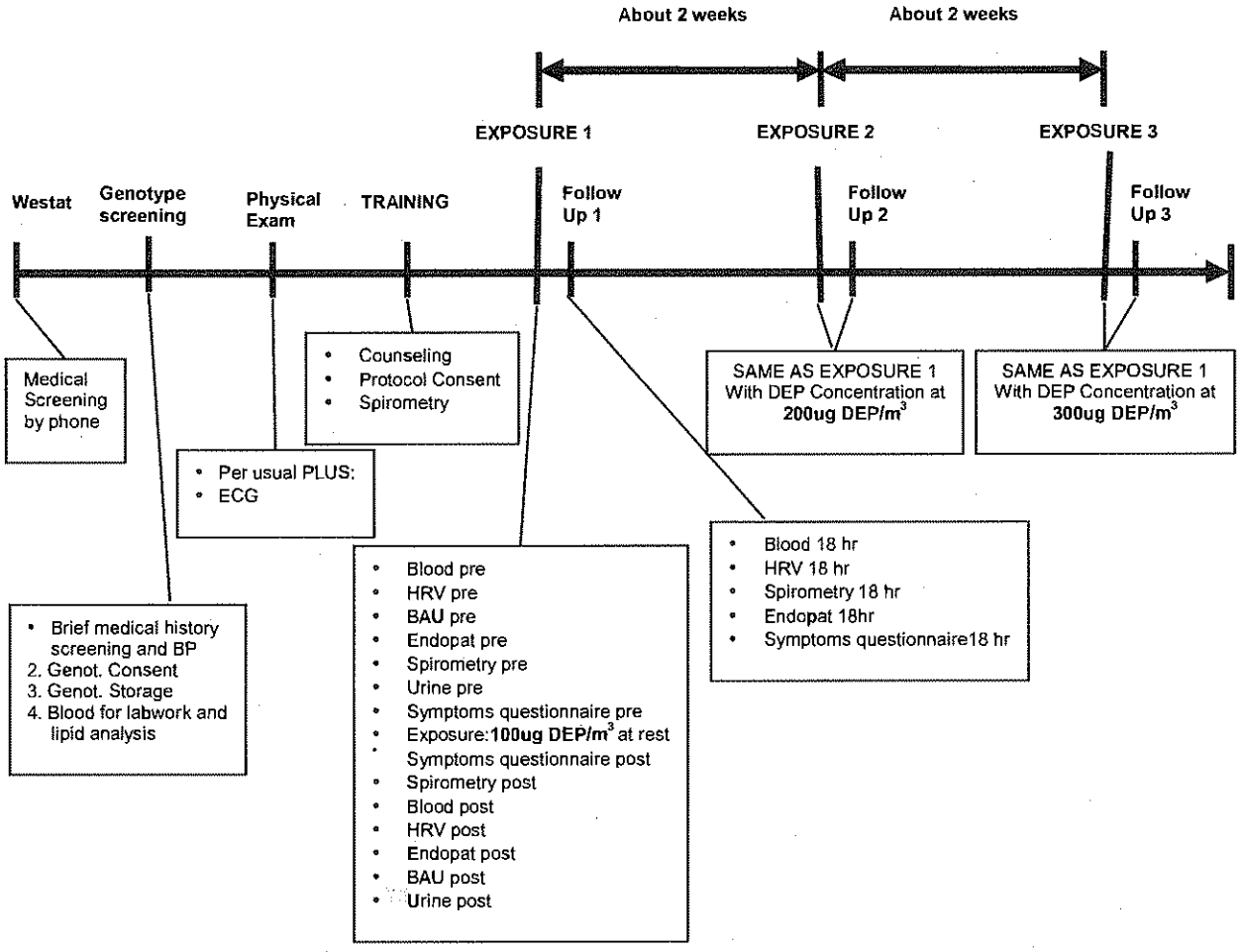
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

__________________________________ ______________
Signature of Research Subject Date

____
Printed Name of Research Subject

__________________________________ ______________
Signature of Person Obtaining Consent Date

____
Printed Name of Person Obtaining Consent



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52, Mason Farm Road
CB #7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

TO: Haiyan Tong
Environmentally Protection Agency, CB# 7315

FROM: Biomedical IRB

Authorized signature on behalf of IRB

APPROVAL DATE: 8/16/2007
EXPIRATION DATE OF APPROVAL: 2/25/2008

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
SUBMISSION TYPE: Modification
EXPEDITED CATEGORY: Minor Change to Previously Approved Research
STUDY #: 07-0190
Other #: GCRC-2579
STUDY TITLE: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal.

Submission Description:
This amendment dated August 15, 2007 replaces the Condensation Particle Counter instrument. Instead, diesel exhausts will be monitored by tapered element oscillating micro-balance. Also, David DeMerini is added to the co-investigator list.

Investigator's Responsibilities
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

CC: James Samet, Environmentally Protection Agency, Faculty Advisor; Wanda Simmons, (GCRC), Non-IRB Review Contact

First Review of IRB Submission

Receipt Date : 8/15/2007 **Submission Type:** Modification **IRB ID :** 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong **IRB:** Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☒ Expedited (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Submitted by: Sonya H Bateman

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults ages 50 to 75 will complete 7 visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☒ Reviewer Checklist completed
- ☒ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter: DM 8-16-07
  - ☒ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 8/16/07 IP)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☒ Hard copy sent

Consent forms attached: ☒

Other attachments: ☒

FINAL ACTIONS:

- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN
RESEARCH ETHICS

MEDICAL SCHOOL BUILDING 52
CAMPUS BOX 7097
CHAPEL HILL, NC 27599-7097

T 919.966.3113
F 919.966.7879
http://ohre.unc.edu

TO: Haiyan Tong
CB# 7315

FROM: Laura Curry
UNC Biomedical IRB Office

DATE: August 2, 2007

SUBJECT: ***Submit materials to GCRC 1st.***

IRB STUDY # 07-0190 (GCRC-2579)
GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

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The UNC Biomedical IRB Office received your Modification for the above titled study. All materials that are related to a GCRC study must be submitted to the GCRC 1st. The GCRC will forward to the IRB Office.

I have forwarded this Modification to the GCRC Office.
Please make sure to always include your contact information and full IRB# on all materials submitted. Example: 2142 (not correct) GCRC-2142 (correct). Please include both the old and new IRB's on correspondence. This helps to avoid delays or mistakes in processing.

Please Note: The directions and forms for IRB submissions are constantly changing. Please get the forms from the IRB webpage for each submission. On the modification, renewal, and application forms it is ok to type the full IRB# in the block marked "for IRB Use."

Please feel free to e-mail me at irb_questions@unc.edu if you have questions regarding this policy.

8-15-07

I checked the "Yes" box on the adverse event. It should be "No", because we have not started the study yet.

This new page will replace the page I submitted to IRB on July 31, 2007.

Thank You
Haiyan Tong

This page replaces the next page. I didn't want to throw away b/c it had IRB stamp on it.

Bevely

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 11-Jul-2007

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining.	2
□	4. The sponsor's document describing the amendment, if any.	1
X	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

Is this modification being submitted in response to an unanticipated problem or adverse event? __yes __X_no

IRB study #: 07-0190 **Date:** August 15, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James M Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Date:
Investigator Brochure version: Date:
Any details you need documented on IRB approval:

List and describe each proposed change:

I. Modification in the IRB protocol.
The Condensation Particle Counter (CPC) instrument will not be used to monitor particle concentration during the diesel exhausts exposures and we will delete the sentence on page 16 in the protocol. Because the CPC instrument is not reliable at diesel exhausts above 100 ug/m3, but the diesel exhausts mass will be monitored by the tapered element oscillating microbalance (TEOM) as stated in the protocol.

II. Modifications to the exclusion criteria in the protocol and consent forms.
Add "Currently have depression and/or are taking anti-depression medications (such as serotonin specific receptors inhibitors)." in the exclusion criteria in both the pilot and main studies. Studies suggest that depression and anti-depression medications affect the endpoints (endothelial function and heart rate variability) that we are looking at in this study.

We add this in the exclusion criteria on page 2 in the **pilot consent form.**

We have decided to drop off adding this exclusion criterion after we submitted this amendment. There will be no change in the protocol and consent forms regarding the exclusion criteria.

III. Add David DeMerini, PhD in the co-investigators list. Please see the attached ethical certification.

Hanja Tony — 8/15/07

Signature of Principal Investigator or designee — Date

Signature of Faculty Advisor (if applicable) — Date

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 11-Jul-2007

RECEIVED
AUG 0 1 2007
IRB UNC-CH

RECEIVED
8/9/07
GCRC

RECEIVED
AUG 1 4 2007
IRB UNC-CH

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining.	2
□	4. The sponsor's document describing the amendment, if any.	1
X	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

Is this modification being submitted in response to an unanticipated problem or adverse event? __x_yes ___no

IRB study #: 07-0190 GCRC-2579 **Date:** July 31, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James M Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Date:
Investigator Brochure version: Date:
Any details you need documented on IRB approval:

List and describe each proposed change:

I. Modification in the IRB protocol.
The Condensation Particle Counter (CPC) instrument will not be used to monitor particle concentration during the diesel exhausts exposures and we will delete the sentence on page 16 in the protocol. Because the CPC instrument is not reliable at diesel exhausts above 100 ug/m3, but the diesel exhausts mass will be monitored by the tapered element oscillating microbalance (TEOM) as stated in the protocol.

II. Modifications to the exclusion criteria in the protocol and consent forms.
Add "Currently have depression and/or are taking anti-depression medications (such as serotonin specific receptors inhibitors)." in the exclusion criteria in both the pilot and main studies. Studies suggest that depression and anti-depression medications affect the endpoints (endothelial function and heart rate variability) that we are looking at in this study.

We add this in the exclusion criteria on page 2 in the **pilot consent form**.

III. Add David DeMerini, PhD in the co-investigators list. Please see the attached ethical certification.

Hassan Tong — 7/31/07

Signature of Principal Investigator or designee — Date

7/31/07

Signature of Faculty Advisor (if applicable) — Date

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Revised amendment dated 8/15/07 Does not required Consent form revision

Biomedical Form—PILOT STUDY

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 31 July, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You have depression and/or are currently taking anti-depression medications (such as serotonin specific receptors inhibitors).
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

Initial/Date _____

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?

You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first

Initial/Date _____

exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30

Initial/Date _____

minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second

Initial/Date _____

exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.

- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).

Initial/Date _____

- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?
You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an

Initial/Date _____

intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your

Initial/Date _____

information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $1953.

Initial/Date _____

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90
Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75
Total for completion of exposure = $1479	
18 hours after exposure (3 hour)	

Initial/Date _____

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $1953

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

Initial/Date _____

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Subject's Agreement:

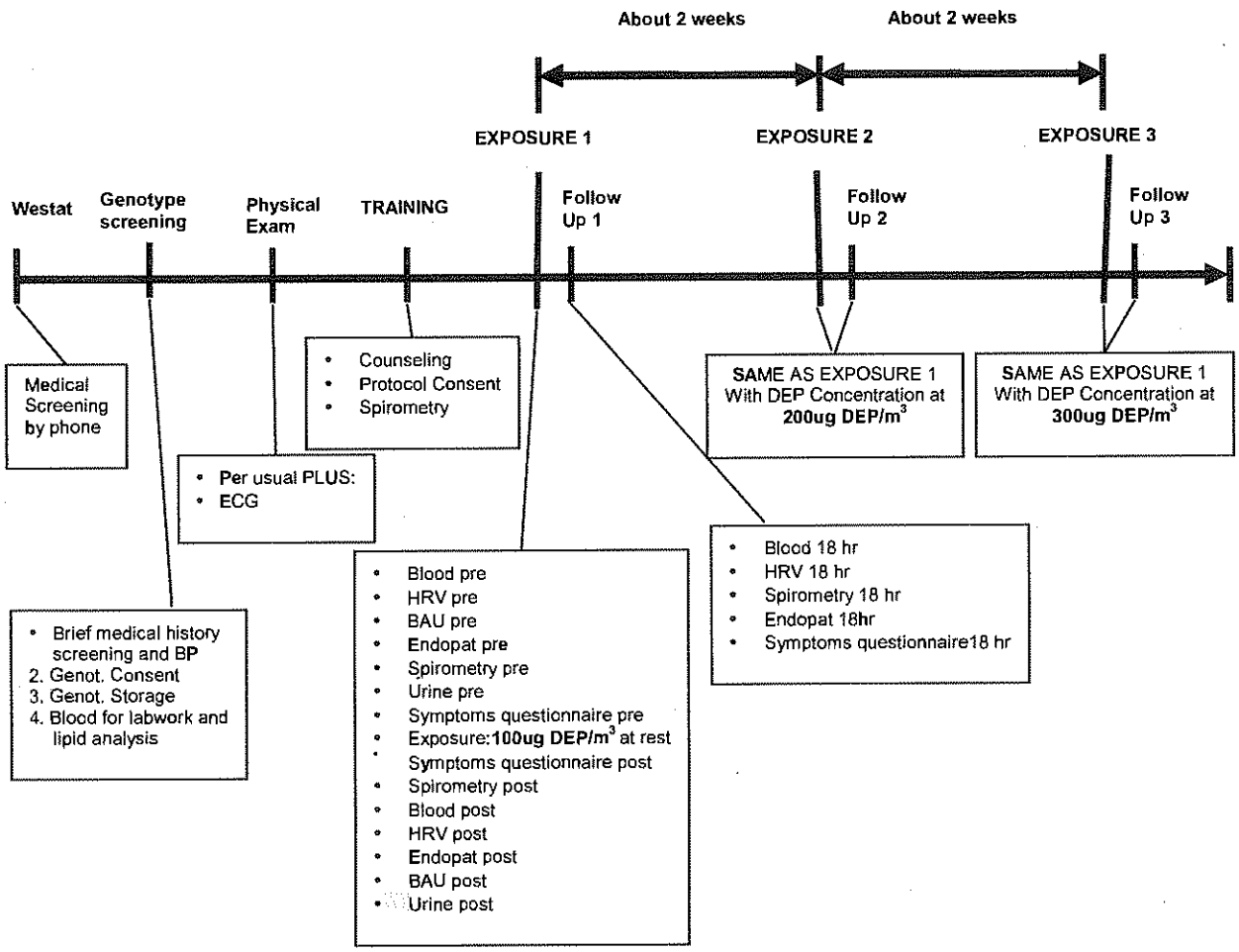
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

______________________________ ____________
Signature of Research Subject Date

____
Printed Name of Research Subject

______________________________ ____________
Signature of Person Obtaining Consent Date

____
Printed Name of Person Obtaining Consent



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
Office of the Vice Chancellor for Research and Economic Development

Campus Box 4100, 301 Bynum Hall
Chapel Hill, NC 27599-4100
(919) 962-7757 FAX: (919) 962-6769
http://research.unc.edu

July 26, 2007

To Whom It May Concern:

In accordance with the National Institutes of Health (NIH) notices OD-00-039 of August 25, 2000 and OD-01-061 of September 5, 2001, The University of North Carolina at Chapel Hill submits the following information concerning the education in the protection of human research participants undertaken by the key personnel involved with this project. The following persons have been trained in the ethical and regulatory requirements for protection of human research participants in compliance with NIH requirements and The University of North Carolina at Chapel Hill "Policy on Education and Certification of Investigators Involved in Human Subjects Research." http://ohre.unc.edu/irbtraining/

Name	Dept	Training
David Demarini		CITI- Data only

Should any additional persons come to work on this project and meet the definition of key personnel, they will be trained similarly; their names and certification of their training in the protection of human research participants will be forwarded.

Sincerely,

James E. Peterson

James E. Peterson, Ph.D.
Associate Vice Chancellor and Director,
Office of Sponsored Research

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52, Mason Farm Road
CB #7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

TO: Haiyan Tong
Environmentally Protection Agency
CB# 7315

FROM: Biomedical IRB

Authorized signature on behalf of IRB

APPROVAL DATE: 9/07/2007
EXPIRATION DATE OF APPROVAL: 2/25/2008

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
SUBMISSION TYPE: Modification
EXPEDITED CATEGORY: Minor Change to Previously Approved Research
STUDY #: 07-0190
Other #: GCRC - GCRC-2579
STUDY TITLE: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal.

Submission Description:

This amendment, dated September 5, 2007 makes minor changes to the "you should not participate if you are unable to comply with the following requirements" section of the consent form.

Investigator's Responsibilities

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

CC:
James Samet, Environmentally Protection Agency, Faculty Advisor
Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 9/7/07 AND 2/25/08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY **3**

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 5 September, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if…

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal

Initial/Date _____

anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?

You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur

Initial/Date _____

about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

Initial/Date _____

- Have about 50 ml blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You

Initial/Date _____

would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.

- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

Initial/Date _____

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes

Initial/Date _____

brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study

Initial/Date _____

personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $1953.

Initial/Date _____

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90
Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75
Total for completion of exposure = $1479	

18 hours after exposure (3 hour)

Initial/Date _____

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $1953

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

Initial/Date _____

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Subject's Agreement:

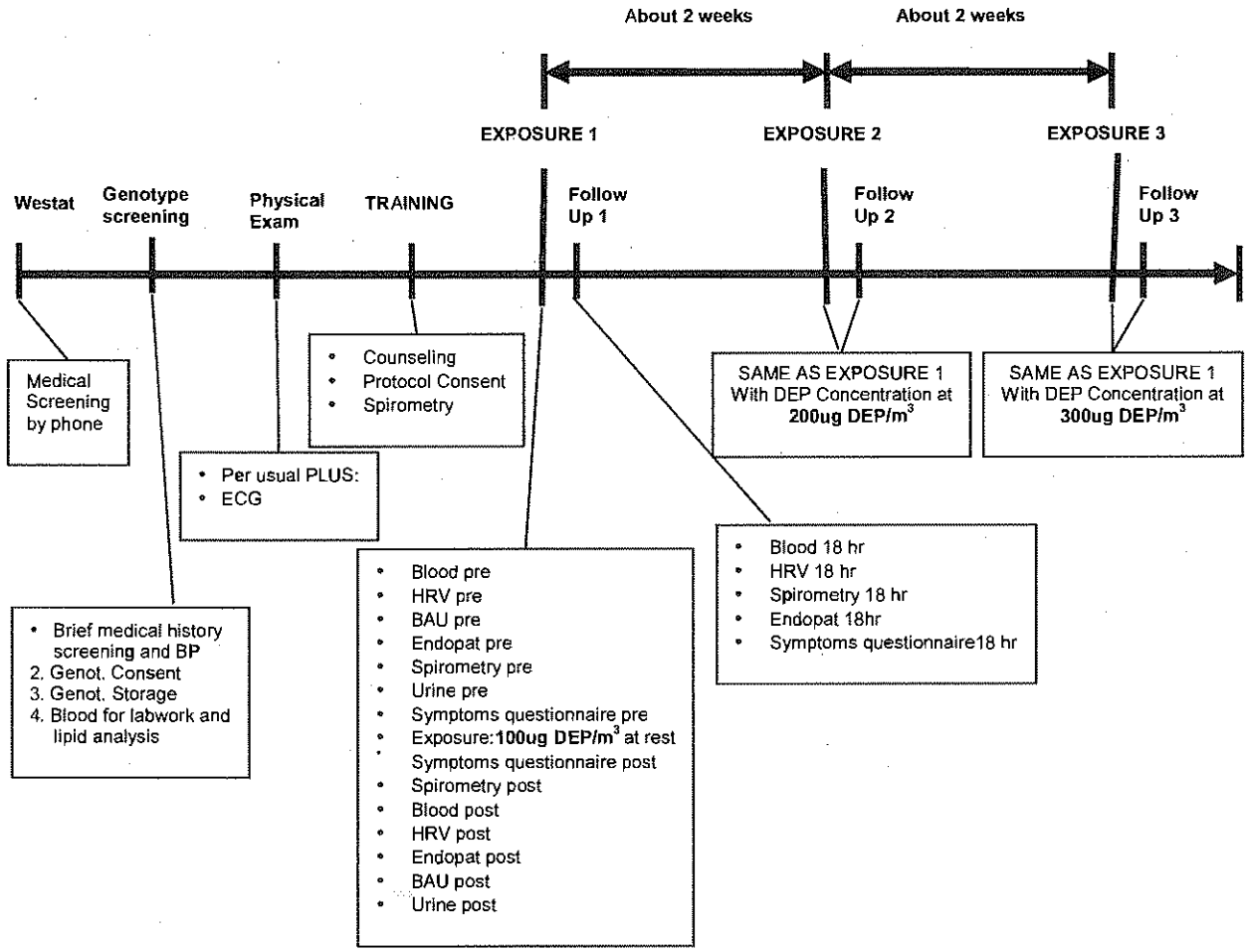
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

______________________________ ______________
Signature of Research Subject Date

____
Printed Name of Research Subject

______________________________ ______________
Signature of Person Obtaining Consent Date

____
Printed Name of Person Obtaining Consent



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

First Review of IRB Submission

Receipt Date : 9/05/2007 **Submission Type:** Modification **IRB ID :** 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong **IRB:** Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☒ Expedited (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Submitted by: Laura O Curry

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults ages 50 to 75 will complete 7 visits over 18-20 weeks.

Submission Description:

They have a Patient scheduled for 9/10/07. Please Review ASAP.

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☑ Draft letter prepared JR 9-7-07
  - ☒ Approved by chair as attached (Initials/Date: 9/7/07 IP)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 1

Other attachments: 0

FINAL ACTIONS:

- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 11-Jul-2007

RECEIVED
SEP 05 2007
IRB UNC-CH

RECEIVED
9/5/07
GCRC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

Is this modification being submitted in response to an unanticipated problem or adverse event? __yes __X_no

IRB study #: 07-0190 GCRC-2579 **Date:** September 5, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James M Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Date:
Investigator Brochure version: Date:
Any details you need documented on IRB approval:

Pt. Scheduled FOR 9/10/07

List and describe each proposed change:

I. Modifications to the exclusion criteria on page 3 in the pilot consent form.

1. Replace the "all study visits" with "the exposure and post-exposure visits"
2. Delete the sentence of "Not eat or drink anything for 2 hours prior to the training day visit."

Hayi Tong — 9/5/07

Signature of Principal Investigator or designee — Date

[signature] — Sep 5, 2007

Signature of Faculty Advisor (if applicable) — Date

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-4105
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

TO: Haiyan Tong
Environmentally Protection Agency, CB# 7315

FROM: Biomedical IRB

Authorized signature on behalf of IRB

APPROVAL DATE: 10/09/2007
EXPIRATION DATE OF APPROVAL: 2/25/2008

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
SUBMISSION TYPE: Modification
EXPEDITED CATEGORY: Minor Change to Previously Approved Research
STUDY #: 07-0190
Other #: GCRC-2579
STUDY TITLE: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal.

Submission Description:
This amendment dated October 4, 2007 adds a 24-hour baseline heart rate viability (HRV) on the training day. For two subjects who have already completed their training session, baseline HRV will be measured approximately two weeks after their first diesel exhaust exposure. Also, Dr. Martha Sue Carraway is added to the study team.

The consent form (dated October 4, 2007) has been revised to reflect this change and an addendum to the consent form for the two subjects who have already completed the training session is submitted.

Investigator's Responsibilities
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

CC: James Samet, Environmentally Protection Agency; Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 10-9-07 AND 2-25-08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
ADDENDUM FOR Consent to Participate in a Research Study
Adult Subjects
Biomedical Form—PILOT STUDY

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 4 October, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

Initial/Date _____

This is a supplement to the consent to participate in the pilot study.

We will collect your baseline heart rate viability (HRV) by a Holter monitor at about 2 weeks after your first diesel exhaust exposure. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

Payment for the Holter monitor will be $100.

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 10-9-07 AND 2-25-08
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY **3**

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 4 October, 2007

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

<u>What are some general things you should know about research studies?</u>
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal

Initial/Date _____

anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

We will collect your baseline heart rate viability (HRV) by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your

Initial/Date _____

heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of

Initial/Date _____

the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

Initial/Date _____

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse

Initial/Date _____

reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?

Initial/Date _____

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?

You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

Initial/Date _____

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $2053.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90

Initial/Date _____

Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75

Total for completion of exposure = $1579

18 hours after exposure (3 hour)

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $2053

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

Initial/Date _____

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

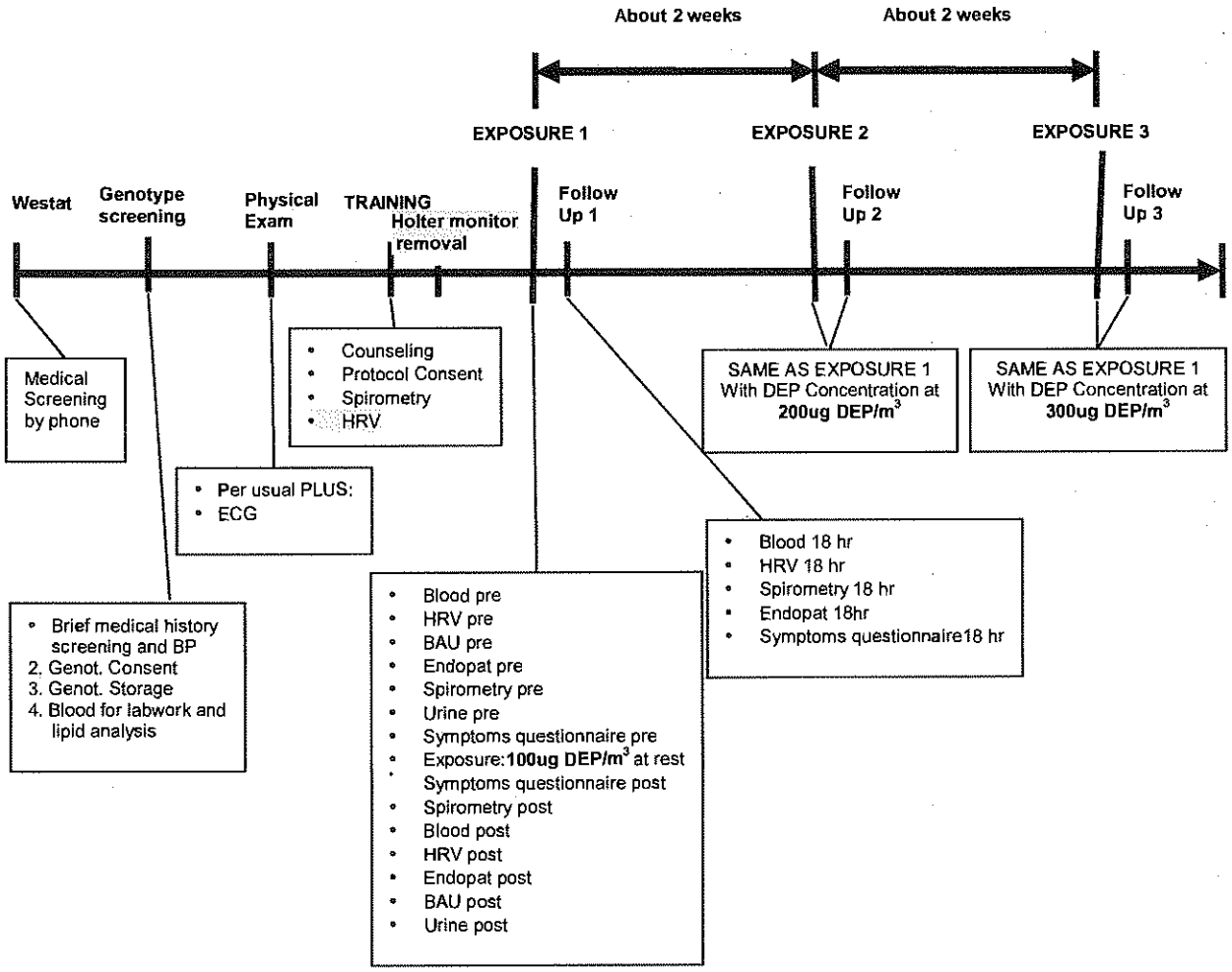
_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

First Review of IRB Submission

Personnel

Full Name	Role	Training
Haiyan Tong	Principal Investigator	
James Samet	Faculty Advisor	Current

Receipt Date : 10/08/2007 Submission Type: **Modification** IRB ID : 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong IRB: Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☐ Expedited (Category:) ☐ Full IRB

Agenda Date _____ Reviewer 1: _____ Reviewer 2: _____

Submitted by: Wyomie Milliken

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults ages 50 to 75 will complete 7 visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date: _____)
  - ☐ Approved by chair, see edits (Initials/Date: _____)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter:
  - ☑ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date: ENK 10-09-07)
  - ☐ Approved by chair, see edits (Initials/Date: _____)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 2

Other attachments: Ø

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 11-Jul-2007

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IRB UNC-CH

RECEIVED
10/4/07
G CKC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
☐	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining.	2
☐	4. The sponsor's document describing the amendment, if any.	1
X	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

Is this modification being submitted in response to an unanticipated problem or adverse event? __yes __X_no

IRB study #: 07-0190 **Date:** October 5, 2007
GCRC-2579
Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James M Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Date:
Investigator Brochure version: Date:
Any details you need documented on IRB approval:

List and describe each proposed change:

(Heart rate & Viability)

I. Modifications to the pilot consent form. All the changes are highlighted.
Because there is no air control exposure in the proposed study, we don't have baseline HRV data before diesel exhaust exposure. Therefore, we will add "Twenty-four-hour baseline HRV will be measured by Holter monitor on the training day". And we will add the detailed description of HRV measurement on page 3. We will change the pilot study diagram on page 14 accordingly. We also add the payment of Holter monitor on page 10-11.

Note: For the first 2 subjects that have finished their training session in the pilot study, we will measure their baseline HRV by Holter monitor at about 2 weeks after their first diesel exhaust exposure. Please see the Addendum of pilot consent.

II. Modifications to the protocol. All the changes are highlighted.
1. We will add "baseline HRV measurement by Holter monitor in the pilot study in the protol.

2. We will add "Blood pressure, heart rate, and oxygen saturation will be measured during the diesel exhaust exposure" in the protocol. These measurements have already been listed in the consent forms.

III. Add Dr. Martha Sue Carraway who recently joined EPA in the protocol and consent forms. Her ethics training certificate is enclosed.

[signature] 10/4/07

Signature of Principal Investigator or designee — Date

[signature] 10/4/07

Signature of Faculty Advisor (if applicable) — Date

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
Office of the Vice Chancellor for Research and Economic Development

Campus Box 4100, 301 Bynum Hall
Chapel Hill, NC 27599-4100
(919) 962-7757 FAX: (919) 962-6769
http://research.unc.edu

October 4, 2007

To Whom It May Concern:

In accordance with the National Institutes of Health (NIH) notices OD-00-039 of August 25, 2000 and OD-01-061 of September 5, 2001, The University of North Carolina at Chapel Hill submits the following information concerning the education in the protection of human research participants undertaken by the key personnel involved with this project. The following persons have been trained in the ethical and regulatory requirements for protection of human research participants in compliance with NIH requirements and The University of North Carolina at Chapel Hill "Policy on Education and Certification of Investigators Involved in Human Subjects Research." http://ohre.unc.edu/irbtraining/

Name	Dept	Training
Martha Carraway		CITI- Biomedical

Should any additional persons come to work on this project and meet the definition of key personnel, they will be trained similarly; their names and certification of their training in the protection of human research participants will be forwarded.

Sincerely,

James E. Peterson

James E. Peterson, Ph.D.
Associate Vice Chancellor and Director,
Office of Sponsored Research

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-4105
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

TO: Haiyan Tong
Environmentally Protection Agency
CB# 7315

FROM: Biomedical IRB

Authorized signature on behalf of IRB

APPROVAL DATE: 10/15/2007
EXPIRATION DATE OF APPROVAL: 2/25/2008

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
SUBMISSION TYPE: Modification
EXPEDITED CATEGORY: Minor Change to Previously Approved Research
STUDY #: 07-0190
Other #: GCRC - GCRC-2579
STUDY TITLE: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal.

Submission Description:

This amendment dated 10/11/07 makes changes to the study recruitment materials since an additional baseline test will now be done.

Investigator's Responsibilities

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

CC:
James Samet, Environmentally Protection Agency
Wanda Simmons, (GCRC), Non-IRB Review Contact

APPROVED - IRB, UNC-CH
OCT 15 2007

OMEGA PILOT STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will be used to determine the type of particular genes you carry. The genes, called glutathione-S-transferase, are responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying an inactive form of one of these genes and a variance of another may be more susceptible to the effects of air pollutants. Therefore, in this pilot research we will study the responses to diesel exhaust in healthy older subjects with very specific genetic characteristics.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $2053 if all study visits are completed.

Procedures:

Volunteers initially undergo a genetic screening visit and blood work, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genetic testing visit and $30 for the additional medical screening. If it is determined that your genotype is not needed for this study, no further visits will be scheduled for the pilot study, but you may be eligible for the full study at a later time.

If you are eligible for the remainder of the pilot study you will have up to 8 visits over approximately 18 to 20 weeks. The goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in the future study. Results will be analyzed after finishing each proposed exposure to decide whether to continue on to the next level of proposed exposure. You may not have all these 3 exposures if this pilot study is terminated at some point based on interim results.

Your participation in this pilot study will include one training session for about 3 hours with a brief follow up visit the following day, up to 3 exposure sessions, each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours. Exposures are separated by about 2 weeks.

Study procedures will include breathing tests, heart rate monitoring, blood draws, and exposures to controlled amounts of diesel exhaust. Even at the highest level of exposure, you will not be exposed to more particles than you might encounter if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure session.

Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In order to participate you must be willing to observe several important restrictions. They are:

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to the exposure and post-exposure visits.
- Due to the overnight heart monitor on training and exposure visits must be willing to refrain from bathing between exposures and follow-ups.

Would you be able to comply with these restrictions? If so, we can schedule you for ~~an office~~ a visit to complete the initial part of the screening.

Study Schedule

Genotyping visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (about 1 hour)

Physical Exam: days and times TBD (2 hours)

Training: days/times TBD (3 hours)
Follow-up day: approximately 21 hours after Training

Exposure Day 1: 9:00 AM days TBD (8 hours)
Follow-up 1: 18 hours after Exposure 1 (3 hours)

Exposure Day 2: 9:00 AM days TBD, about 2 weeks after Exposure 1 (8 hours)
Follow-up 2: 18 hours after Exposure 2 (3 hours)

Exposure Day 3: 9:00 AM days TBD, about 2 weeks after Exposure 2 (8 hours)
Follow-up 3: 18 hours after Exposure 3 (3 hours)

The US Environmental Protection Agency is seeking

ADULT VOLUNTEERS

Ages 50 to 75 for Research

APPROVED - IRB, UNC-CH

OCT 15 2007

This is a pilot study about genetics and exposure to diesel exhaust.

We are looking for healthy older adults with a very specific genotype to study the effects of diesel exhaust exposure on heart and lung function.

If you qualify for this study, you will complete screening and up to 8 visits over about 18-20 weeks. Total time commitment after screening is about 37 hours. You will receive payment for screening, the study, and out of town travel. Parking is provided.

1-888-279-9353 or
919-966-0604
www.epastudies.org

WESTAT • EPA Recruiting • In care of the environment

The Human Studies Facility is located on the UNC-CH campus

APPROVED - IRB, UNC-CH

OCT 15 2007

Web Site Advertising: www.epastudies.org

OMEGA PILOT: Elder Diesel Pilot Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of particular genes you carry. The genes, glutathione-S-transferase (GSTM1 and GSTP1) are genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying an inactive form of GSTM1 and a variance of GSTP1 may be more susceptible to the effects of air pollutants. Therefore, in this pilot study, we will study the responses to diesel exhaust in healthy older subjects with very specific genetic characteristics.

If you are eligible for the remainder of the study after screening you will have up to 8 visits over approximately 18 to 20 weeks. Total time commitment after eligibility screening is about 37 hours. Procedures involved include breathing tests, heart rate monitoring, blood tests, and exposures to controlled amounts of diesel exhaust. Even at the highest level of exposure, you will not be exposed to more particles than you might encounter if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day. Compensation is provided.

OMEGA PILOT STUDY: RETURN HOLTER MONITORING FOR SUBJECTS WHO COMPLETED EXPOSURE 1

IRB # 07-0190 GCRC #2579

The OMEGA study investigators have added an overnight heart rate monitor to the training session for this study. However, because you already completed the training without this baseline reading, they would like for you to return to wear the monitor for about 24 hours at least two weeks after your recent exposure. It will take about 15 minutes to apply the monitor and to remove it the next day, about 24 hours later. You will wear it just as you did during your first exposure session, again without bathing or showering until it is removed. You will be paid $100 for this additional monitoring session.

Purpose:
Because the OMEGA study does not have an air day, the investigators would like to see what your heart rate and rhythm are like during your normal activities. This will serve as a comparison to the ECG readings that they got (and will be getting) on your exposure days. They are interested in what your heart does while at work, or home and at rest.

First Review of IRB Submission

Personnel

Full Name	Role	Training
Haiyan Tong	Principal Investigator	
James Samet	Faculty Advisor	Current

Receipt Date : 10/15/2007 **Submission Type:** **Modification** **IRB ID :** 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong **IRB:** Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☑ Expedited (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Submitted by: Laura O Curry

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):
- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☑ Draft letter prepared JR 10-15-07
  - ☑ Approved by chair as attached (Initials/Date: ENK 10-15-07)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 0
Other attachments: 4

FINAL ACTIONS:
- ☑ Approved
- ☑ Approved with Minor Stipulations
- ☑ NHSR
- ☑ Return to sender
- ☑ Termination

APPROVED - IRB. UNC-CH
OCT 15 2007

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA PILOT STUDY**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information provided here as available.

May be folded here for display

OMEGA Pilot Study Guidelines
For All Study Visits

Illness. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores.

No active allergies. If you have questions about allergies, please call the EPA medical station at 966-6232.

Medications

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- No caffeine for 12 hours prior to the exposure and post-exposure visits.

Food

- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.

Exercise. Clothing

- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the training session and follow up visit and between the exposure and follow-up visits.

If you are unable to keep your appointment, please call Recruitment. Please be on time!

Thank You!
Westat EPA Support Services

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 11-Jul-2007

RECEIVED
10/11/07
G uc

RECEIVED
OCT 15 2007
IRB UNC-CH

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

Is this modification being submitted in response to an unanticipated problem or adverse event? __yes __X_no

IRB study #: 07-0190 **Date:** October 11, 2007
GCRC-2579
Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James M Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Date:
Investigator Brochure version: Date:
Any details you need documented on IRB approval:

List and describe each proposed change:

I. Modifications to the recruitment materials of pilot study. All the changes are highlighted.

Because there is no air control exposure in the proposed study, we have added baseline HRV data collection before diesel exhaust exposure in the consent form modified on Oct 4. All the recruitment materials are changed accordingly.

Hai Tong

Signature of Principal Investigator or designee

10/11/07

Date

Signature of Faculty Advisor (if applicable)

Date

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 2/19/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Full Board Review
Submission Type: Renewal
Study #: 07-0190
Other #: GCRC - 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated.

Study Description:
The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Investigator's Responsibilities
Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Should any adverse event or unanticipated problem involving risks to subjects or others occur it must be reported immediately to the IRB using the adverse event form at the same web site.

CC: James Samet, Environmentally Protection Agency; Wanda Simmons, (GCRC)

Verne S. Caviness

General Clinical Research Center

RECEIVED
FEB 26 2008
IRB UNC-CH

School of Medicine
The University
of
North Carolina
at
Chapel Hill
&
UNC
Hospitals

Campus Box #7600
Room 3005 APCF
Chapel Hill, NC
27599-7600

Program Director
Paul B. Watkins, MD
(919) 966-1435

Assistant Director
Marybeth Lister, MPH
(919) 966-4767

Associate Directors
Michael W. Fried, MD
(919) 966-2511

Joseph J. Eron, MD
(919) 843-2722

James D. Beck, PhD
(919) 843-2787

Protocol Coordinator
Wanda C. Simmons
(919) 966-5759

Director of Nursing
Nancy J. Cozart, MPA, RN
(919) 843-0560

Bionutrition Core Director
Marjorie G. Busby, MPH, RD
(919) 966-4746

Informatics Core Director
Clarence W. Potter, MS
(919) 966-4802

Biostatistician
Paul W. Stewart, PhD
(919) 966-7276

Director of Hispanic Outreach
Paul Camarena, MA, CHES
(919) 843-0726

**Training &
Career Development**
Susan N. Pusek, MPH
(919) 966-0128

**Office of
Research Subject Advocacy**
David J. Weber, MD
Director
(919) 843-0726

Marie H. Zeldin, RN, BSN
Assistant Director
(919) 966-6844

MEMORANDUM

TO: Dr. Haiyan Tong

FROM: Dr. Paul B. Watkins, Director

DATE: February 21, 2008

SUBJECT: **GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust**
IRB Study #: 07-0190

Both the Clinical Research Advisory Committee and the Committee on the Protection of the Rights of Human Subjects have reviewed this proposal and have approved it until **February 9, 2009.** Approximately two months prior to its expiration date, you will receive a renewal form.

REMEMBER:
This approval is contingent on your citing the GCRC grant number (RR00046) in any publications that might ensue from the work conducted on this NIH funded center.

Inpatient admissions should be scheduled with Ms. Nancy Cozart (843-0560) and outpatient visits with Mrs. Annie Johnson (966-1437). The approved consent form must be signed by each patient before any studies are done. A copy of this signed consent form must be left at the Nursing Station, and one should be placed in the UNC Hospitals record.

Should any significant side effects occur in any volunteer participating in this study, you are expected to inform the Director of the General Clinical Research Center and the Chairman of the Human Rights Committee immediately.

Paul

Paul B. Watkins, MD, Director

Enclosure

cc: IRB Chair

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 2-19-08 AND 2-9-09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information--PILOT STUDY **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 19, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?

Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 50 ml of blood for genotyping and blood analysis. If you are re-contacted from the previous study, you will not need genotype screening but you will need to get blood drawn for other blood analysis. At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office.

How will the blood sample be collected?
You will have about 50 ml (about 3 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should

Initial/Date _____

not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers and lipid lowering statins. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical staff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?

Initial/Date _____

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board,

Initial/Date _____

University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 2-19-08 AND 2-9-09 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

2

University of North Carolina at Chapel Hill

Consent for Storing Biological Specimens With Identifying Information

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IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 18 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to the effect of air pollutants such as ozone and diesel exhaust. The purpose of this research study is to examine the role of the GSTM1 gene in mediating the cardiovascular effects following acute diesel exhaust exposure in GSTM1 null and GSTM1 sufficient healthy older adults.

Diesel exhaust exposure has been found to cause a range of lung and systemic changes in normal subjects. We will be collecting blood samples which will help us to further study this condition. In order to do so, we will need to collect and store blood. Blood samples will be collected in order to look for but not limited to indicators of inflammation due to the diesel exhaust exposure. We may further isolate RNA from blood for genetic analysis. RNA may be isolated for genetic analysis to see if there is a difference between pre- and post diesel exhaust exposures.

All samples will be stored where only project members will have access to the samples. There is a need to store samples in such a repository because this will be an ongoing study where samples from subjects will be collected over an extended period of time. Storing of samples allows for all samples to be processed at the same time and also allows our scientist the opportunity to further study these samples with as yet unknown questions and techniques.

How will the specimen be collected?
The description of the samples to be collected and the manner in which this will be done have been described in the main study consent.

What will happen to the specimen?
Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the diesel exhaust exposure. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens (or data) for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens (or data) will be provided without identifiers to these other researchers by employing a data use agreement.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding the cardiovascular effects of diesel exhaust exposure in older population.

What are the possible risks or discomforts involved with being in this study?

Initial/Date _____

This study might involve the following risks and/or discomforts to you:

1. Blood sampling will be performed by medical personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you for storage of the specimens?
There will be no cost to you for the storage and use of the specimens for research purposes.

Will you receive anything for being in this study?
You will not receive anything for taking part in this study with regard to storage of excess specimens. Reimbursement for participation in the main study is addressed in the detailed consent for that study.

Who owns the specimens?
Any blood, body fluids, or tissue specimens obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires

Initial/Date _____

the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the specimens from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss

Initial/Date _____

or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 2-19-08 AND 2-9-09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

3

Biomedical Form—PILOT STUDY

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: February 19, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal

Initial/Date _____

anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

We will collect your baseline heart rate viability (HRV) by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your

Initial/Date _____

heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of

Initial/Date _____

the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 3 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

Initial/Date _____

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 3 1/3 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse

Initial/Date _____

reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?

Initial/Date _____

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

Initial/Date _____

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $2053.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90

Initial/Date _____

Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75

Total for completion of exposure = $1479

18 hours after exposure (3 hour)

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $2053

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

Initial/Date _____

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ____________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

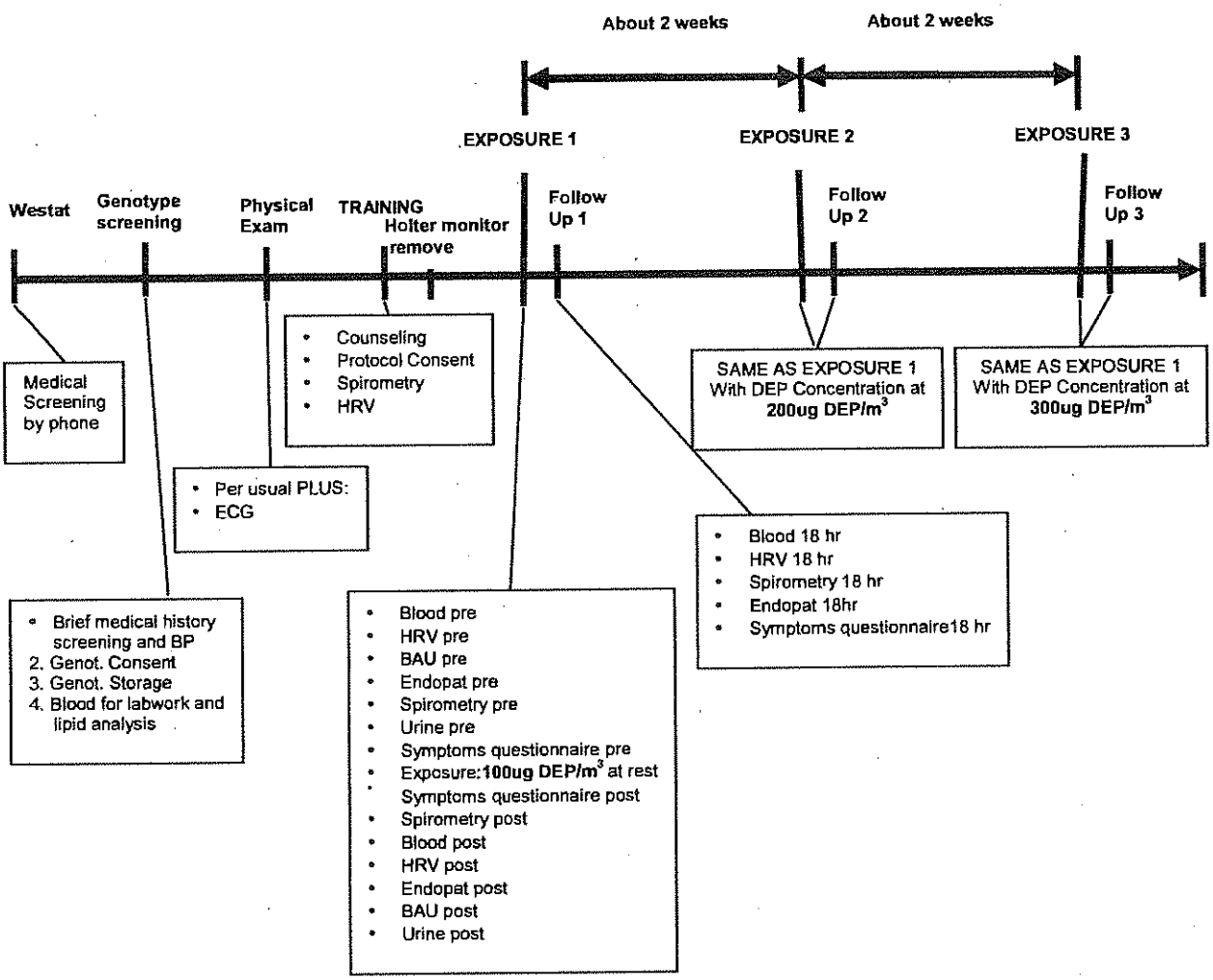
_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 2.19.08 AND 2.9.09 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotype Screening <u>With</u> Identifying Information—MAIN STUDY **4**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 19, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

<u>What are some general things you should know about this research study?</u>
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to diesel exhaust. We will have equal numbers of subjects with GSTM1 sufficient gene or GSTM1 null gene enrolled in this study. The purpose of this screening session is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 60 participants in this study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and you will be instructed to mail the FFQ. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Initial/Date _____

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol) and lipid lowering statins. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Initial/Date _____

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Initial/Date _____

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 2-19-08 AND 2-9-09 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—MAIN STUDY

5

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: February 19, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, and chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.

Initial/Date _____

- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Use olive oil for cooking, dressings, and sauces during the study.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 60 people in this research study.

How long will your participation in this study last?

You will have up to 3 visits to the research facility over approximately 5-6 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) two-week of fish oil/olive oil treatments part, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

Initial/Date _____

What will happen if you take part in the study?

You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a 15-day fish oil or olive oil supplementation and the exposure session. You will be given a 15-day supply of either fish oil or olive oil tablets. Please take 2 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next two weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this 15-day of supplementation period. We will give you another 15-day supply of either fish oil or olive oil tablets and you will need to restart the pills again.

In the morning of your last pill of supplements, you will come to the HSF for the baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel

Initial/Date _____

exhaust causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF in the next morning for the exposure and the monitor will be removed.

Exposure day
We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat before midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we will perform on your last pill day).
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- We will bring you back to the HSF where we may also measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT) at HSF and we will compare the findings with ultrasound measurement of the artery in your arm. We will place two small PAT probes to both of your index fingers. The procedure is similar to that of ultrasound measurement and we will only place a blood pressure cuff on one of your arm. We will obtain recordings

Initial/Date _____

for 5 minutes at rest, 5 minutes during the blood pressure cuff inflated, and 5 minutes after the blood pressure cuff released.

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust (the concentration will be determined based on the pilot study) for 2 hours in a small exposure chamber. This particle concentration is representative of diesel exhaust particle (DEP) levels which you would inhale if you were occupationally exposed to DEP, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over a period at a busy intersection in a polluted city (such as Los Angeles) where DEP concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will obtain another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)

Initial/Date _____

You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: The concentration of diesel exhaust in this study has been chosen based on a pilot study in susceptible older subjects. Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in

Initial/Date _____

this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing these exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Finger Endo-PAT: Similar to the measurement by brachial artery ultrasound, there are no significant risks associated with index fingers arteries measurement.
Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Initial/Date _____

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1195.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml) (genotyping)	$30

Pre-study qualification total = $60

Initial/Date _____

Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Brachial artery ultrasound (2@ $50 each)	$100
Finger Endo-PAT	$20
Time (8h @$12/h)	$96
Food record (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $823

18 hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3 hrs @$12/h)	$36
Finger Endo-PAT	$10

Day 3 total for completion of post-exposure = $76

Protocol Completion Bonus $100

Approximate TOTAL for completion of the study = $1195

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been

Initial/Date _____

stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

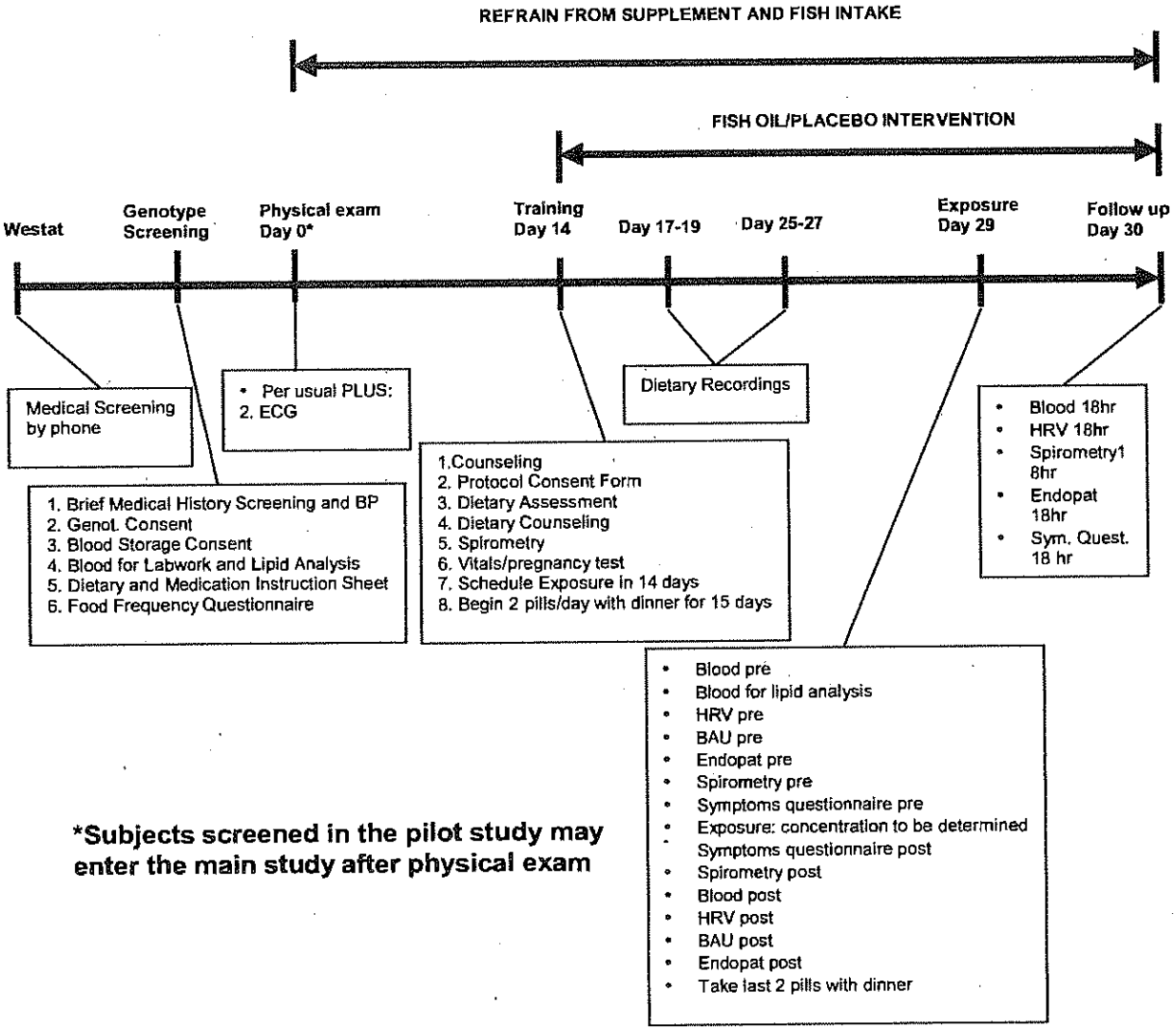
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



Initial/Date _____

APPROVED - IRB UNC-CH
FEB 19 2008

SUBJ #_____ DATE_____

SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4

Response to Minor Stipulations

Receipt Date : 1/22/2008 Submission Type: Renewal IRB ID : 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong IRB: Biomedical Coordinator: Joe Andrews

☑ Full Board Review ☐ Not Full Board Review

Study Description :

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description :

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter:
  - ☑ Draft letter prepared 2/19/08
  - ☑ Approved by chair as attached (Initials/Date: EWK 2-21-08)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 5

Other attachments: 1

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to Full IRB
- ☐ Return to sender
- ☐ Termination

R-13

Full Committee Review

Receipt Date : 1/22/2008 Submission Type: **Renewal** IRB ID : 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong IRB: Biomedical

Agenda Date 2/11/2008 Reviewer 1: **Rocamora, Nancy** Reviewer 2: Kraybill, Ernest N. (chair)

Additional Findings and/or Special Populations: ☐ Children ☐ Prisoners ☐ Pregnant Women

☐ Sensitive Info ☑ IND ☐ IDE ☐ DNA/Gene transfer ☑ Genetics ☑ GCRC ☐ ORC ☐ LAR

Meeting Notes and Required Findings :

OK

A.4.9. – collect SSNs

all CFs except #2

have orphan pages.

Marked CFs.

PROCESSING STEPS (OFFICE USE ONLY):

☐ Reviewer Checklist completed

☑ Minor Stipulation letter:

- ☑ Draft letter prepared 2/12/08
- ☑ Approved by chair as attached (Initials/Date: ENK 2-12-08)
- ☐ Approved by chair, see edits (Initials/Date:)
- ☐ Email copy sent
- ☐ Hard copy sent

☐ Approval letter:

- ☐ Draft letter prepared
- ☐ Approved by chair as attached (Initials/Date:)
- ☐ Approved by chair, see edits (Initials/Date:)
- ☑ Email copy sent
- ☑ Hard copy sent

Consent forms attached: 4 marked

Other attachments: Ø

FINAL ACTIONS:

☐ Approved

☐ Approved with Minor Stipulations

☑ NHSR

☐ Deferred

☐ Disapproved

☐ Return to sender

☐ Termination

RECEIVED
2/19/08
GCRC

To: Biomedical IRB
CB# 7097

From: Haiyan Tong
Environmental Protection Agency
CB# 7315

RECEIVED
FEB 19 2008
IRB UNC-CH

Date: 2/19/08

Study #: 07-0190
GCRC#: 2579
Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Dear IRB review committee,

Thanks for the Full Board Review for the above study! Enclosed are the revised sbumission in response to the "Contingencies Approval on 2/11/08".

To clearify the study specific details:

1. There is no requirement for Social Security Numbers collection in EPA studies. Please see the enclosed memo from Daniel Nelson regarding this issue.
2. The study title and Principle Investigator's name have been added on the consent signature pages. Please see the highlighted changes.
3. All Co-Investigators are now listed on the consent forms. Please see the highlighted changes.
4. All the requested marks have been accepted and highlighted on the consent forms.

Please let me know if you have any questions regarding this revision.

Sincerely,

Haiyan Tong

Haiyan Tong

Daniel Nelson
<daniel_nelson@unc.edu>
11/08/2007 09:56 AM

To Robert Truckner/RTP/USEPA/US@EPA, Andrea Ware/RTP/USEPA/US@EPA
cc
bcc
Subject [Fwd: no need to collect SSN in EPA studies]

Bob and Andrea:

I have distributed this message to our IRB chairs and staff. I hope this is clear, and meshes with your understanding?

Please feel free to circulate within your research groups, and to keep on file for times when this nuance slips through the cracks during IRB review.

Dan

-------- Original Message --------
Subject: no need to collect SSN in EPA studies
Date: Thu, 08 Nov 2007 09:52:35 -0500
From: Daniel Nelson <daniel_nelson@unc.edu>
To: IRBs at UNC <irb@listserv.unc.edu>

OHRE:

One thing that hasn't come up during our many discussions on the Social Security Number (SSN) policy is how to handle studies where payments are made by someone/something OTHER THAN UNC. One immediate and ongoing example is the EPA, but there may occasionally be others, where we review studies for non-university entities.

In these cases, we should remember that this is not an IRB policy, but is a UNC policy, driven by the university's IRS reporting obligations. If someone else is paying subjects in a study that we happen to be reviewing, the SSN collecting and IRS reporting become their concerns... but UNC policy (on this topic) should not apply. Using the EPA example, Westat is responsible for paying subjects in EPA studies, and they look after IRS reporting, following their own policies. Reducing it to an equation:

No university funds disbursed = No IRS reporting requirement (for UNC) = No need (for UNC) to collect SSN.

So there is no confusion.... whether subjects are being paid too much in any given study much remains our concern, because that is an IRB issue. But we're only worried about SSN collection when it's our money being paid.

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information--PILOT STUDY **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 19, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 50 ml of blood for genotyping and blood analysis. If you are re-contacted from the previous study, you will not need genotype screening but you will need to get blood drawn for other blood analysis. At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office.

How will the blood sample be collected?
You will have about 50 ml (about 3 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should

Initial/Date _____

not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers and lipid lowering statins. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical staff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?

Initial/Date _____

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?

You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?

By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?

Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?

If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board,

Initial/Date _____

University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

University of North Carolina at Chapel Hill

Consent for Storing Biological Specimens With Identifying Information 2

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 18 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to the effect of air pollutants such as ozone and diesel exhaust. The purpose of this research study is to examine the role of the GSTM1 gene in mediating the cardiovascular effects following acute diesel exhaust exposure in GSTM1 null and GSTM1 sufficient healthy older adults.

Diesel exhaust exposure has been found to cause a range of lung and systemic changes in normal subjects. We will be collecting blood samples which will help us to further study this condition. In order to do so, we will need to collect and store blood. Blood samples will be collected in order to look for but not limited to indicators of inflammation due to the diesel exhaust exposure. We may further isolate RNA from blood for genetic analysis. RNA may be isolated for genetic analysis to see if there is a difference between pre- and post diesel exhaust exposures.

All samples will be stored where only project members will have access to the samples. There is a need to store samples in such a repository because this will be an ongoing study where samples from subjects will be collected over an extended period of time. Storing of samples allows for all samples to be processed at the same time and also allows our scientist the opportunity to further study these samples with as yet unknown questions and techniques.

How will the specimen be collected?
The description of the samples to be collected and the manner in which this will be done have been described in the main study consent.

What will happen to the specimen?
Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the diesel exhaust exposure. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens (or data) for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens (or data) will be provided without identifiers to these other researchers by employing a data use agreement.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding the cardiovascular effects of diesel exhaust exposure in older population.

What are the possible risks or discomforts involved with being in this study?

Initial/Date _____

This study might involve the following risks and/or discomforts to you:

1. Blood sampling will be performed by medical personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you for storage of the specimens?
There will be no cost to you for the storage and use of the specimens for research purposes.

Will you receive anything for being in this study?
You will not receive anything for taking part in this study with regard to storage of excess specimens. Reimbursement for participation in the main study is addressed in the detailed consent for that study.

Who owns the specimens?
Any blood, body fluids, or tissue specimens obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires

Initial/Date _____

the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the specimens from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss

Initial/Date _____

or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY 3

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: February 19, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
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Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

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What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal

Initial/Date _____

anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

We will collect your baseline heart rate viability (HRV) by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your

Initial/Date _____

heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of

Initial/Date _____

the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 3 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

Initial/Date _____

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 3 1/3 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse

Initial/Date _____

reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?

Initial/Date _____

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

Initial/Date _____

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $2053.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total =	$60
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90

Initial/Date _____

Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75

Total for completion of exposure = $1479

18 hours after exposure (3 hour)

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $2053

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

Initial/Date _____

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

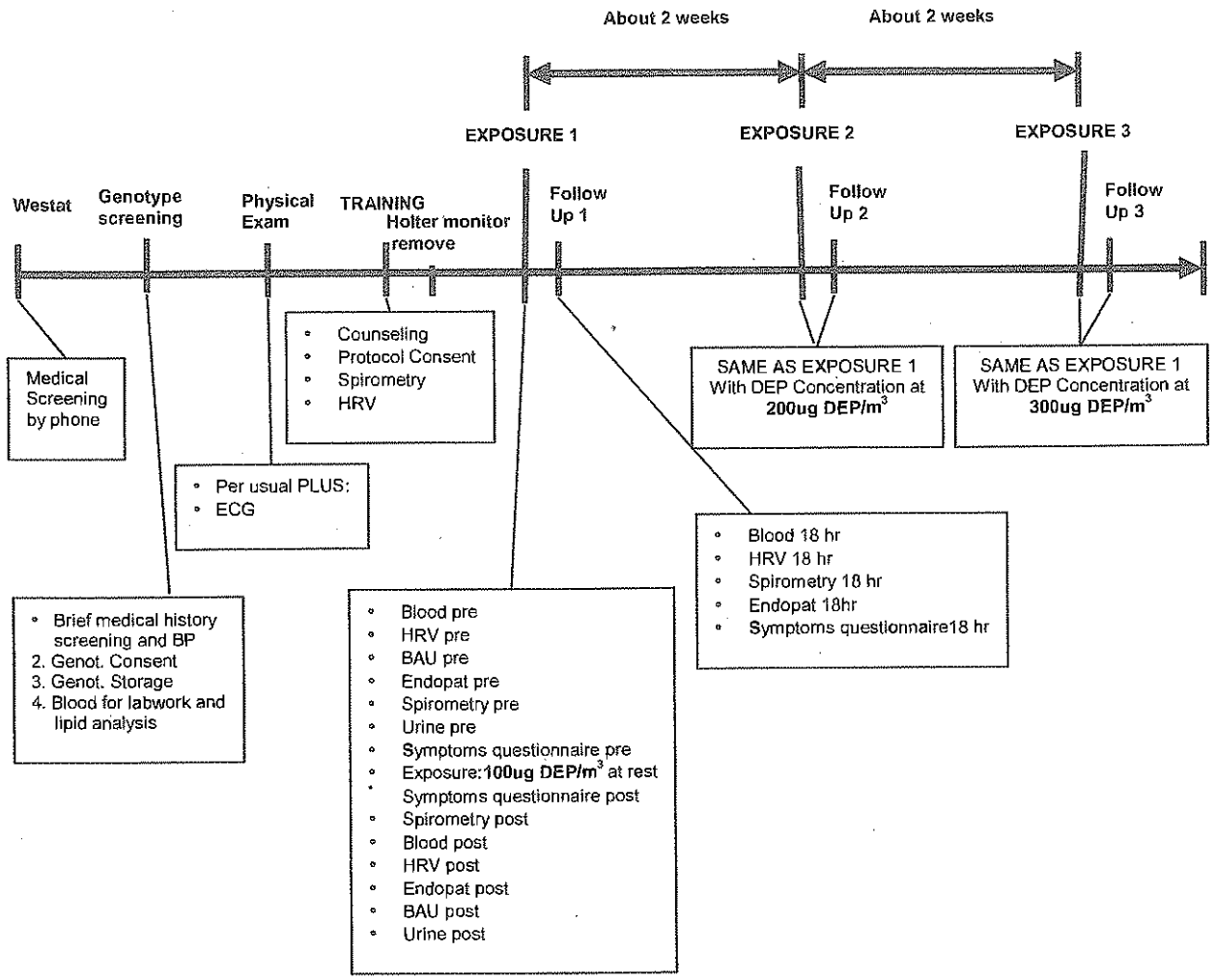
_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information—MAIN STUDY 4

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 19, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
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Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to diesel exhaust. We will have equal numbers of subjects with GSTM1 sufficient gene or GSTM1 null gene enrolled in this study. The purpose of this screening session is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 60 participants in this study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and you will be instructed to mail the FFQ. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Initial/Date _____

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol) and lipid lowering statins. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Initial/Date _____

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Initial/Date _____

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—MAIN STUDY 5

IRB Study #____**07-0190**____ **GCRC #: 2579**
Consent Form Version Date: February 19, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, and chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.

Initial/Date _____

- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should NOT participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Use olive oil for cooking, dressings, and sauces during the study.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 60 people in this research study.

How long will your participation in this study last?

You will have up to 3 visits to the research facility over approximately 5-6 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) two-week of fish oil/olive oil treatments part, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

Initial/Date _____

What will happen if you take part in the study?

You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a 15-day fish oil or olive oil supplementation and the exposure session. You will be given a 15-day supply of either fish oil or olive oil tablets. Please take 2 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next two weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this 15-day of supplementation period. We will give you another 15-day supply of either fish oil or olive oil tablets and you will need to restart the pills again.

In the morning of your last pill of supplements, you will come to the HSF for the baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel

Initial/Date _____

exhaust causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF in the next morning for the exposure and the monitor will be removed.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat before midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we will perform on your last pill day).
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- We will bring you back to the HSF where we may also measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT) at HSF and we will compare the findings with ultrasound measurement of the artery in your arm. We will place two small PAT probes to both of your index fingers. The procedure is similar to that of ultrasound measurement and we will only place a blood pressure cuff on one of your arm. We will obtain recordings

Initial/Date _____

for 5 minutes at rest, 5 minutes during the blood pressure cuff inflated, and 5 minutes after the blood pressure cuff released.

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust (the concentration will be determined based on the pilot study) for 2 hours in a small exposure chamber. This particle concentration is representative of diesel exhaust particle (DEP) levels which you would inhale if you were occupationally exposed to DEP, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over a period at a busy intersection in a polluted city (such as Los Angeles) where DEP concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will obtain another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)

Initial/Date _____

You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: The concentration of diesel exhaust in this study has been chosen based on a pilot study in susceptible older subjects. Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in

Initial/Date _____

this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing these exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Finger Endo-PAT: Similar to the measurement by brachial artery ultrasound, there are no significant risks associated with index fingers arteries measurement.
Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Initial/Date _____

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1195.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml) (genotyping)	$30

Pre-study qualification total = $60

Initial/Date _____

Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Brachial artery ultrasound (2@ $50 each)	$100
Finger Endo-PAT	$20
Time (8h @$12/h)	$96
Food record (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $823

18 hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3 hrs @$12/h)	$36
Finger Endo-PAT	$10

Day 3 total for completion of post-exposure = $76

Protocol Completion Bonus $100

Approximate TOTAL for completion of the study = $1195

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been

Initial/Date _____

stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ____________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

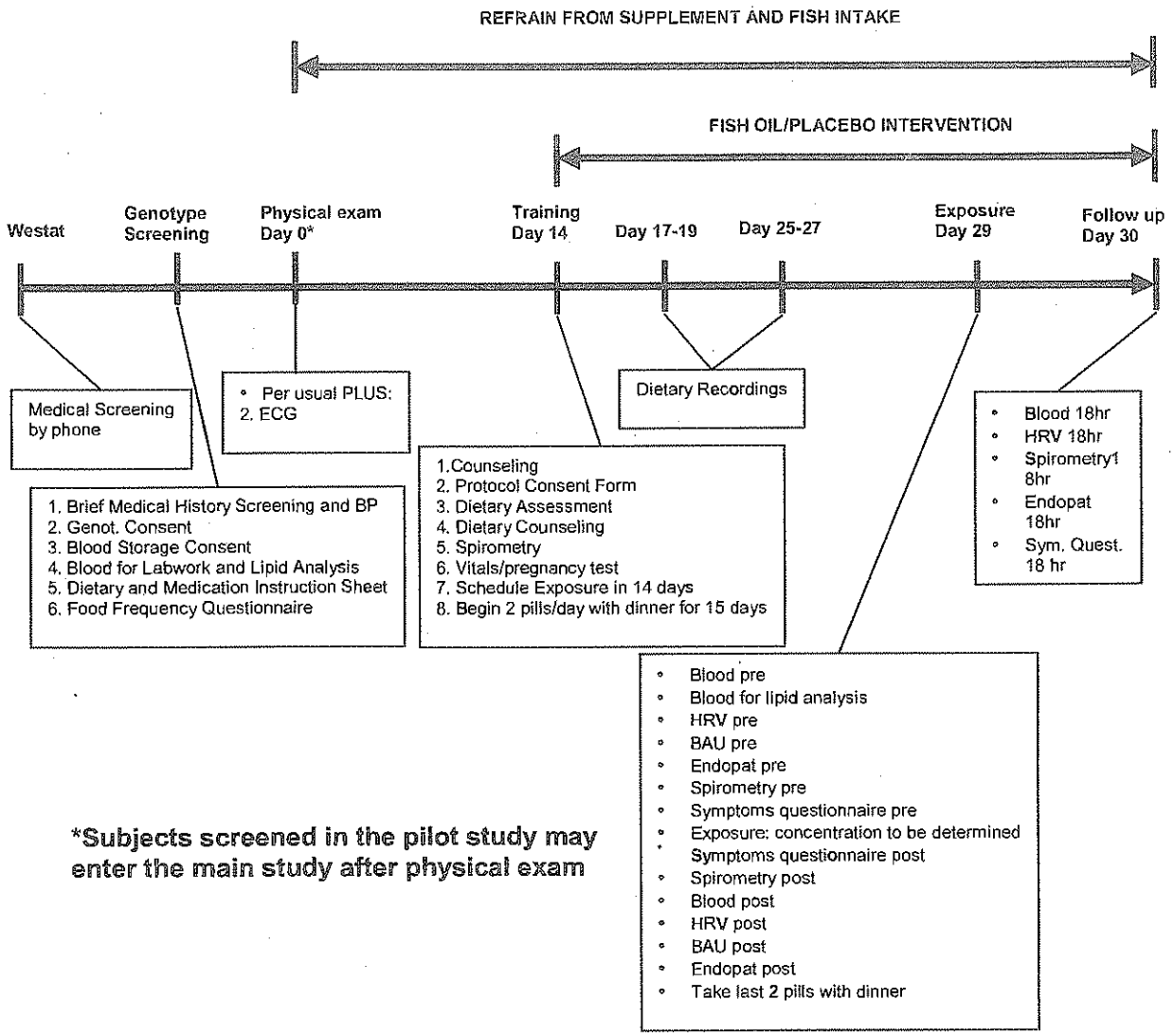
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



Initial/Date _____

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB EWK

Date: 2/12/2008

RE: Minor Contingencies to be addressed following Full Board IRB Review on 2/11/2008
Submission Type: Renewal
Study #: 07-0190
Other #: GCRC - 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been reviewed by the above IRB at a convened meeting. This is not an IRB approval. You may not implement the research activities described in your submission until you have received a memo indicating final IRB approval. The IRB determined that this submission MAY BE APPROVED, pending stipulated changes and/or clarifications as detailed below:

Study Specific Details:

1. Please indicate, in section A.4.9. of the application, that Social Security Numbers will be collected.
2. Please ensure that the study title and Principal Investigator's name appear on the consent signature pages.
3. Please ensure that all Co-Investigators are listed on the consent forms.
4. See marked consent form(s) for requested changes.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the item(s) listed above. Any additional changes (including new materials) must also be listed and discussed in the memo. You will need to return 2 copies of all revised materials to the IRB, one in which all changes are underlined and the other a "clean" copy with no underlining.

CC: James Samet, Environmentally Protection Agency; Wanda Simmons, (GCRC), Non-IRB Review Contact

Marked

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information--PILOT STUDY **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: 1 June, 2007 ← Update

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Maryann Bassett, RN; Andrew Ghio MD; Debbie Levin, BSN; Tracey Montilla, RN; Michael Schmitt MS
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 50 ml of blood for genotyping and blood analysis. If you are re-contacted from the previous study, you will not need genotype screening but you will need to get blood drawn for other blood analysis. At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office.

How will the blood sample be collected?
You will have about 50 ml (about 5 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers and lipid lowering statins. You need to

Initial/Date _____

Staff

be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

Initial/Date _____

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Initial/Date _____

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

No Title on Signature page. Please correct.

Initial/Date _____

Marked

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY **3**

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: 4 October, 2007 ← update

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal

Initial/Date _____

anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

We will collect your baseline heart rate viability (HRV) by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your

Initial/Date _____

heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will occur about 2 weeks after the second exposure. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. We will analyze the results after finishing each proposed exposure and decide whether we will continue on the next level of proposed exposure. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of

Initial/Date _____

the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

Initial/Date _____

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons). $3\frac{1}{3}$ T
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 cc or about 5 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse

Initial/Date _____

reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

<u>What if we learn about new findings or information during the study?</u>

Initial/Date _____

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?

You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

Initial/Date _____

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $2053.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90

Initial/Date _____

Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75

Total for completion of exposure = $1479

18 hours after exposure (3 hour)

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $2053

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

Initial/Date _____

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ______________

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

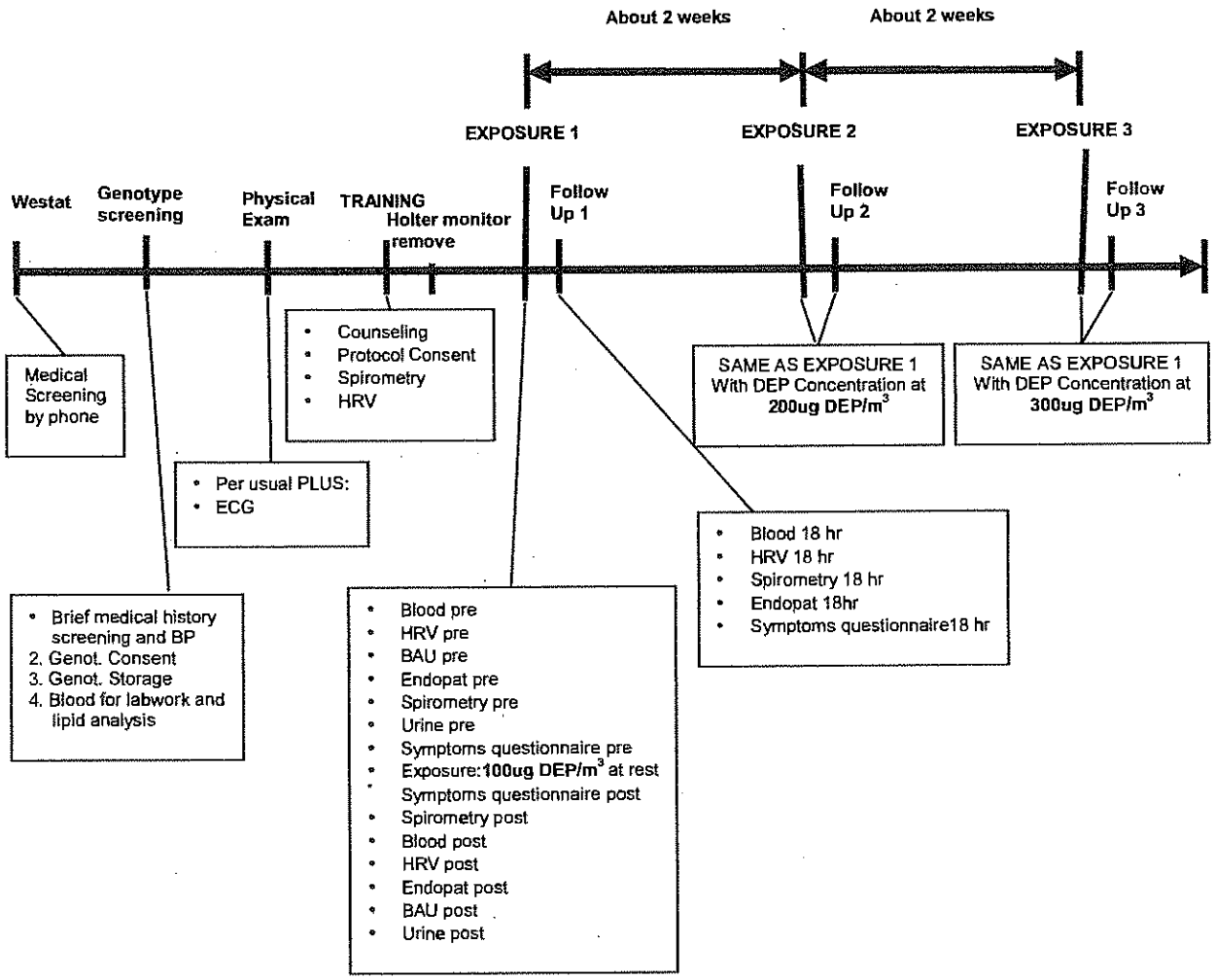
Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

No Title on signature page. Please correct.

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

Marked

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information—MAIN STUDY 4

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: 1 June 2007 ← up date

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Maryann Bassett, RN; Andrew Ghio MD; Debbie Levin, BSN; Tracey Montilla, RN; Michael Schmitt MS
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to diesel exhaust. We will have equal numbers of subjects with GSTM1 sufficient gene or GSTM1 null gene enrolled in this study. The purpose of this screening session is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 60 participants in this study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and you will be instructed to mail the FFQ. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about ~~8~~ 5 2/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should

Initial/Date _____

not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol) and lipid lowering statins. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Initial/Date _____

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Initial/Date _____

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from - ~~form~~ having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

No Title on signature page. Please correct.

Initial/Date _____

Marked

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—MAIN STUDY **5**

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: 29 May, 2007 ← update

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; Andrew Ghio MD; Milan Hazucha, MD, PhD; Margaret Herbst, RN, MSN; Alan Hinderliter, MD; Fernando Holguin, MD; Sally Ivins, BA; Howard Kehrl, MD; Debbie Levin, BSN; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, and chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking medications for lowering the lipid level in your blood such as lovestatin.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You are currently taking ACE inhibitors (such as captopril, enalapril, and lisinopril).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.

Initial/Date _____

- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Use olive oil for cooking, dressings, and sauces during the study.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 60 people in this research study.

How long will your participation in this study last?

You will have up to 3 visits to the research facility over approximately 5-6 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) two-week of fish oil/olive oil treatments part, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

Initial/Date _____

What will happen if you take part in the study?

You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a 15-day fish oil or olive oil supplementation and the exposure session. You will be given a 15-day supply of either fish oil or olive oil tablets. Please take 2 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next two weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this 15-day of supplementation period. We will give you another 15-day supply of either fish oil or olive oil tablets and you will need to restart the pills again.

In the morning of your last pill of supplements, you will come to the HSF for the baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel

Initial/Date _____

exhaust causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF in the next morning for the exposure and the monitor will be removed.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat before midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we will perform on your last pill day).
- Have about 80 ml blood drawn (about 8 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- We will bring you back to the HSF where we may also measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT) at HSF and we will compare the findings with ultrasound measurement of the artery in your arm. We will place two small PAT probes to both of your index fingers. The procedure is similar to that of ultrasound measurement and we will only place a blood pressure cuff on one of your arm. We will obtain recordings

Initial/Date _____

for 5 minutes at rest, 5 minutes during the blood pressure cuff inflated, and 5 minutes after the blood pressure cuff released.

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust (the concentration will be determined based on the pilot study) for 2 hours in a small exposure chamber. This particle concentration is representative of diesel exhaust particle (DEP) levels which you would inhale if you were occupationally exposed to DEP, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over a period at a busy intersection in a polluted city (such as Los Angeles) where DEP concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will obtain another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 cc or about ~~8~~ 5⅓ tablespoons). ccml?
- Fill out a symptom score questionnaire.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

Initial/Date _____

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 cc or about 8 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: The concentration of diesel exhaust in this study has been chosen based on a pilot study in susceptible older subjects. Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health

Initial/Date _____

effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing these exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from healthy food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Finger Endo-PAT: Similar to the measurement by brachial artery ultrasound, there are no significant risks associated with index fingers arteries measurement.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal

Initial/Date _____

remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1095.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml) (genotyping)	$30
Pre-study qualification total =	$60
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36

Initial/Date _____

Exposure sessions (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Brachial artery ultrasound (2@ $50 each)	$100
Finger Endo-PAT	$20
Time (8h @$12/h)	$96
Food record (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $823

18 hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3 hrs @$12/h)	$36
Finger Endo-PAT	$10

Day 3 total for completion of post-exposure = $76

Protocol Completion Bonus $100

Approximate TOTAL for completion of the study = $1095

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Initial/Date _____

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ____________

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____
Signature of Research Subject Date

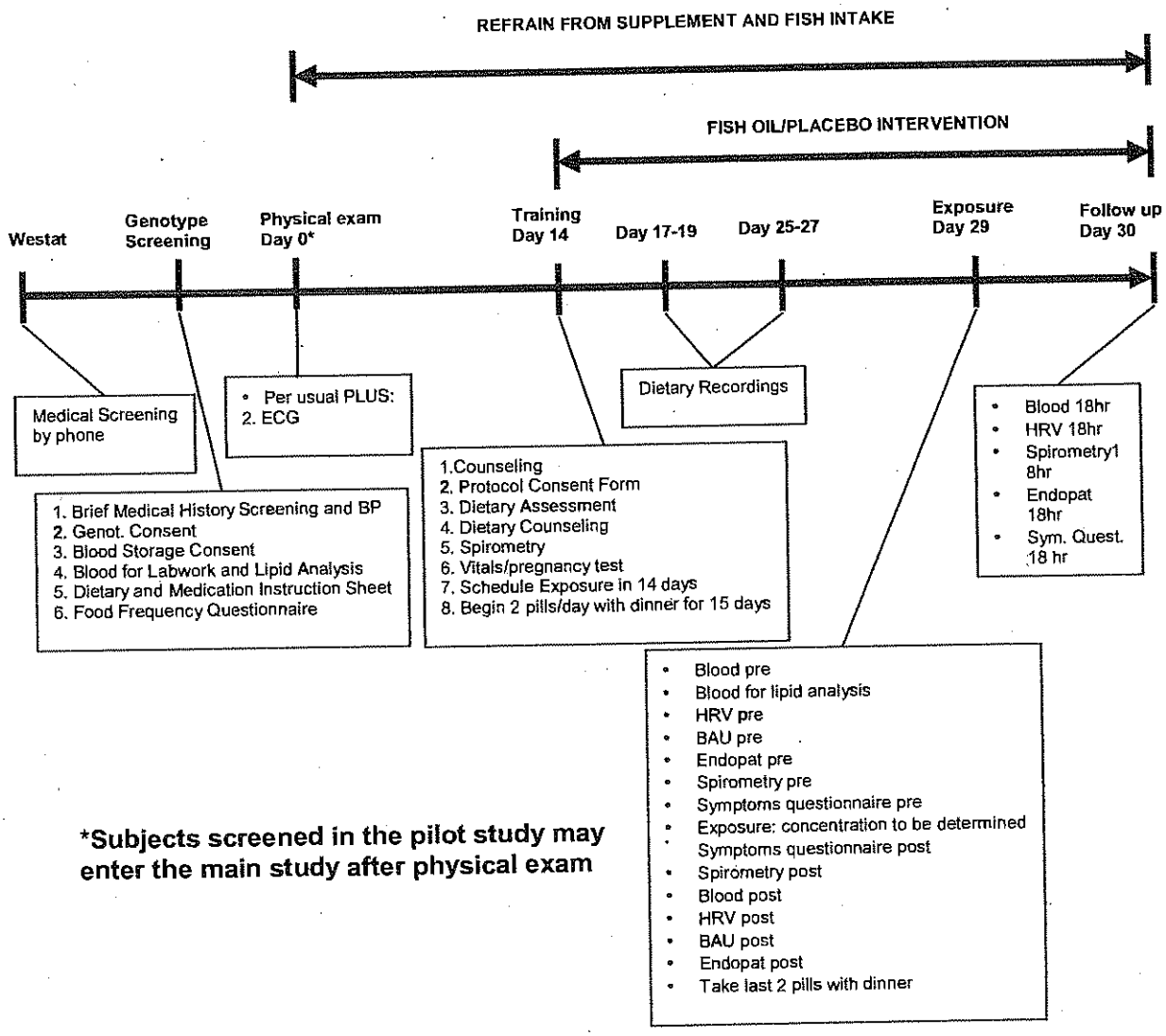
_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

No Title on signature page. Please Correct.

Initial/Date _____



***Subjects screened in the pilot study may enter the main study after physical exam**

Initial/Date _____

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
RENEWAL OR TERMINATION OF
IRB APPROVAL OF HUMAN SUBJECTS RESEARCH (PROGRESS REPORT)
version 3-Oct-2007

RECEIVED
JAN 22 2008
IRB UNC-CH

If the research is continuing:

- Check the relevant items.
- Include two collated sets of copies (sorted in the order listed) of checked items.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This form (renewal or termination).	2
□	2. Any items specifically requested in questions # 4 through 9 (in that order).	2
x	3. The most recent application submitted for IRB approval updated to include any modifications since the study was initially approved or last renewed. **If there are any modifications included with this renewal, highlight the proposed modifications by underlining.**	2
x	4. Clean copies of all consent document(s) to be used in the upcoming approval period, for stamping.	2
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

IRB study #: #: 07-0190 GCRC #: 2579

Date: 1/15/08

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD
Faculty advisor (if applicable): James M Samet, PhD, MPH
For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

1. In a few sentences, describe the past year's work, and describe what you plan for the upcoming year, including data analysis, if relevant.

We have modified the IRB protocol and consent forms in the past year. We started to recruit subjects for the pilot phase in July 2007 and we are continuing the recruitment process to reach our goal of 6 subjects. Four subjects have been exposed to 100 ug/m^3 of diesel exhaust. We will finish the exposure and data analysis for the pilot phase in the next 5-6 months then start the main phase which will last about 11/2 to 2 years.

2. Number of subjects involved through direct contact or use of their data (for multi-site studies, include only subjects covered by this IRB) (Note: b+d should not be larger than a)
   a. Total projected number as approved by IRB: 6 subjects in the pilot phase and 60 subjects in the main phase.
   b. Total number of subjects involved to date: 4 subjects in the pilot phase.

c. Number of subjects added since last renewal: N/A
d. Number to be included in upcoming year: 2 subjects in the pilot phase and 60 subjects in the main phase.

Answer the following questions based on information since initial approval or last renewal. Only include subjects covered by this IRB.

3. Have there been any modifications approved since the last review? If research is continuing, be sure all changes are reflected in the attached revised application for IRB approval. _x yes __ no
4. Have any subjects withdrawn voluntarily or been withdrawn from the study? **If yes, explain**; give number and reasons for withdrawals. __ yes x_ no
5. Have there been any findings (e.g., publications) that alter the risk/benefit ratio or otherwise impact the study? **If yes, explain.** __ yes x_ no
6. Does this study have a Data and Safety Monitoring Committee (DSMC or DSMB)? **If yes, provide a report** from the DSMC. __ yes _x no
7. Have there been unanticipated problems or serious adverse events since the last renewal? **If yes, include all copies** of local Adverse Event reports with this submission. __ yes _x no
8. Has this study been audited by external sponsor or monitor since approved or last renewed? **If yes, include a copy** of the audit report. __ yes _x no
9. Are you requesting any changes? **If yes, include the form** requesting *Modification of Approved Human Subjects Research* and underline the proposed change in the updated application. __ yes x_ no
10. Will you be enrolling, consenting or re-consenting subjects in the upcoming approval period? **If yes, include clean copies** of consents/assents/fact sheets to receive a new stamp. **Include any new recruitment materials** to be used with subjects. _x yes __ no

Action requested by Principal Investigator (choose only one):

Renew approval:

Study has always involved *only* analysis of existing data or specimens.

__ Continue as approved.

Study involves(ed) direct interaction/intervention or contact with subjects:

_x_ Enrollment of new subjects continues.

__ Enrollment of new subjects closed; interaction/intervention with previously enrolled subjects continues.

__ Direct interaction with subjects completed but subsequent monitoring or follow up continues.

__ Subjects' involvement completed but renewal is requested for data analysis.

Terminate approval because:

__ Research completed: Identifiable data or human biological specimens are stored according to plan already approved by the IRB.

__ Research completed: All data or human biological specimens are deidentified.

__ Lack of funding or other *(specify)*:

[signature] 1/16/08

Signature of Principal Investigator Date

[signature] 1/16/08

Signature of Faculty Advisor (if applicable) Date

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF HUMAN SUBJECTS RESEARCH
Version 2-Nov-2006

RECEIVED
JAN 22 2008
IRB UNC-CH

For IRB Use
Behav Biomed PH-Nurs
IRB Study #_____
Rec'd_____
Full Expedited Exempt

Part A.1. Contact Information, Agreements, and Signatures

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Date: 4 October, 2007
IRB #: **07-0190** 07-0190 Biomedical
GCRC #: 2579

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH
Co- Principal Investigator: Robert Devlin, PhD.
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov
For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: James M Samet, PhD, MPH
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
Phone #: (919) 966-0665 Fax #: (919) 966-6271 Email Address: samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor:
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): **Sponsor or award number**: EPA Intramural Federal Research

Include following items with your submission, where applicable.
- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.

- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. This application. One copy must have original PI signatures.	3
X	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
X	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
X	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
□	6. Protocol, grant application or proposal supporting this submission; (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal).	3
X	7. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
X	10. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist

Are the following involved?	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	X
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X	__
A.2.3. Videotaping, audiotaping, filming of subjects (newly collected or existing)?	__	X
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	X	
b. Non-English-speaking?		X
c. Decisionally impaired?		X
d. Patients?	X	
e. Prisoners, others involuntarily detained or incarcerated, or parolees?		X
f. Pregnant women?		X
g. Minors (less than 18 years)? ***If yes***, give age range: to years		X
A.2.5. a. Is this a multi-site study (sites outside UNC-CH engaged in the research)?		X
b. Is UNC-CH the sponsor or lead coordinating center?		X
If yes, include the ***Addendum for Multi-site Studies where UNC-CH is the Lead Coordinating Center***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, provide contact information for the foreign IRB.		
A.2.6. Will there be a data and safety monitoring committee (DSMB or DSMC)?	__	X
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	X
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	X
A.2.8. a. Investigational drugs? (provide **IND #** _____)		X
b. Approved drugs for "non-FDA-approved" conditions?		X
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	X	
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #** _____)	__	X
A.2.11. Fetal tissue?	__	X
A.2.12. Genetic studies on subjects' specimens?	X	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for* ***Consent for Stored Samples***.	X	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	X
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	X
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the* ***Oncology Protocol Review Committee***.	__	X
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	X	__

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	X no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the Sponsor of this study for the use or benefit of any member of the research team?	__ yes	X no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	X no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. The first part of this protocol is a pilot study to identify the optimum diesel exhaust concentration to investigate the cardiovascular effects in healthy older subjects. The main part of this proposal is to examine the health effects of diesel exhaust exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust.
Participants: Approximately 6 healthy 50-75 year-old male and female subjects with both GSTM1 null and GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val; n=2 each) for the pilot study. Sixty healthy 50-75 year-old male and female subjects (30 subjects are GSTM1 positive genotype and 30 subjects are GSTM1 null) for the main study.
Procedures (methods): In the pilot study, subjects will have 3 sequential exposures to the diesel exhausts at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours with a about 2 weeks of interval between exposures. In the main study, GSTM1 positive and GSTM1 null genotype subjects will be exposed to the optimum concentration (will be determined based on the pilot study) of diesel exhaust for 2 hours after 15 days of omega-3 fatty acid fish oil or olive oil supplementations.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from

cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13).

A recent epidemiological study involving eight European cities suggested that the primary effect of PM air pollution on cardiac admissions was likely attributable to diesel exhaust inhalation (14). Diesel exhaust is a component of air pollution in urban settings, and contributes both PM and gaseous phase compounds to the atmosphere. Diesel exhaust fumes are made up largely of fine and ultrafine carbonaceous particulates generated by incomplete combustion of fuel. The U.S. EPA and the National Research Council (NRC) PM Research Priorities currently include identification of components of PM (including PM size and chemical composition) that contribute to the reported increased morbidity and mortality (NRC, 1998; EPA, 1997). In addition, both the EPA and NRC have stressed the importance of examining gaseous co-pollutants that may also affect PM toxicity. Diesel exhaust (i.e., gas phase constituents and diesel exhaust particles (DEP)) is ideal for examining the role of these factors in inducing human health effects for the following reasons: (1) Because of the relatively high content of organic compounds on DEP (usually 10-30% by weight), DEP are ideal for examining the role of organics in inducing health effects; (2) The exhaust is a mixture of PM and gaseous pollutants including some (e.g., aldehydes, NOx, carbon monoxide) that are known to produce human toxicity in high enough concentrations that may potentially augment PM-induced effects; (3) the size of the DEP vary depending on engine type and other factors, allowing assessment of PM size on the induction of human health effects. Few controlled human exposures to diesel exhaust have been performed. One hour exposure to 300 $\mu g/m^3$ diesel exhaust with intermittent exercise in healthy human volunteers resulted in marked systemic and redox-sensitive pulmonary inflammatory responses (15, 16), and vascular dysfunction and impaired endogenous fibrinolysis that links diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (17). Despite a decade of intensive studies, much about the PM health effects problem, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that diesel exhaust exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of diesel exhaust exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (18)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (19-21). Fish oil

supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (22). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (23). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (24). Another enzyme called GSTP1 is also associated with anti-oxidants effects and we will determine the optimal concentration of diesel exhaust in the pilot study in the most vulnerable subgroup with GDTM1 null and 3 GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val). Therefore, the role of GSTs in mediating the cardiovascular effects following acute diesel exhaust exposure will also be studied in the main protocol in GSTM1 null and GSTM1 positive healthy older adults.

U.S. EPA regulations concerning diesel emissions, mandated by the Clean Air Act, have resulted in dramatic decreases in emissions from diesel engines since 1988, with the last set of significant engine modifications occurring in 1995 (EPA, 1998). The respiratory effects of diesel exhaust have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to diesel exhaust in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to diesel exhaust in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.

12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Le Tertre, A., Medina, S., Samoli, E., Forsberg, B., Michelozzi, P., Boumghar, A., Vonk, J.M., Bellini, A., Atkinson, R., Ayres, J.G., Sunyer, J., Schwartz, J. and Katsouyanni, K. (2002). Short-term effects of particulate air pollution on cardiovascular diseases in eight European cities. J Epidemiol Community Health 56:773-779.
15. Salvi, S., Blomberg, A., Rudell, B., Kelly, F., Sandstrom, T., Holgate, S.T. and Frew, A. (1999). Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. American journal of respiratory and critical care medicine 159:702-709.
16. Behndig, A.F., Mudway, I.S., Brown, J.L., Stenfors, N., Helleday, R., Duggan, S.T., Wilson, S.J., Boman, C., Cassee, F.R., Frew, A.J., Kelly, F.J., Sandstrom, T. and Blomberg, A. (2006). Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Respir J 27:359-365.
17. Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstrom, T. and Newby, D.E. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112:3930-3936.
18. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
19. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
20. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
21. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
22. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
23. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
24. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.
25. Lauer, M.S., Pothier, C.E., Chernyak, Y.B., Brunken, R., Lieber, M., Apperson-Hansen, C. and Starobin, J.M. (2006). Exercise-induced QT/R-R-interval hysteresis as a predictor of myocardial ischemia. Journal of electrocardiology 39:315-323.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal includes approximately 6 subjects with both GSTM1 null and GSTP1 codon 105 (Ile/Ile, Ile/Val, and Val/Val; n=2 each) genotypes for the pilot study and 60 subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) for the main study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria (pilot study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Currently taking ACE inhibitors.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Have an allergy to latex.

Exclusion criteria (main study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin.

- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Currently taking ACE inhibitors.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Female subjects currently taking estrogen replacement therapy.
- Because of reported cardioprotective effects of red wine, subjects will be prohibited from drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Have an allergy to latex.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

Pilot study: Approximately 6 subjects will undergo 3 exposures to air containing approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts sequentially for 2 hours in an exposure chamber. There will be a 2-week interval between exposures. We anticipate that there will be 6 subjects at each concentration of diesel exhaust exposure during the course of this pilot study. However, circumstances beyond our control may arise (i.e. subject/subjects might drop off due to illness and/or other reasons during the study) which may prevent finishing the proposed 3

exposures, and then we will have to add new subject/subjects at that point. Each new subject will have 3 exposures at approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts. Subjects who withdraw from the study will be compensated for all procedures and time completed to that point (See the pilot study design flow chart).

The purpose of the pilot study is to identify safe and measurable cardiovascular effects of diesel concentration. Our primary endpoints in the pilot study include HRV, pulmonary function, and biomarkers such as neutrophils, platelets, fibrinogen, c-reactive protein, lactate, ferretin, plasminogen activator inhibitor, and α1-antitrypsin. We will also assess the endothelial cell function by brachial artery ultrasound and finger artery Endo-Pat. We will analyze these endpoints after finishing each concentration of diesel exposure and determine whether we will continue the next higher concentration exposure. For example, if we find effects at approximately 100 $\mu g/m^3$ of diesel concentration then we will use that concentration for our main study. We may not need to test subjects at the next higher proposed concentrations. We will carefully monitor the symptoms that subjects may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, dyspnea, pallor, ataxia, and significant cardiac arrhythmias. In addition, analyses of blood samples taken after exposure will be scrutinized for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. Symptoms and/or changes detected in the blood samples that are considered clinically significant could prompt the study physicians to stop the study. Therefore, we will select the diesel concentration based solely on safety considerations.

Main study: This will be a randomized double-blinded study. Sixty study subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) will be given, in a double-blind fashion, a container filled with enteric-coated either 2g/day of fish oil or 2g/day of olive oil capsules for 15 days. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 25-35% of eicosapentaenoic acid (EPA) and 30-65% of docosahexaenoic acid (DHA). Each 1000 mg of these olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. There will be four groups of subjects in this study with 15 subjects in each group: the first group is fish oil supplement in GSTM1 positive subjects, the second group is fish oil supplement in GSTM1 null subjects, the third group is olive oil supplement in GSTM1 positive subjects, and the fourth group is olive oil supplement in GSTM1 null subjects. In the exposure study, each subject will be exposed to air containing approximately optimum diesel exhaust for 2 hours in an exposure chamber. (See the main study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for a genetic screening appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotype screening informed consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 26, methods of recruiting) will not need genotype screening. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 50 ml of blood collected in the pilot study and approximately 80 ml of blood collected in the main study. A portion of the blood

will be used for genetic screening of GSTM1 and/or GSTP1 Ile105 Val polymorphism and another portion of the blood will be used for the cholesterol levels, fatty acid analysis, and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back after they are selected on the genotype.

- ***GSTM1/GSTP1 genotype screening***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1 and GSTP1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene and GSTP1 Ile105Val polymorphism. Since the pilot study only seeks people that are GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotypes, and since the joint GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotype is present in about 8%, 17%, and 14% respectively of the population, it is likely that about 80-90 % of the subjects will be excluded in this pilot study but they will be informed to participate in our future study which will enroll both GSTM1 positive and null genotypes. Those subjects with GSTM1 positive will not be asked to continue with the study and will be compensated for their time. Since the main study seeks to enroll equal numbers of people that are GSTM1 positive and null, it is likely that as the study nears completion, we will still be seeking people with the null allele but not with the sufficient allele. If that is the case, those subjects will not be asked to continue with the study and will be compensated for their time.

- ***Food frequency questionnaire in the main study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the university of south carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the genetic screening will be scheduled for a physical examination in the HSF. Those subjects who are genetically screened but are not eligible for the pilot study, may be included in the main study if they are interested in participating. During this visit, subjects will sign an informed consent for a physical and the medical history form completed that contains information on general personal and family medical history. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam and genetic screening will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the main study:*** Subjects will be given an approximately 15 days supply of either fish oil or olive oil supplements randomly assigned. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of energy and nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record per week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 15 day of supplementation period. We will give the subjects another 15 days supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

- ***Baseline HRV measurement*:** In the pilot and main study, subjects will have their baseline 24-hour heart rate viability (HRV) measured by Holter monitor on the training day. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Subjects will be released while wearing the Holter monitor and return to the HSF the next day to get the Holter monitor removed.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (blood pressure, HRV, endothelial cell function by brachial artery ultrasound (BAU) and possibly by Endo-PAT, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement in both pilot and main studies*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained.
- ***Brachial artery ultrasound in both the pilot and main studies:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.
- ***Peripheral endothelial function by Endo-PAT in both pilot and main studies***: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be

displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).

- ***Pulmonary function in both pilot and main studies*** will be measured by spirometry.
- ***Blood sampling in both pilot and main studies***: approximately 50 ml of blood samples will then be collected in the pilot study and approximately 80 ml of blood samples will then be collected in the main study.
- ***Urine sampling in both the pilot and main studies:*** approximately 30 ml of urine samples will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

In the pilot study: Subjects will then enter the diesel exposure chamber for 3 sequentially exposures at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours each with a about 2-week interval between exposures. In the main study: Subjects will have only one exposure at an optimum concentration of diesel exhaust that will be identified from the pilot study.

Exposure: All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. The diesel exhaust will be generated from a 6-cylinder, 205 hp, 5.9 L displacement diesel engine (Cummins, Columbus IN) mounted on a vehicle located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean filtered and humidified air by approximately 1/30th to give a chamber concentration of approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$. The monitoring analyzer registers a concentration greater than 100 ug/m^3 above the target concentration as the exposure limit. Specifically, exposures will be terminated at values $\geq$200, 300 or 400 during runs in which the exposure targets are 100, 200 and 300 ug/m^3, respectively. The running sum of the 2-minute average concentrations displayed by the monitoring analyzer during the 2 hr exposure period exceeds 18,000 ug/m^3. This safety limit is specifically designed to address exposures for which the target concentration is 300 ug/m^3. It will prevent an inhalational exposure greater than 600 ug, which assumes that the resting subject will inhale 2 m^3 during the 120 min exposure. It is expected that 75 to 95% of the diesel exhaust PM from this type of engine will be centered around approximately 0.1-0.2 μm. Preliminary testing has shown the mean particle size to be approximately 0.14 μm. Levels of carbon monoxide, and oxides of nitrogen (NOx; mainly NO, and some NO2), will be kept under 10 ppm CO, 15 ppm NO, and 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) for these substances are: carbon monoxide=50 ppm; NO2=5 ppm; NO=25 ppm; The American Conference of Governmental Industrial Hygienists (ACGIH) 8 hr threshold limit values (TLVs) are: NO=25 ppm, NO2=3 ppm, and CO=25 ppm]. Diesel fuel used for the study will be purchased as a certified fuel (Chevron Phillips Chemical Co., Borger TX, 0.05 LS Certification Fuel, type II). This certified fuel represents diesel fuel composition that is typically available at gas stations for most regions of the country except for California. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured with a tapered element oscillating microbalance (TEOM) and/or a Data-RAM, and chamber PM concentrations during exposure further validated by weighing filters obtained from versatile air pollution samplers (VAPS) impacters. Particle size distribution will be determined by scanning mobility particle size (SMPS) not during the subject exposure but at regular intervals (e.g., monthly) to show that the

size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. The minute ventilation during exposure will be measured. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 50 ml of blood and 30 ml of urine samples will be collected in the pilot study and approximately 80 ml of blood will be collected in the main study. Approximately 30 ml of urine samples will be collected in both the pilot and main studies.

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 50 ml of blood will be collected in the pilot study and approximately 80 ml of blood will be taken in the main. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound and finger arteries Endo-PAT). Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood (about 50 ml in the pilot study about 80 ml in the main study) will be drawn by standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In the main study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to a diesel exhaust and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of diesel exhaust on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound and finger arteries Endo-PAT:** There are no known risks associated with imaging of the brachial artery and index finger arteries. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Diesel exposure** The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban areas. Diesel particulate concentrations proposed for the pilot study will be 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 μg/m3 and the optimum concentration will be used for the main study. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it

exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. During exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have EKG leads attached during the exposure to monitor cardiac function during exercise. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for their participation up to that point. The investigators or duty physician will end the exposure if the subject is found to be suffering from any significant adverse effect. The small pilot study will allow us to monitor the adverse effects of diesel exhaust closely and we will determine whether we will go to the next higher concentration based on the analysis. We will be able to identify a safe concentration of diesel exhaust that induces a measurable change in our study endpoints. No major adverse health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

- **Confidentiality** Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test our hypothesis that diesel exhaust causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust. We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to diesel will also be measured (e.g., blood pressure, HRV, brachial arterial blood flow rate, change in blood coagulation factors, platelets, fibrinogen, c-reactive protein, lactate, plasminogen activator inhibitor, ferretin, and α1-antitrypsin). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 was derived using a power of 0.8 and an α of 0.05.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to diesel exhaust compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial blood flow rate, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial blood flow rate. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of diesel exhaust on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. **X** Names
b. **X** Telephone numbers
c. **X** Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. **X** Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. **X** Electronic mail addresses
g. __ Social security numbers
h. **X** Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, characteristic or code, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.11. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

X No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:

__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.12. **Data security for storage and transmission**. Please check all that apply.

For electronic data:

X Secure network X Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question 7 above)
__ Locked suite or office
X Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.13. **Post-study disposition of identifiable data or human biological materials**. Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects.** If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent.** *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true:

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study involves sensitive data that could be damaging if disclosed). ___ yes ___ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). ___ yes ___ no
Explain.

If you checked "yes" to either, will consent be oral? Will you give out a fact sheet? Use an online consent form, or include information as part of the survey itself, etc?

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ *If this does not apply to your study, do not submit this section.*

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with your IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Will the information collected be limited only to that necessary to contact the subjects to ask if they are interested in participating in the study?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the pilot study will be approximately 3 months and the main study will take approximately 18 months. Participant recruitment and screening is expected to be continuous throughout the study until the intended number of participants is reached.

If the subject is eligible for the pilot study they will have 9 visits to the HSF over approximately 18-20 weeks. Subjects will have about 2 weeks break between 2 exposures. If the subject is eligible for the main study of diesel exhaust exposure the individual will have 5 visits to the research facility over approximately 5-6 weeks. The pre-enrollment genotyping and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after genotyping of the blood sample. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment for the main study) will require approximately 3 hours. Exposure day will last approximately 8 hours for the pilot and main studies. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours for the pilot and the main study.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Include food or refreshments that may be provided.

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be paid for the entire study, excluding completion bonus.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled, and will be paid 50% up to a total maximum of $100 for canceled procedures. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study.

1. If following genotyping of the blood samples on screening day, it is determined that the individual's genotype is not needed for completion of the study, then that subject may be informed that his/her participation in this study is no longer necessary. The subject will be notified by phone and the total compensation for this study will be approximately $60.
2. If the subject is qualified and finished the entire pilot study, the total compensation for completion of this study will be approximate $2053.
3. If the subject is qualified and finished the entire main study, the total compensation for completion of this study will be approximate $1195.

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml of pilot and ~80ml of main study; genotyping)	$30
Pre-study qualification total =	$60

Subjects based on their genotype assessment may not be asked to further participate in the study.

Training day (3 hours)

Time (3h @$12/h)	$36
Holter monitor	$100

Pilot study:

Exposure (8 hours)

Venipuncture (~50ml, pre; 3@$30 each)	$90
Holter monitor (3 @$100 each)	$300
Chamber exposure (3 @$72 each)	$216
Venipuncture (~50ml, post; 3@$30 each)	$90
Time (3X8h @$12/h)	$228
Finger artery Endo-PAT (6 @$10 each)	$60
Brachial artery ultrasound (6@$50 each)	$300
On-time bonus (3@$25 each)	$75

Urine samplings (6 @$10 each)	$60

Total for completion of exposure = $1579

Eighteen hours after exposure (3 hour)

Venipuncture (~50ml; 3@$30 each)	$90
Time (3X3h @$12/h)	$108
Finger artery Endo-PAT (3 @$10 each)	$30

Total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each)	$150

Approximate TOTAL for completion of pilot study = $2053

Main study:

Exposure (8 hours)

Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Finger artery Endo-PAT (2 @$10 each)	$20
Food records (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $823

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Finger artery Endo-PAT	$10

Total for completion of post-exposure = $76

Protocol Completion Bonus	$100

Approximate TOTAL for completion of main study = $1195

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Data, Records or Human Biological Specimens without Direct Contact with Subjects

→ *If this does not apply to your study, do not submit this section.*

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

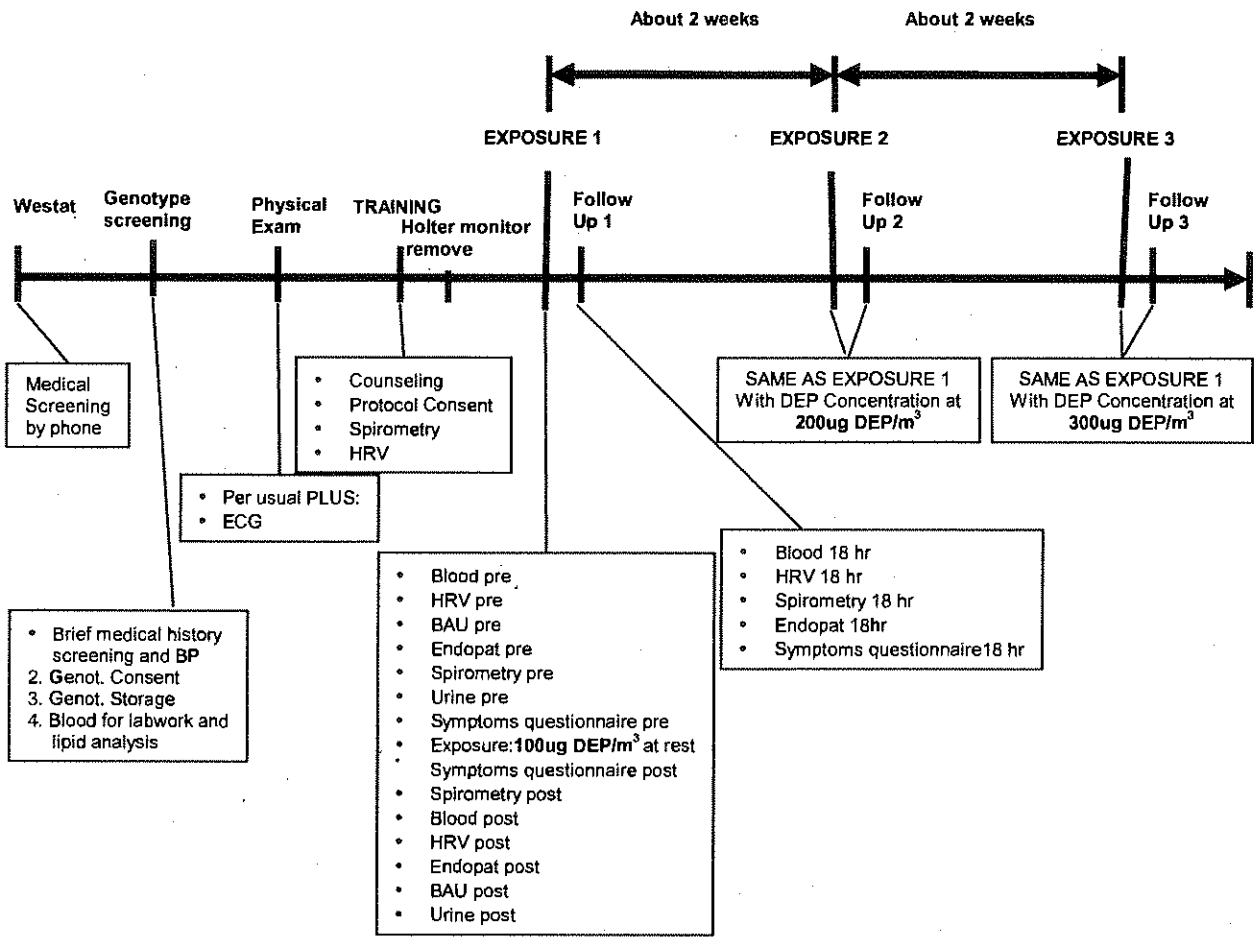
C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

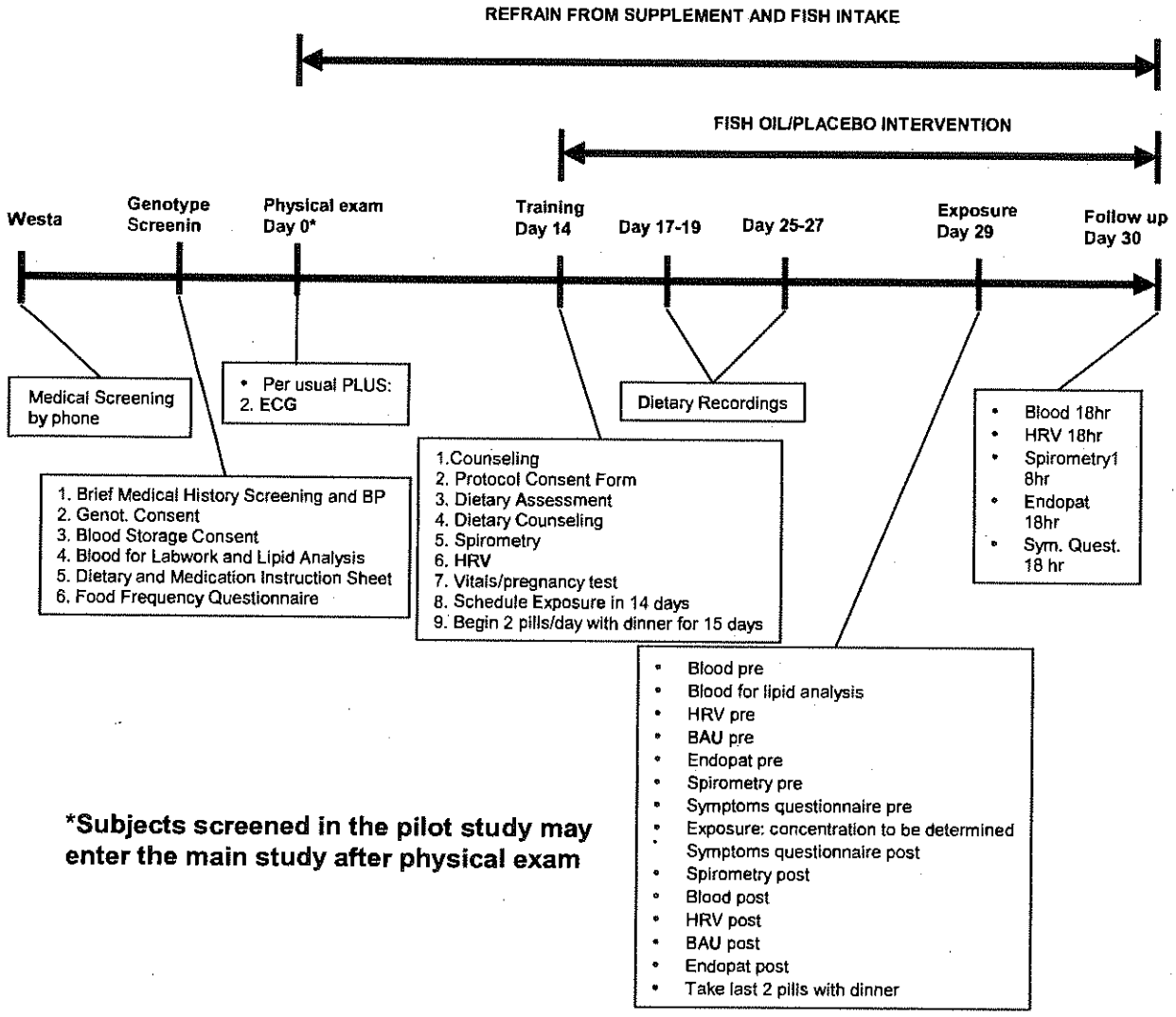
__ yes __ no __ not applicable (explain)

C.6. Do all of these data records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain



PILOT PHASE FLOW DIAGRAM



MAIN STUDY FLOW DIAGRAM

School of Medicine
The University
of
North Carolina
at
Chapel Hill
&
UNC
Hospitals

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27599-7600

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Clarence W. Potter, MS
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Biostatistician
Paul W. Stewart, PhD
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**Training &
Career Development**
Susan N. Pusek, MPH
(919) 966-0128

**Office of
Research Subject Advocacy**
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Marie H. Zeldin, RN, BSN
Assistant Director
(919) 966-6844

Verne S. Caviness

General Clinical Research Center

RECEIVED
JAN 22 2008
IRB UNC-CH

MEMORANDUM

TO: Stephen Bernard, MD, Chairman
Committee on the Protection of the
Rights of Human Subjects

FROM: Dr. Paul B. Watkins
Director, GCRC

DATE: January 16, 2008

SUBJECT: **RENEWAL: GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust**
IRB Study #: 07-0190

PI: **Haiyan Tong, MD**

The Clinical Research Advisory Committee has now received the enclosed project from the Principal Investigator requesting renewal. Copies of the project are enclosed for action by your Committee.

Enclosures

IRB
please call Haiyan
Tong at 966-4993
when it's ready!
Thank you!
Haiyan Tong

RECEIVED
JAN 22 2008
IRB UNC-CH

GCRC RENEWAL

Instructions to Reviewer: Return this form to the Committee office (GCRC, CB #7600) along with a brief report specifying the basis for your recommendations. Please feel free to contact the P.I. directly if you need additional information, or to enlist the help of any colleagues needed.

GCRC Project **GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust**
IRB Study #: 07-0190

P.I.: **Dr. Haiyan Tong**

Original Approval: 2/25/07

Project Expiration: **2/25/08**

Date Submitted: **1/16/08**

RECOMMENDATION OF REVIEWER:

✓ Approval.

_____ Approval, with modifications stated in accompanying report.

_____ Returned for re-submission.

_____ Disapproval.

DURATION OF APPROVAL

12 months. (usually 12 months, never longer)

_____ Contingent on review or progress after:

Should reapproval be submitted to Committee for new date? (Disapproval must be voted on by full committee.)

_____ Yes
✓ No

Marie Zeldin, RN or Dr. David Weber — 1/18/08 (Date)

Dr. Paul Watkins — (Date)

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 3/04/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: GCRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal.

Submission Description:

This modification, dated January 28, 2008, changes the inclusion/exclusion criteria so that statins and ACE inhibitors are no longer exclusionary. In addition, subjects will now advance from the pilot phase of this study to the second exposure.

Investigator's Responsibilities

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 3/4/08 AND 2/9/09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—MAIN STUDY

5

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: February 22, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, diesel exhaust, elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by diesel exhaust. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, and chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.

Initial/Date _____

- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Use olive oil for cooking, dressings, and sauces during the study.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not eat or drink anything for 2 hours prior to the training day visit.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 60 people in this research study.

How long will your participation in this study last?

You will have up to 3 visits to the research facility over approximately 5-6 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) two-week of fish oil/olive oil treatments part, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a 15-day fish oil or olive oil supplementation and the exposure session. You will be given a 15-day supply of either fish oil or olive oil tablets. Please take 2 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next two weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this 15-day of supplementation period. We will give you another 15-day supply of either fish oil or olive oil tablets and you will need to restart the pills again.

In the morning of your last pill of supplements, you will come to the HSF for the baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF in the next morning for the exposure and the monitor will be removed.

Initial/Date _____

Exposure day
We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat before midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we will perform on your last pill day).
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- We will bring you back to the HSF where we may also measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT) at HSF and we will compare the findings with ultrasound measurement of the artery in your arm. We will place two small PAT probes to both of your index fingers. The procedure is similar to that of ultrasound measurement and we will only place a blood pressure cuff on one of your arm. We will obtain recordings for 5 minutes at rest, 5 minutes during the blood pressure cuff inflated, and 5 minutes after the blood pressure cuff released.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust (the concentration will be determined based on the pilot study) for 2 hours in a small exposure chamber. This particle concentration is representative of diesel exhaust particle (DEP) levels which you would inhale if you were occupationally exposed to DEP, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over a period at a busy intersection in a polluted city (such as Los Angeles) where DEP concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will obtain another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: The concentration of diesel exhaust in this study has been chosen based on a pilot study in susceptible older subjects. Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause

Initial/Date _____

people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing these exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Finger Endo-PAT: Similar to the measurement by brachial artery ultrasound, there are no significant risks associated with index fingers arteries measurement.
Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be

Initial/Date _____

exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss

Initial/Date _____

or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1195.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	

Initial/Date _____

Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Brachial artery ultrasound (2@ $50 each)	$100
Finger Endo-PAT	$20
Time (8h @$12/h)	$96
Food record (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $823

18 hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3 hrs @$12/h)	$36
Finger Endo-PAT	$10

Day 3 total for completion of post-exposure = $76

Protocol Completion Bonus $100

Approximate TOTAL for completion of the study = $1195

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

Initial/Date _____

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ________________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

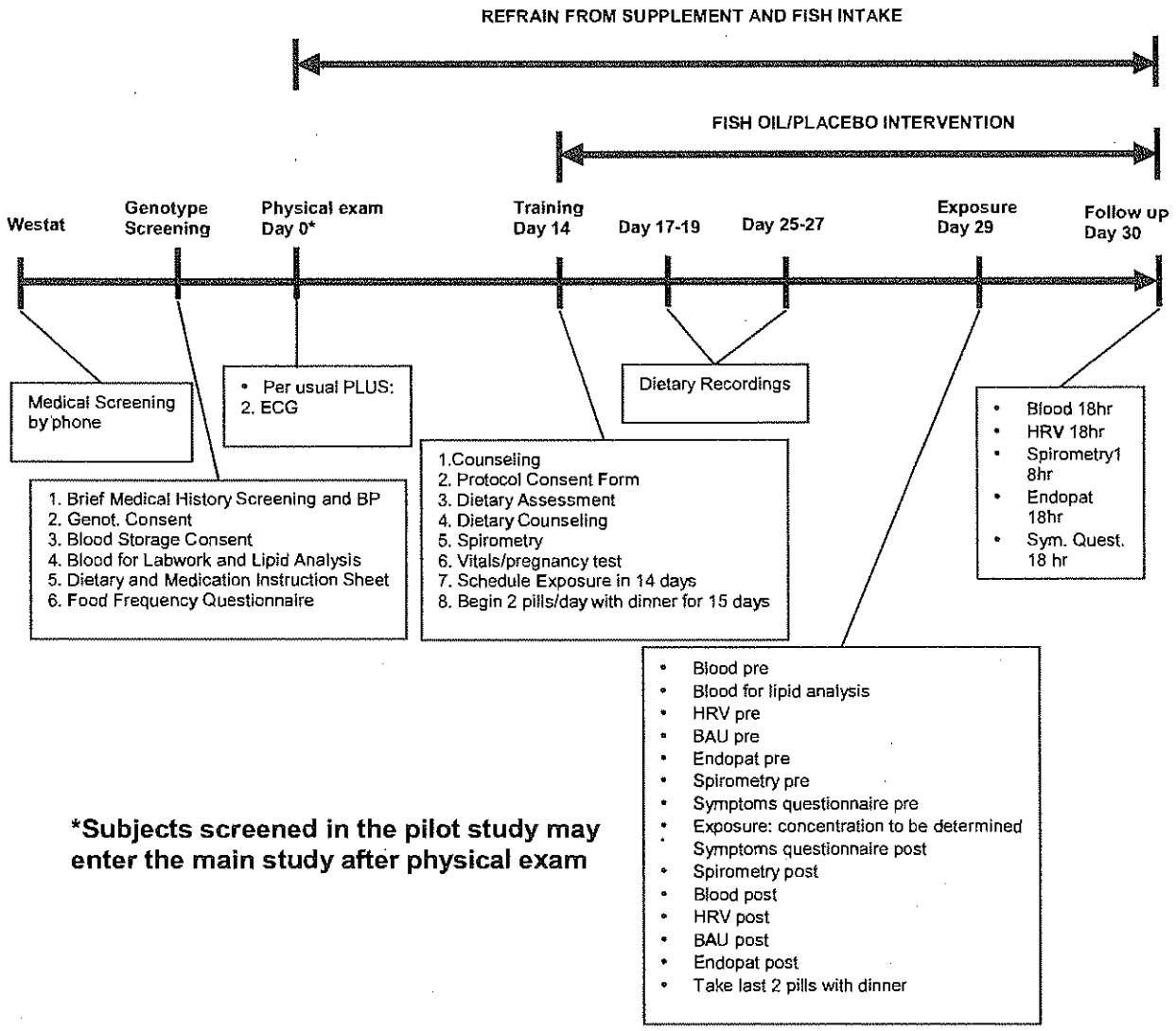
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



***Subjects screened in the pilot study may enter the main study after physical exam**

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 3/4/08 AND 2/8/09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information--PILOT STUDY **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 22, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?

Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 50 ml of blood for genotyping and blood analysis. If you are re-contacted from the previous study, you will not need genotype screening but you will need to get blood drawn for other blood analysis. At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office.

How will the blood sample be collected?
You will have about 50 ml (about 3 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should

Initial/Date _____

not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical staff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these

Initial/Date _____

specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB

Initial/Date _____

7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 3/4/08 AND 2/9/09 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY

3

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: February 22, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.

Initial/Date _____

- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

We will collect your baseline heart rate viability (HRV) by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

Initial/Date _____

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. We will analyze the results after everyone finishing the 200 $\mu g/m^3$ exposure and decide whether we will continue on the 300 $\mu g/m^3$ exposure. If we decided to continue, you will have the third exposure at approximately 300 $\mu g/m^3$ for 2 hours that will occur about 2 weeks later. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart

Initial/Date _____

rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 3 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel

Initial/Date _____

exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.

- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 ml or about 3 1/3 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.

Initial/Date _____

- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 ml or about 3 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

Initial/Date _____

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?

Initial/Date _____

You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

Initial/Date _____

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $2053.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90
Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75

Initial/Date _____

Total for completion of exposure = $1479

18 hours after exposure (3 hour)

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $2053

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

Initial/Date _____

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ____________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

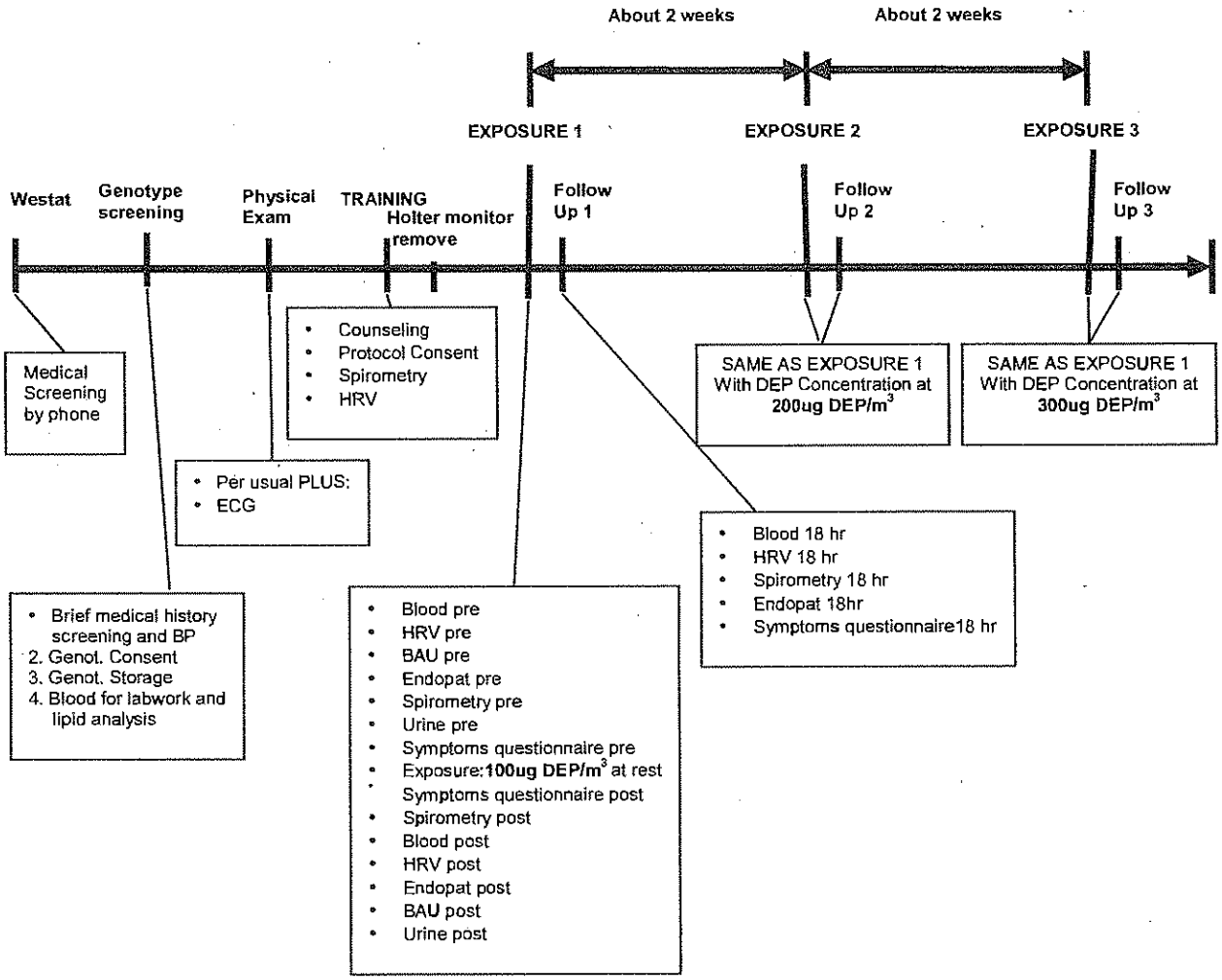
_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

Response to Minor Stipulations
Modification

Receipt Date : 2/04/2008 | Previous Review Type: **Renewal Full Board** | IRB ID : 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong | IRB: Biomedical | Coordinator: Astrid Ertola

☐ Full Board Review ☑ Not Full Board Review

Study Description :

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description :

This modification, dated January 28, 2008, changes the inclusion/exclusion criteria so that statins and ACE inhibitors are no longer exclusionary. In addition, subjects will now advance from the pilot phase of this study to the second exposure.

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date: 3/5/08)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached: 3

Other attachments: 0

FINAL ACTIONS:

- ☐ Approved 3/5/08
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to Full IRB
- ☐ Return to sender
- ☐ Termination

First Review of IRB Submission

Pending Reviews

Submission Type	Date Submitted	Approval Date	IRB Coordinator	Review Type
Renewal	1/22/2008	Submitted		Full Board

Training Not Met (Haiyan Tong)

Receipt Date : 2/04/2008 Submission Type: **Modification** IRB ID : 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong IRB: Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ☑ Full IRB

Agenda Date Reviewer 1: ENK 2-21-08 Reviewer 2:

Entered by: Laura O Curry

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):
- ☑ Reviewer Checklist completed
- ☑ Minor Stipulation letter:
  - ☑ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☑ Hard copy sent
- ☑ Approval letter:
  - ☑ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☑ Hard copy sent

Consent forms attached:
Other attachments:

FINAL ACTIONS:
- ☑ Approved
- ☑ Approved with Minor Stipulations
- ☑ NHSR
- ☑ Return to sender
- ☑ Termination

RECEIVED
MAR 03 2008
IRB UNC-CH

To: Biomedical IRB
CB# 7097

From: Haiyan Tong
Environmental Protection Agency
CB# 7315

Date: 3/3/08

Study #: 07-0190
GCRC#: 2579
Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Re: Contingencies to be addressed on 2-22-08 review

Dear IRB review committee,

Thanks for the Review of the Modification submited on 1-28-08. Enclosed is the updated IRB application which includes the prpposed changes in response to the "Contingencies Approval on 2/22/08".

Enclosed please also find the CLEAN consent forms which are printed on the new IRB consent forms.

Please let me know if you have any questions regarding this revision.

Sincerely,

Haiyan Tong

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Date: 2/22/2008

RE: Contingencies to be addressed following IRB Review
Submission Type: Modification
Study #: 07-0190
Other #: GCRC - 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been reviewed by the above IRB. This is not an IRB approval. You may not implement the research activities described in your submission until you have received a memo indicating final IRB approval. The IRB determined that this submission MAY BE APPROVED, pending stipulated changes and/or clarifications as detailed below.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the item(s) listed above. **When you respond, please be sure to cite the IRB study number noted above, and indicate that you are responding to this memo as dated above; failure to link your response to this study, in general, and this memo, in particular, may result in delays.** Any additional changes (including new materials) must also be listed and discussed in the memo. If the application or any study materials must be revised, please submit 2 copies of all revised materials, one in which all changes are underlined and the other a "clean" copy with no underlining.

Study Specific Details:

1. Please provide a copy of the updated IRB application which includes these changes.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (GCRC), Non-IRB Review Contact

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To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB EWK

Date: 2/22/2008

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James Samet, Environmentally Protection Agency
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Wanda Simmons, (GCRC), Non-IRB Review Contact

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 3-Oct-2007

RECEIVED
FEB 04 2008
IRB UNC-CH

RECEIVED
1/31/08
GCRC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes _X__no

IRB study #: 07-0190_____ GCRC #: 2579 **Date:** January 28, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James M Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

List and describe each proposed change: (all the changes are highlighted)

I. Modification of exclusion criteria of the Pilot and Main phases of the study,

1. Use of the cholesterol control medication class known as statins will no longer be considered as an exclusion criterion for enrollment in the study. The reason for this change is based on new information from relevant studies indicating that statin use is not a factor that alters human responsiveness to ambient particulate matter exposure. Accordingly, we have deleted statin use from the list of exclusion criteria on page 2 in the consent form and page 2 of the genotype screening consent.
2. Similarly, ACE inhibitor use will be allowed in the study, as there is no indication that these medications will interfere with our ability to measure endpoints of interest. Accordingly, we have deleted the ACE inhibitors from the list of exclusion criteria on page 2 in the consent form.

II. Modify the pilot exposure schedule in order to retain the subjects.

The current protocol calls for subjects to undergo 3 incremental diesel exhaust exposures, consisting of exposure to 100 ug/m^3, 200 ug/m^3 and 300 ug/m^3. The approved protocol further specifies that subjects will progress through the exposures as a group. However, unforeseen delays in the recruitment of qualified volunteers now threaten the retention of enrolled subjects who have already undergone exposure to the first dose of diesel exhaust. In order to avoid unnecessarily prolonging enrollment in the study, subjects who have completed the first exposure will now be allowed to progress to the second exposure while exposures to the first dose are still being conducted. Four of the 6 subjects planned for the pilot phase have completed the 100 ug/m^3 exposure and have been evaluated. No effects have been observed. These subjects will now be scheduled for the second exposure while recruitment/enrollment continues. All 6 subjects will complete the 200 ug/m^3 exposure before the 300 ug/m^3 exposures are started. These changes are highlighted on page 4 in the consent form.

Signature of Principal Investigator or designee	1/28/08 Date
Signature of Faculty Advisor (if applicable)	1-28-08 Date

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TO: Haiyan Tong
Environmentally Protection Agency
CB# 7315

FROM: Biomedical IRB

DATE: 2/04/2008

RE: **Study #**: 07-0190
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

The UNC IRB Office has received your Modification dated 1/28/2008. However, it can not be processed until the previous action (Renewal) has been approved. In the future, please wait to submit an action until the previous action has been approved. This will help avoid delays and errors in processing.

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 19-Feb-2008

07-0190

RECEIVED
MAR 03 2008
IRB UNC-CH

Part A.1. Contact Information, Agreements, and Signatures

Date: March 3, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH
Co- Principal Investigator: Robert Devlin, PhD.
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: James M Samet, PhD, MPH
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315
Phone #: (919) 966-0665 Fax #: (919) 966-6271 Email Address: samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. This application. One copy must have original PI signatures.	3
☐	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
☐	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
☐	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
☐	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator — Date: 3/3/08

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor — Date: 3/3/08

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee — Date

Print Name of Department Chair or designee — Department

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	_X_
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	_X_	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_X_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_X_	__
b. Non-English-speaking?	__	_X_
c. Decisionally impaired?	__	X__
d. Patients?	_X_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_X_
f. Pregnant women?	__	_X_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_X_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_X_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_X_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_X_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_X_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_X_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_X_
b. Approved drugs for "non-FDA-approved" conditions?	__	_X_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	__	_X_
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_X_
A.2.11. Fetal tissue?	__	_X_
A.2.12. Genetic studies on subjects' specimens?	_X_	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for* ***Consent for Stored Samples***.	_X_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	_X_
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	_X_
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_X_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_X_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_X_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_X no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_X no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. The first part of this protocol is a pilot study to identify the optimum diesel exhaust concentration to investigate the cardiovascular effects in healthy older subjects. The main part of this proposal is to examine the health effects of diesel exhaust exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust.
Participants: Approximately 6 healthy 50-75 year-old male and female subjects with both GSTM1 null and GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val; n=2 each) for the pilot study. Sixty healthy 50-75 year-old male and female subjects (30 subjects are GSTM1 positive genotype and 30 subjects are GSTM1 null) for the main study.
Procedures (methods): In the pilot study, subjects will have 3 sequential exposures to the diesel exhausts at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours with a about 2 weeks of interval between exposures. In the main study, GSTM1 positive and GSTM1 null genotype subjects will be exposed to the optimum concentration (will be determined based on the pilot study) of diesel exhaust for 2 hours after 15 days of omega-3 fatty acid fish oil or olive oil supplementations.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from

cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13).

A recent epidemiological study involving eight European cities suggested that the primary effect of PM air pollution on cardiac admissions was likely attributable to diesel exhaust inhalation (14). Diesel exhaust is a component of air pollution in urban settings, and contributes both PM and gaseous phase compounds to the atmosphere. Diesel exhaust fumes are made up largely of fine and ultrafine carbonaceous particulates generated by incomplete combustion of fuel. The U.S. EPA and the National Research Council (NRC) PM Research Priorities currently include identification of components of PM (including PM size and chemical composition) that contribute to the reported increased morbidity and mortality (NRC, 1998; EPA, 1997). In addition, both the EPA and NRC have stressed the importance of examining gaseous co-pollutants that may also affect PM toxicity. Diesel exhaust (i.e., gas phase constituents and diesel exhaust particles (DEP)) is ideal for examining the role of these factors in inducing human health effects for the following reasons: (1) Because of the relatively high content of organic compounds on DEP (usually 10-30% by weight), DEP are ideal for examining the role of organics in inducing health effects; (2) The exhaust is a mixture of PM and gaseous pollutants including some (e.g., aldehydes, NOx, carbon monoxide) that are known to produce human toxicity in high enough concentrations that may potentially augment PM-induced effects; (3) the size of the DEP vary depending on engine type and other factors, allowing assessment of PM size on the induction of human health effects. Few controlled human exposures to diesel exhaust have been performed. One hour exposure to 300 $\mu g/m^3$ diesel exhaust with intermittent exercise in healthy human volunteers resulted in marked systemic and redox-sensitive pulmonary inflammatory responses (15, 16), and vascular dysfunction and impaired endogenous fibrinolysis that links diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (17). Despite a decade of intensive studies, much about the PM health effects problem, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that diesel exhaust exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of diesel exhaust exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (18)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (19-21). Fish oil

supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (22). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (23). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (24). Another enzyme called GSTP1 is also associated with anti-oxidants effects and we will determine the optimal concentration of diesel exhaust in the pilot study in the most vulnerable subgroup with GDTM1 null and 3 GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val). Therefore, the role of GSTs in mediating the cardiovascular effects following acute diesel exhaust exposure will also be studied in the main protocol in GSTM1 null and GSTM1 positive healthy older adults.

U.S. EPA regulations concerning diesel emissions, mandated by the Clean Air Act, have resulted in dramatic decreases in emissions from diesel engines since 1988, with the last set of significant engine modifications occurring in 1995 (EPA, 1998). The respiratory effects of diesel exhaust have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to diesel exhaust in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to diesel exhaust in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.

12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Le Tertre, A., Medina, S., Samoli, E., Forsberg, B., Michelozzi, P., Boumghar, A., Vonk, J.M., Bellini, A., Atkinson, R., Ayres, J.G., Sunyer, J., Schwartz, J. and Katsouyanni, K. (2002). Short-term effects of particulate air pollution on cardiovascular diseases in eight European cities. J Epidemiol Community Health 56:773-779.
15. Salvi, S., Blomberg, A., Rudell, B., Kelly, F., Sandstrom, T., Holgate, S.T. and Frew, A. (1999). Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. American journal of respiratory and critical care medicine 159:702-709.
16. Behndig, A.F., Mudway, I.S., Brown, J.L., Stenfors, N., Helleday, R., Duggan, S.T., Wilson, S.J., Boman, C., Cassee, F.R., Frew, A.J., Kelly, F.J., Sandstrom, T. and Blomberg, A. (2006). Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Respir J 27:359-365.
17. Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstrom, T. and Newby, D.E. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112:3930-3936.
18. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
19. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
20. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
21. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
22. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
23. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
24. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.
25. Lauer, M.S., Pothier, C.E., Chernyak, Y.B., Brunken, R., Lieber, M., Apperson-Hansen, C. and Starobin, J.M. (2006). Exercise-induced QT/R-R-interval hysteresis as a predictor of myocardial ischemia. Journal of electrocardiology 39:315-323.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal includes approximately 6 subjects with both GSTM1 null and GSTP1 codon 105 (Ile/Ile, Ile/Val, and Val/Val; n=2 each) genotypes for the pilot study and 60 subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) for the main study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria (pilot study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin. (delete)
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Currently taking ACE inhibitors. (delete)
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Have an allergy to latex.

Exclusion criteria (main study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin. (delete)

- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Currently taking ACE inhibitors.(delete)
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Female subjects currently taking estrogen replacement therapy.
- Because of reported cardioprotective effects of red wine, subjects will be prohibited from drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Have an allergy to latex.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

Pilot study: Approximately 6 subjects will undergo 3 exposures to air containing approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts sequentially for 2 hours in an exposure chamber. There will be a 2-week interval between exposures. We anticipate that there will be 6 subjects at each concentration of diesel exhaust exposure during the course of this pilot study. However, circumstances beyond our control may arise (i.e. subject/subjects might drop off due to illness and/or other reasons during the study) which may prevent finishing the proposed 3

exposures, and then we will have to add new subject/subjects at that point. Each new subject will have 3 exposures at approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts. Subjects who withdraw from the study will be compensated for all procedures and time completed to that point (See the pilot study design flow chart).

The purpose of the pilot study is to identify safe and measurable cardiovascular effects of diesel concentration. Subjects will have the second exposure in about two weeks after the first exposure. All six subjects will complete the 200 $\mu g/m^3$ exposure before the 300 $\mu g/m^3$ exposures are started. Our primary endpoints in the pilot study include HRV, pulmonary function, and biomarkers such as neutrophils, platelets, fibrinogen, c-reactive protein, lactate, ferretin, plasminogen activator inhibitor, and α1-antitrypsin. We will also assess the endothelial cell function by brachial artery ultrasound and finger artery Endo-Pat. We will analyze these endpoints after finishing the 200 $\mu g/m^3$ diesel exposures and determine whether we will continue the 300 $\mu g/m^3$ concentration exposure. For example, if we find effects at approximately 200 $\mu g/m^3$ of diesel concentration then we will use that concentration for our main study. We may not need to test subjects at the next higher proposed concentrations. We will carefully monitor the symptoms that subjects may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, dyspnea, pallor, ataxia, and significant cardiac arrhythmias. In addition, analyses of blood samples taken after exposure will be scrutinized for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. Symptoms and/or changes detected in the blood samples that are considered clinically significant could prompt the study physicians to stop the study. Therefore, we will select the diesel concentration based solely on safety considerations.

Main study: This will be a randomized double-blinded study. Sixty study subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) will be given, in a double-blind fashion, a container filled with enteric-coated either 2g/day of fish oil or 2g/day of olive oil capsules for 15 days. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 25-35% of eicosapentaenoic acid (EPA) and 30-65% of docosahexaenoic acid (DHA). Each 1000 mg of these olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. There will be four groups of subjects in this study with 15 subjects in each group: the first group is fish oil supplement in GSTM1 positive subjects, the second group is fish oil supplement in GSTM1 null subjects, the third group is olive oil supplement in GSTM1 positive subjects, and the fourth group is olive oil supplement in GSTM1 null subjects. In the exposure study, each subject will be exposed to air containing approximately optimum diesel exhaust for 2 hours in an exposure chamber. (See the main study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for a genetic screening appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotype screening informed consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 26, methods of recruiting) will not need genotype screening. Subjects will then undergo a brief medical history screening and blood

pressure measurement. Subjects will then have approximately 50 ml of blood collected in the pilot study and approximately 80 ml of blood collected in the main study. A portion of the blood will be used for genetic screening of GSTM1 and/or GSTP1 Ile105 Val polymorphism and another portion of the blood will be used for the cholesterol levels, fatty acid analysis, and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back after they are selected on the genotype.

- ***GSTM1/GSTP1 genotype screening***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1 and GSTP1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene and GSTP1 Ile105Val polymorphism. Since the pilot study only seeks people that are GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotypes, and since the joint GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotype is present in about 8%, 17%, and 14% respectively of the population, it is likely that about 80-90 % of the subjects will be excluded in this pilot study but they will be informed to participate in our future study which will enroll both GSTM1 positive and null genotypes. Those subjects with GSTM1 positive will not be asked to continue with the study and will be compensated for their time. Since the main study seeks to enroll equal numbers of people that are GSTM1 positive and null, it is likely that as the study nears completion, we will still be seeking people with the null allele but not with the sufficient allele. If that is the case, those subjects will not be asked to continue with the study and will be compensated for their time.

- ***Food frequency questionnaire in the main study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the university of south carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the genetic screening will be scheduled for a physical examination in the HSF. Those subjects who are genetically screened but are not eligible for the pilot study, may be included in the main study if they are interested in participating. During this visit, subjects will sign an informed consent for a physical and the medical history form completed that contains information on general personal and family medical history. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam and genetic screening will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the

training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the main study:*** Subjects will be given an approximately 15 days supply of either fish oil or olive oil supplements randomly assigned. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of energy and nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record per week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 15 day of supplementation period. We will give the subjects another 15 days supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

- ***Baseline HRV measurement*:** In the pilot and main study, subjects will have their baseline 24-hour heart rate viability (HRV) measured by Holter monitor on the training day. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Subjects will be released while wearing the Holter monitor and return to the HSF the next day to get the Holter monitor removed.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (blood pressure, HRV, endothelial cell function by brachial artery ultrasound (BAU) and possibly by Endo-PAT, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement in both pilot and main studies*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined

- ***Brachial artery ultrasound in both the pilot and main studies:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Peripheral endothelial function by Endo-PAT in both pilot and main studies***: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one

arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be induced by sudden cuff deflation. The PWA recordings will be measured continuously during suprasystolic occlusion and deflation reactive hyperemia. PAT measurement will be displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).

- ***Pulmonary function in both pilot and main studies*** will be measured by spirometry.
- ***Blood sampling in both pilot and main studies***: approximately 50 ml of blood samples will then be collected in the pilot study and approximately 80 ml of blood samples will then be collected in the main study.
- ***Urine sampling in both the pilot and main studies:*** approximately 30 ml of urine samples will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

In the pilot study: Subjects will then enter the diesel exposure chamber for 3 sequentially exposures at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours each with a about 2-week interval between exposures. In the main study: Subjects will have only one exposure at an optimum concentration of diesel exhaust that will be identified from the pilot study.

Exposure: All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. The diesel exhaust will be generated from a 6-cylinder, 205 hp, 5.9 L displacement diesel engine (Cummins, Columbus IN) mounted on a vehicle located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean filtered and humidified air by approximately 1/30th to give a chamber concentration of approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$. The monitoring analyzer registers a concentration greater than 100 ug/m^3 above the target concentration as the exposure limit. Specifically, exposures will be terminated at values $\geq$200, 300 or 400 during runs in which the exposure targets are 100, 200 and 300 ug/m^3, respectively. The running sum of the 2-minute average concentrations displayed by the monitoring analyzer during the 2 hr exposure period exceeds 18,000 ug/m^3. This safety limit is specifically designed to address exposures for which the target concentration is 300 ug/m^3. It will prevent an inhalational exposure greater than 600 ug, which assumes that the resting subject will inhale 2 m^3 during the 120 min exposure. It is expected that 75 to 95% of the diesel exhaust PM from this type of engine will be centered around approximately 0.1-0.2 μm. Preliminary testing has shown the mean particle size to be approximately 0.14 μm. Levels of carbon monoxide, and oxides of nitrogen (NOx; mainly NO, and some NO2), will be kept under 10 ppm CO, 15 ppm NO, and 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) for these substances are: carbon monoxide=50 ppm; NO2=5 ppm; NO=25 ppm; The American Conference of Governmental Industrial Hygienists (ACGIH) 8 hr threshold limit values (TLVs) are: NO=25 ppm, NO2=3 ppm, and CO=25 ppm]. Diesel fuel used for the study will be purchased as a certified fuel (Chevron Phillips Chemical Co., Borger TX, 0.05 LS Certification Fuel, type II). This certified fuel represents diesel fuel composition that is typically available at gas stations for most regions of the country except for California. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured with a tapered element oscillating microbalance (TEOM) and/or a Data-RAM, and chamber PM concentrations

during exposure further validated by weighing filters obtained from versatile air pollution samplers (VAPS) impacters. Particle size distribution will be determined by scanning mobility particle size (SMPS) not during the subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. The minute ventilation during exposure will be measured. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 50 ml of blood and 30 ml of urine samples will be collected in the pilot study and approximately 80 ml of blood will be collected in the main study. Approximately 30 ml of urine samples will be collected in both the pilot and main studies.

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 50 ml of blood will be collected in the pilot study and approximately 80 ml of blood will be taken in the main. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound and finger arteries Endo-PAT). Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood (about 50 ml in the pilot study about 80 ml in the main study) will be drawn by standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following:

omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In the main study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to a diesel exhaust and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of diesel exhaust on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single

inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound and finger arteries Endo-PAT:** There are no known risks associated with imaging of the brachial artery and index finger arteries. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Diesel exposure** The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban areas. Diesel particulate concentrations proposed for the pilot study will be 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m3$ and the optimum concentration will be used for the main study. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the

exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. During exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have EKG leads attached during the exposure to monitor cardiac function during exercise. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for their participation up to that point. The investigators or duty physician will end the exposure if the subject is found to be suffering from any significant adverse effect. The small pilot study will allow us to monitor the adverse effects of diesel exhaust closely and we will determine whether we will go to the next higher concentration based on the analysis. We will be able to identify a safe concentration of diesel exhaust that induces a measurable change in our study endpoints. No major adverse health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

- **Confidentiality** Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her

personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test our hypothesis that diesel exhaust causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust. We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to diesel will also be measured (e.g., blood pressure, HRV, brachial arterial blood flow rate, change in blood coagulation factors, platelets, fibrinogen, c-reactive protein, lactate, plasminogen activator inhibitor, ferretin, and α1-antitrypsin). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 was derived using a power of 0.8 and an α of 0.05.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to diesel exhaust compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial blood flow rate, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial blood flow rate. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of diesel exhaust on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _X_ Names
b. _X_ Telephone numbers
c. _X_ Any elements of dates (other than year) for dates directly related to an

individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older

d. _X_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code

e. __ Fax numbers

f. _X_ Electronic mail addresses

g. __ Social security numbers

h. _X_ Medical record numbers

i. __ Health plan beneficiary numbers

j. __ Account numbers

k. __ Certificate/license numbers

l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers

m. __ Device identifiers and serial numbers (e.g., implanted medical device)

n. __ Web universal resource locators (URLs)

o. __ Internet protocol (IP) address numbers

p. __ Biometric identifiers, including finger and voice prints

q. __ Full face photographic images and any comparable images

r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data.** Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _X_ no

A.4.11. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

_X_ No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_X_ Secure network _X_ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_X_ Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). ___ yes ___ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). ___ yes ___ no
Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the pilot study will be approximately 3 months and the main study will take approximately 18 months. Participant recruitment and screening is expected to be continuous throughout the study until the intended number of participants is reached.

If the subject is eligible for the pilot study they will have 9 visits to the HSF over approximately 18-20 weeks. Subjects will have about 2 weeks break between 2 exposures. If the subject is eligible for the main study of diesel exhaust exposure the individual will have 5 visits to the research facility over approximately 5-6 weeks. The pre-enrollment genotyping and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after genotyping of the blood sample. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment for the main study) will require approximately 3 hours. Exposure day will last approximately 8 hours for the pilot and main studies. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours for the pilot and the main study.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $40.00 at one time or cumulatively more than $200.00 per year, collection of subjects' Social Security Number is required (University policy) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be paid for the entire study, excluding completion bonus.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled, and will be paid 50% up to a total maximum of $100 for canceled procedures. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study.

1. If following genotyping of the blood samples on screening day, it is determined that the individual's genotype is not needed for completion of the study, then that subject may be informed that his/her participation in this study is no longer necessary. The subject will be notified by phone and the total compensation for this study will be approximately $60.
2. If the subject is qualified and finished the entire pilot study, the total compensation for completion of this study will be approximate $2053.
3. If the subject is qualified and finished the entire main study, the total compensation for completion of this study will be approximate $1195.

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml of pilot and ~80ml of main study; genotyping)	$30

Pre-study qualification total = $60

Subjects based on their genotype assessment may not be asked to further participate in the study.

Training day (3 hours)

Time (3h @$12/h)	$36
Holter monitor	$100

Pilot study:

Exposure (8 hours)

Venipuncture (~50ml, pre; 3@$30 each)	$90
Holter monitor (3 @$100 each)	$300
Chamber exposure (3 @$72 each)	$216
Venipuncture (~50ml, post; 3@$30 each)	$90
Time (3X8h @$12/h)	$228
Finger artery Endo-PAT (6 @$10 each)	$60
Brachial artery ultrasound (6@$50 each)	$300
On-time bonus (3@$25 each)	$75
Urine samplings (6 @$10 each)	$60

Total for completion of exposure = $1579

Eighteen hours after exposure (3 hour)

Venipuncture (~50ml; 3@$30 each)	$90
Time (3X3h @$12/h)	$108
Finger artery Endo-PAT (3 @$10 each)	$30

Total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each)	$150

Approximate TOTAL for completion of pilot study = $2053

Main study:

Exposure (8 hours)

Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Finger artery Endo-PAT (2 @$10 each)	$20
Food records (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $823

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Finger artery Endo-PAT	$10

Total for completion of post-exposure = $76

Protocol Completion Bonus $100

Approximate TOTAL for completion of main study = $1195

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Data, Records or Human Biological Specimens without Direct Contact with Subjects

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do all of these data records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

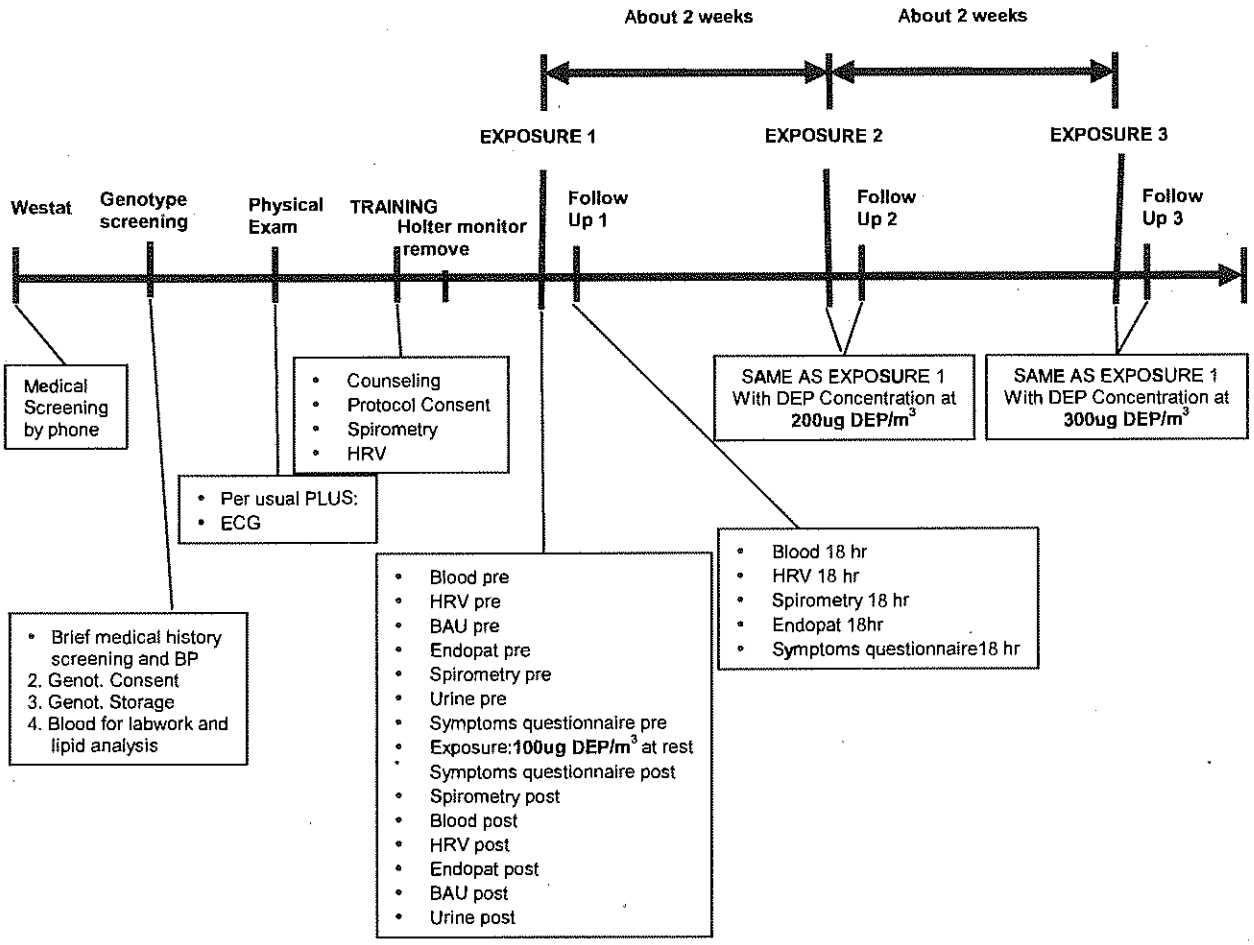
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

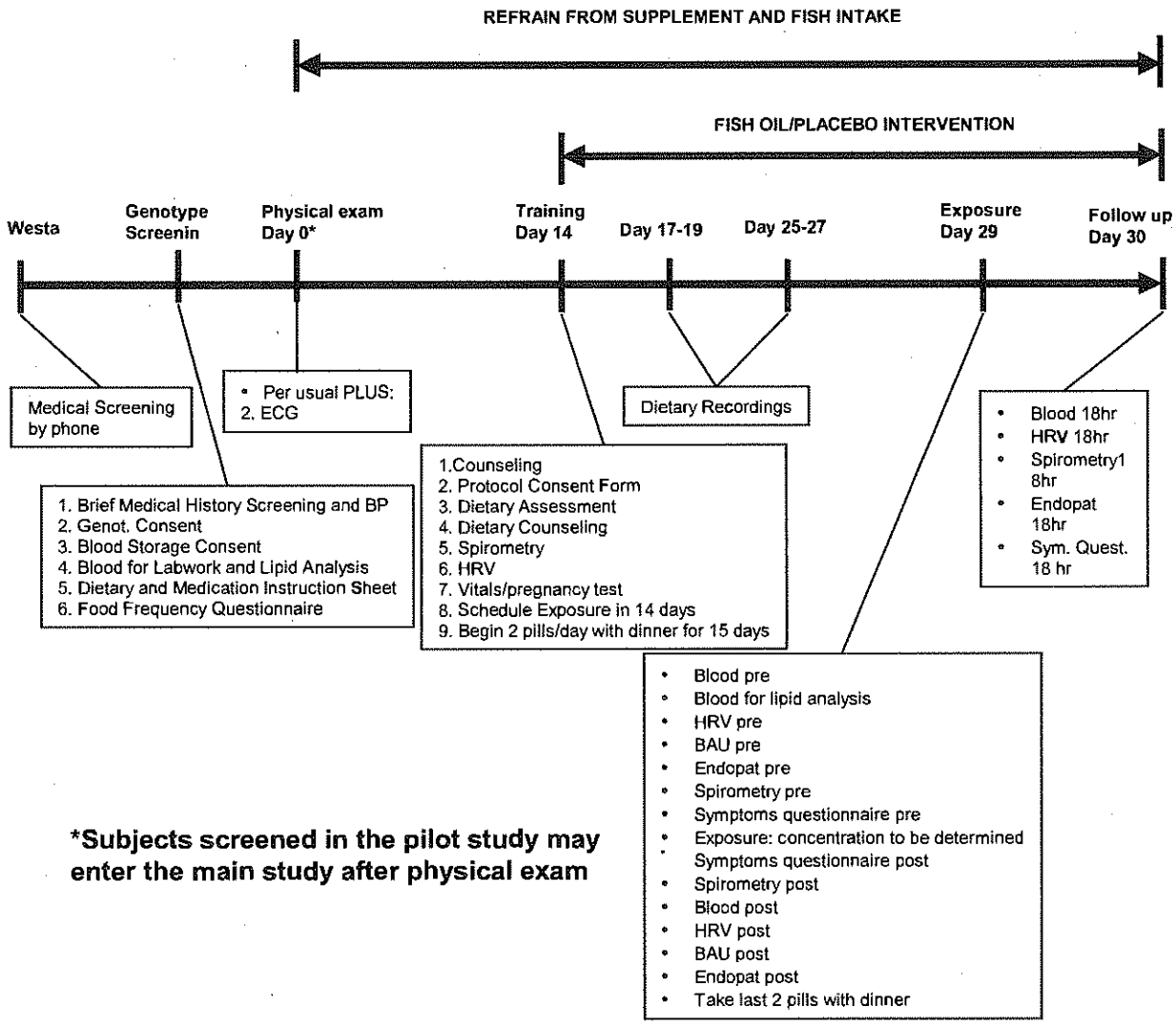
INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	**NONE**	**TRACE**	**MILD**	**MODERATE**	**SEVERE**
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



PILOT PHASE FLOW DIAGRAM



MAIN STUDY FLOW DIAGRAM

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 3/05/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: GCRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal.

Submission Description:

This modification, dated February 25, 2008 allows researchers to recruit 6 additional subjects as stand by subjects to replace those who may drop out. If not enrolled, they will be given $50 as compensation.

Investigator's Responsibilities

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (GCRC), Non-IRB Review Contact

RECEIVED
FEB 25 2008
IRB UNC-CH

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 19-Feb-2008b

RECEIVED
3/5/08
G clc

RECEIVED
MAR 05 2008
IRB UNC-CH

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
☒	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
☒	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
☐	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
☐	4. The sponsor's document describing the amendment, if any.	1
☐	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 **List and describe each proposed change:** We are requesting permission to recruit a maximum of six (6) pre-qualified subjects at the screening stage to replace enrolled subjects that drop out or are otherwise disqualified from completing the pilot phase of this study. In addition to the standard reimbursement for screening procedures, these pre-qualified subjects will be compensated $50.00 for standing-by until the end of the pilot phase in the event that they are not called to participate. If called to participate, a subject would enroll in the usual manner.

2. **Is this modification being submitted in response to an unanticipated problem or adverse event?** ___yes _Xno

3. **Do any of the proposed changes increase risk?** ___yes X_no If yes, explain.

IRB study #: 07-0190 GCRC 2579 **Date:** February 25, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Signature of Principal Investigator or designee — Date: February 25, 2008

Signature of Faculty Advisor (if applicable) — Date: February 25, 2008

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 3/5/08 AND 2/9/09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information--PILOT STUDY **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 25, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 50 ml of blood for genotyping and blood analysis. If you are re-contacted from the previous study, you will not need genotype screening but you will need to get blood drawn for other blood analysis. At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office.

How will the blood sample be collected?
You will have about 50 ml (about 3 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should

Initial/Date _____

not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical staff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these

Initial/Date _____

specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB

Initial/Date _____

7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

ADDENDUM

Recruitment and Stand-By Status of Pre-Qualified Subjects During Pilot Study

- You are asked to participate as a Pre-Qualified subject in this study. Participation as a Pre-Qualified subject is designed to provide a mechanism for faster completion of this study.
- In order to become a Pre-Qualified subject, you will undergo the genetic screening procedure(s) described in this Consent Form but you will not proceed to the Pilot phase unless you are needed.
- You might be called to proceed to the Pilot phase if one of the subjects already enrolled in the study drops out, is disqualified, or for some other reason.
- Once you have been Pre-Qualifed you will be in a Stand-By status for the duration of the Pilot phase of this study. This could mean a period of several months.
- While on Stand-By status you will not be asked to do anything in conjunction with this study. You will be called only if you are needed.
- If you are not called to participate in the Pilot study, upon completion of the Pilot phase of the study, you will receive a payment of $50.00 for being on the Stand-By list.
- If you are called to participate in the Pilot phase, you will enter the study as described earlier in this consent form.

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

OMEGA PILOT STUDY
IRB # 07-0190 GCRC #2579

APPROVED - IRB, UNC-CH
MAR 0 5 2008

Purpose:

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will be used to determine the type of particular genes you carry. The genes, called glutathione-S-transferase, are responsible for protecting your body against oxidants such as diesel exhaust, and some recent studies have shown that people carrying an inactive form of one of these genes and a variance of another may be more susceptible to the effects of air pollutants. Therefore, in this pilot research we will study the responses to diesel exhaust in healthy older subjects with very specific genetic characteristics.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $2053 if all study visits are completed.

Procedures:

Volunteers initially undergo a genetic screening visit and blood work, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genetic testing visit and $30 for the additional medical screening. If it is determined that your genotype is not needed for this study, no further visits will be scheduled for the pilot study, but you may be eligible for the full study at a later time. If it is determined that you are eligible for this study but the study is full, you may be asked to remain pre-qualified for the study but on stand-by status in the event that another participant does not finish. Pre-qualified volunteers who are willing to be on the stand-by list but are not called to participate will be paid $50 at the end of the pilot study

If you are eligible for the remainder of the pilot study you will have up to 8 visits over approximately 18 to 20 weeks. The goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in the future study. Results will be analyzed after finishing each proposed exposure to decide whether to continue on to the next level of proposed exposure. You may not have all these 3 exposures if this pilot study is terminated at some point based on interim results.

Your participation in this pilot study will include one training session for about 3 hours with a brief follow up visit the following day, up to 3 exposure sessions, each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours. Exposures are separated by about 2 weeks.

Study procedures will include breathing tests, heart rate monitoring, blood draws, and exposures to controlled amounts of diesel exhaust. Even at the highest level of exposure, you will not be exposed to more particles than you might encounter if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure session.

Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In order to participate you must be willing to observe several important restrictions. They are:

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to the exposure and post-exposure visits.
- Due to the overnight heart monitor on training and exposure visits must be willing to refrain from bathing between exposures and follow-ups.

Would you be able to comply with these restrictions? If so, we can schedule you for a visit to complete the initial part of the screening.

Study Schedule

Genotyping visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (about 1 hour)

Physical Exam: days and times TBD (2 hours)

Training: days/times TBD (3 hours)
Follow-up day: approximately 21 hours after Training

Exposure Day 1: 9:00 AM days TBD (8 hours)
Follow-up 1: 18 hours after Exposure 1 (3 hours)

Exposure Day 2: 9:00 AM days TBD, about 2 weeks after Exposure 1 (8 hours)
Follow-up 2: 18 hours after Exposure 2 (3 hours)

Exposure Day 3: 9:00 AM days TBD, about 2 weeks after Exposure 2 (8 hours)
Follow-up 3: 18 hours after Exposure 3 (3 hours)

First Review of IRB Submission
Modification

Training Not Met (Haiyan Tong)

Receipt Date : 3/05/2008 Previous Review Type: **Renewal Full Board** IRB ID : 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

PI: Haiyan Tong IRB: Biomedical

☐ Not-HSR ☐ Exempt (Category:) ☐ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Sonya H Bateman

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):
- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 3/5/08 HB)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached: 1

Other attachments: 01

FINAL ACTIONS:
- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information--PILOT STUDY **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: February 25, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
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Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
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What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 50 ml of blood for genotyping and blood analysis. If you are re-contacted from the previous study, you will not need genotype screening but you will need to get blood drawn for other blood analysis. At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office.

How will the blood sample be collected?
You will have about 50 ml (about 3 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?
You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should

Initial/Date _____

not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers. You need to be non-smoker for at least 1 year prior to the study or smoked only little earlier in life. The investigators and medical staff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these

Initial/Date _____

specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB

Initial/Date _____

7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

ADDENDUM

Recruitment and Stand-By Status of Pre-Qualified Subjects During Pilot Study

- You are asked to participate as a Pre-Qualified subject in this study. Participation as a Pre-Qualified subject is designed to provide a mechanism for faster completion of this study.
- In order to become a Pre-Qualified subject, you will undergo the genetic screening procedure(s) described in this Consent Form but you will not proceed to the Pilot phase unless you are needed.
- You might be called to proceed to the Pilot phase if one of the subjects already enrolled in the study drops out, is disqualified, or for some other reason.
- Once you have been Pre-Qualifed you will be in a Stand-By status for the duration of the Pilot phase of this study. This could mean a period of several months.
- While on Stand-By status you will not be asked to do anything in conjunction with this study. You will be called only if you are needed.
- If you are not called to participate in the Pilot study, upon completion of the Pilot phase of the study, you will receive a payment of $50.00 for being on the Stand-By list.
- If you are called to participate in the Pilot phase, you will enter the study as described earlier in this consent form.

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_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 4/25/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: GCRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal.

Submission Description:

This modification, dated April 24, 2008 adds omega-3 and antioxidant supplements to the exclusion criteria. Dietary changes consistent with these new exclusion criteria have also been made.

Investigator's Responsibilities:

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 4-25-08 AND 2-9-09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

3

Biomedical Form—PILOT STUDY

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: April 24, 2008

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Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are a smoker or have a smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before the exposure.

Initial/Date _____

- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before the exposure.
- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

We will collect your baseline heart rate viability (HRV) by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of

Initial/Date _____

your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. We will analyze the results after everyone finishing the 200 $\mu g/m^3$ exposure and decide whether we will continue on the 300 $\mu g/m^3$ exposure. If we decided to continue, you will have the third exposure at approximately 300 $\mu g/m^3$ for 2 hours that will occur about 2 weeks later. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording

Initial/Date _____

devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 3 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 ml or about 3 1/3 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.

Initial/Date _____

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 ml or about 3 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort, eye irritation, and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. You will wear protective goggles during the exposure time to reduce the eye irritation. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular

Initial/Date _____

diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Initial/Date _____

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents

Initial/Date _____

from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $2053.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90

Initial/Date _____

Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90
Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75

Total for completion of exposure = $1479

18 hours after exposure (3 hour)

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $2053

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up

Initial/Date _____

care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

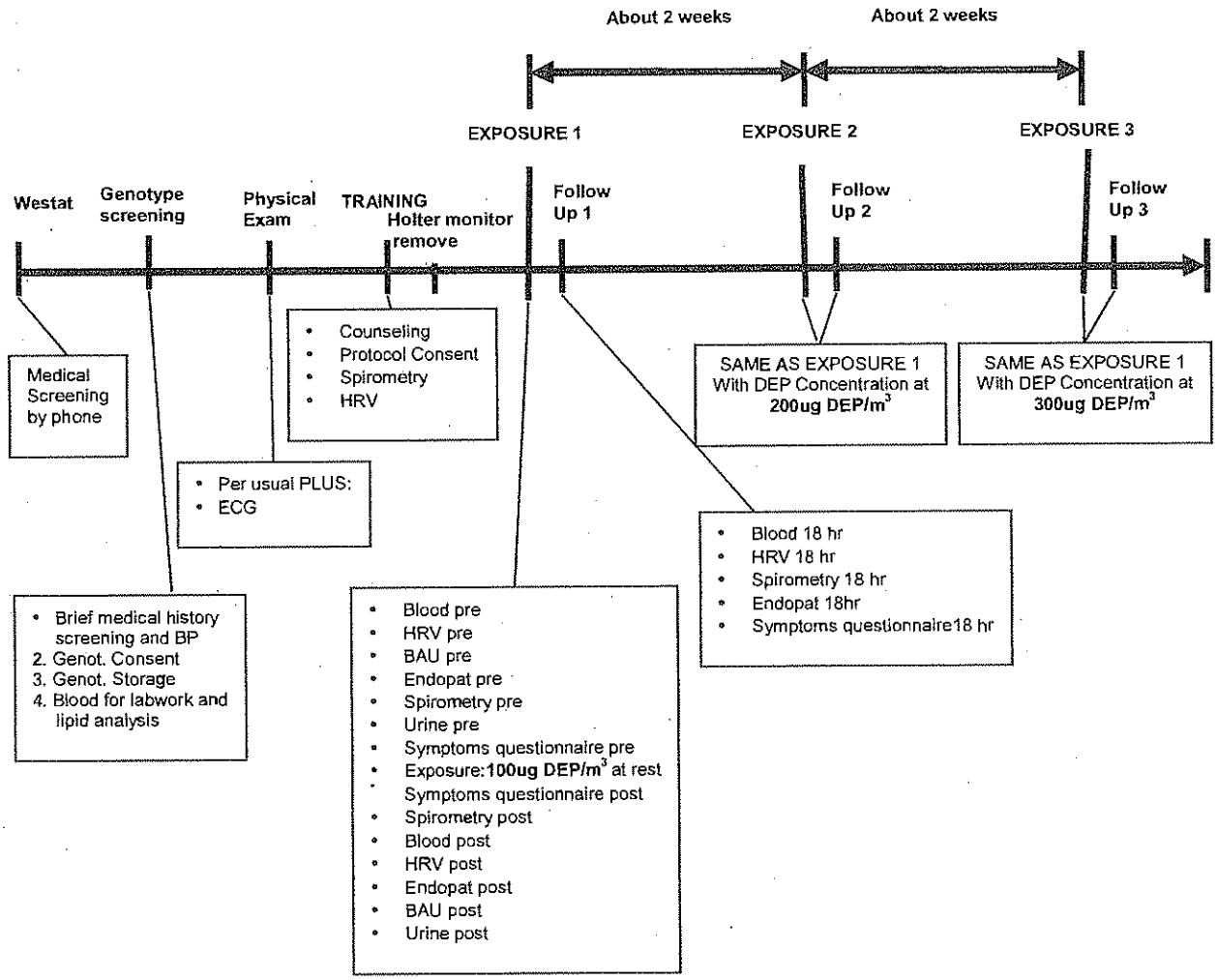
_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

First Review of IRB Submission
Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Tracey Montilla, David Demarini, Martin Case, Wayne Cascio, Mary Bassett)

Receipt Date : 4/24/2008 Expiration Date : Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☐ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Wyomie Milliken

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 4/25/08 HP)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- Consent forms attached: 1
- Other attachments: 0

FINAL ACTIONS:

- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 19-Feb-2008b

07-0190
RECEIVED
4/24/08
GCRC

RECEIVED
APR 24 2008
ONE HRPO

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change: (all the changes are highlighted)

I. Harmonization of exclusion criteria in the Pilot and Main Studies.

In order to control potential confounding effects of dietary supplementation use in the pilot phase, we are requesting an amendment to harmonize restrictions in the pilot phase with those already approved in the main study.

1. Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before the exposure. Accordingly, we have added the omega-3 fatty acids in the list of exclusion criteria on page 2 in the consent form.

2. Subjects will be required to avoid taking antioxidant supplements (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before the exposure. Accordingly, we have added this requirement in the list of exclusion criteria on page 3 in the consent form.

II. Steps to avoid eye discomfort during diesel exposures.

Two subjects have reported "moderate" eye irritation following diesel exhaust exposure. The symptom score was 3 to 4. In both instances, eye irritation reportedly resolved within in a few hours after the exposure. In order to prevent or minimize subject discomfort during the exposure and the period following exposure, subjects will be provided with chemical safety goggles to be worn during all diesel exposures. Goggles with indirect venting have been selected for maximum comfort for eyeglass wearers. Each subject will receive his/her own pair to wear. The modification has been added on page 7.

2. **Is this modification being submitted in response to an unanticipated problem or adverse event?** ___yes _X__no

3. **Do any of the proposed changes increase risk?** ___yes _X__no If yes, explain.

IRB study #: 07-0190_____ GCRC #: 2579 **Date:** April 24, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James M Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

H Tong 4/24/08

Signature of Principal Investigator or designee Date

4-24-08

Signature of Faculty Advisor (if applicable) Date

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 7/03/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: GCRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment 6/27/08 addresses changes to the pilot consent & genotyping consent forms:

1. the new exclusion criteria for the smoking history defined as: subjects are currently smoking or have a smoking history within 1 year of the study or have a greater than/equal to a 5 pack year smoking history

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, Environmentally Protection Agency; Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 7/3/08 AND 2/9/09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotype Screening With Identifying Information--PILOT STUDY **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: June 27, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants such as ozone and diesel exhaust. Another gene called GSTP1 gene is also associated with a significant risk to the effects of air pollutants. The purpose of this pilot study is to look at the cardiovascular and respiratory effects of diesel exhaust in older subjects who do not carry GSTM1 and have different GSTP1 genotypes (Ile/Ile, Ile/Val, and Val/Val). The purpose of this screening session is to determine whether you carry a GSTM1 gene and your GSTP1 genotypes in order for us to make selection.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 6 participants who will complete in this pilot study.

How long will your participation last?
Participation in this genetic screening session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 50 ml of blood for genotyping and blood analysis. If you are re-contacted from the previous study, you will not need genotype screening but you will need to get blood drawn for other blood analysis. At the end, we will give you a copy of the Medical History Form and please hold the form until you hear from us that you are qualified for the study, and then fill it out and mail it back to the Westat recruitment office.

How will the blood sample be collected?
You will have about 50 ml (about 3 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
The blood sample collected will be used to look at the GSTM1 gene and GSTP1 genotypes. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?

- You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you

Initial/Date _____

should not have chronic diseases including active allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are currently taking β-blockers. You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history. The investigators and medical staff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 and GSTP1 genes and this information will determine if you will be qualified for the further diesel exposure study.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting and infection is minimized by the use of sterile technique. If you do have signs of infection ant the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this screening procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law.

Who owns the blood samples?

Initial/Date _____

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?

You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?

By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?

Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?

If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board,

Initial/Date _____

University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury form having your specimen collected. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. **Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care.** If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 7/3/08 AND 2/9/09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY

3

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: June 27, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
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Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

<u>What are some general things you should know about research studies?</u>
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before the exposure.

Initial/Date _____

- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before the exposure.
- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

We will collect your baseline heart rate viability (HRV) by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of

Initial/Date _____

your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. We will analyze the results after everyone finishing the 200 $\mu g/m^3$ exposure and decide whether we will continue on the 300 $\mu g/m^3$ exposure. If we decided to continue, you will have the third exposure at approximately 300 $\mu g/m^3$ for 2 hours that will occur about 2 weeks later. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. You may not have all these 3 exposures if we determine to terminate this pilot study at some point based on interim results. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording

Initial/Date _____

devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 3 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 ml or about 3 1/3 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.

Initial/Date _____

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 ml or about 3 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort, eye irritation, and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. You will wear protective goggles during the exposure time to reduce the eye irritation. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular

Initial/Date _____

diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Initial/Date _____

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents

Initial/Date _____

from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $2053.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total =	$60
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90

Initial/Date _____

Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90
Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75

Total for completion of exposure = $1479

18 hours after exposure (3 hour)

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $2053

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up

Initial/Date _____

care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

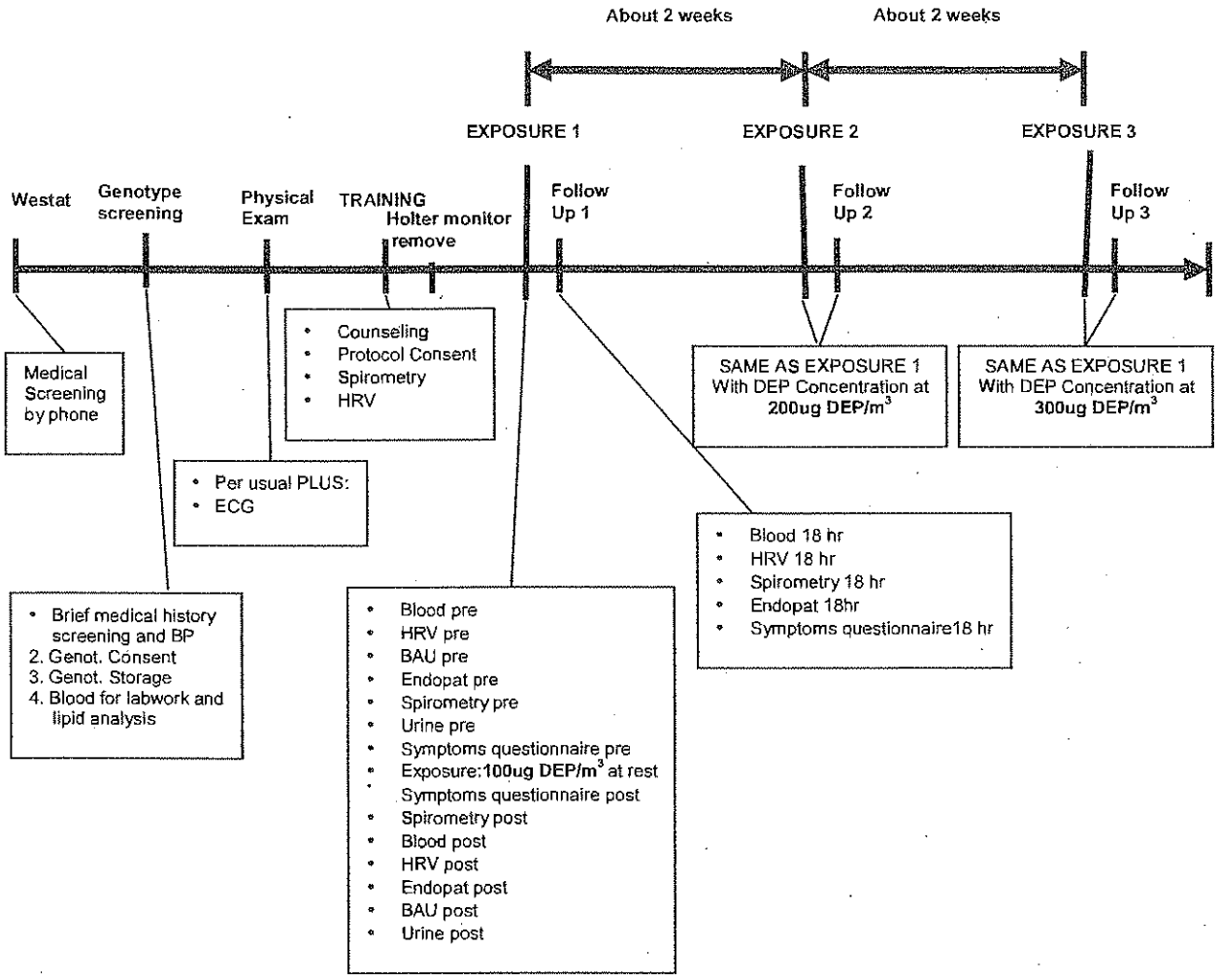
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

First Review of IRB Submission
Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Tracey Montilla, David Demarini, Martin Case, Wayne Cascio, Mary Bassett)

Receipt Date : 7/03/2008 **Expiration Date :** 2/09/2009 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Laura O Curry

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):
- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter: for 7/3/08
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☐ Hard copy sent

Consent forms attached: 3
Other attachments: 0

FINAL ACTIONS:
- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

RECEIVED
JUL 03 2008
IRB UNC-CH

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

Modify the exclusion criteria of subjects smoking history in the study. The changes are highlighted in the pilot consent and genotyping consent forms.

The new exclusion criteria for the smoking history defined as: subjects are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: June 27, 2008**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James M Samet, PhD (if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:

Investigator Brochure version #:

Any other details you need documented on IRB approval:

Version date:

Signature of Principal Investigator or designee

Date: 6/27/08

Signature of Faculty Advisor (if applicable)

Date

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 7/11/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: GCRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:

This modification, dated July 8, 2008, allows researchers to start the third exposure before all 6 participants have completed the second exposure. No effects were observed from the first two exposures.

Investigator's Responsibilities:

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 7/11/08 AND 2/09/09 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form—PILOT STUDY

3

IRB Study #__07-0190_____ GCRC #: 2579
Consent Form Version Date: July 8, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH;
Co-PI: Robert Devlin, PhD.
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-6255
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor:
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-0665 (James M Samet)
Study Contact email: samet.james@epa.gov

<u>What are some general things you should know about research studies?</u>
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this pilot study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

You are being asked to be in this pilot study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this pilot study if...

- You have a history of chest pain, irregular heart beats, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of lung disease and/or active allergy including: hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer.
- You are currently taking estrogen replacement therapy.
- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.

You should **NOT** participate if you are unable to comply with the following requirements:

- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before the exposure.

Initial/Date _____

- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before the exposure.
- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to the exposure and post-exposure visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to the exposure and post-exposure visits.

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 6 people who will complete this pilot research study.

How long will your participation in this study last?
You will have up to 7 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached pilot study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 3 exposure sessions each of which will last approximately 8 hours, and another 3 sessions which will occur 18 hours after each exposure and last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?
You should have already undergone a genetic screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

We will collect your baseline heart rate viability (HRV) by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of

Initial/Date _____

your chest where these leads will be placed. The leads will be connected to a monitor (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. The monitor will be kept on you for about 24 hours until you return the next day to get the monitor removed.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will be scheduled for your first exposure at diesel exhaust concentration of approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. You will have the third exposure at approximately 300 $\mu g/m^3$ for 2 hours that will occur about 2 weeks later. You may terminate your participation from this study at any time. Our goal of this pilot study is to find an optimal concentration of diesel exhaust to be used in a future study. In this pilot study, you will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, difficult to breathe, light-headness, pale skin color, unstable step, and significant irregular heart beats. In addition, analyses of blood samples taken after exposure will be monitored for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Exposure day

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. Please report any pollutant exposure to the study personnel so you can be rescheduled if necessary. You will be rescheduled if you have experienced a respiratory tract illness within the past 4 weeks or any other illness within the past week. You should not eat pan fried or grilled meat before midnight of the exposure day (we will provide you with nutrition guidelines).

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. You will need to wear comfortable clothes and shoes.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your baseline heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day.

Initial/Date _____

You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if diesel exhaust causes small changes in the ability of your nervous system to regulate how your heart beats.

- Have about 50 ml blood drawn (about 3 1/3 tablespoons). We will test this blood to see if diesel exhaust affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT). We will place two small PAT probes to both of your index fingers and a tourniquet on your arm, much like a cuff used to measure blood pressure. First, you will be asked to rest quietly for 10 minutes, and then we will obtain recordings for 5 minutes at rest. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. We will obtain 5 minutes recording during the blood pressure cuff inflated. Another 5 minutes recording will be obtained after the blood pressure cuff is released.
- We will then take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have about 30 ml of urine sample.
- You will then enter the exposure chamber and be exposed to diesel exhaust.

During the exposure, you will:

Initial/Date _____

- Have an exposure to diesel exhaust at approximately 100 $\mu g/m^3$ for 2 hours in a small exposure chamber. About 2 weeks later you will have another diesel exposure at approximately 200 $\mu g/m^3$ for 2 hours. The third exposure at approximately 300 $\mu g/m^3$ for 2 hours will be about 2 weeks after the second exposure. This particle concentration is representative of diesel levels which you would inhale if you were occupationally exposed to diesel, such as being a truck driver, but less than sites in some mines that utilize diesel-generated power. You would also be exposed to a similar total amount over about 5 hr, 10 hr or 15 hr at a busy intersection in a polluted city (such as Los Angeles) where diesel concentrations have been measured at 22 $\mu g/m^3$.
- You will be occasionally asked to breathe into a mouthpiece so that your rate of breathing can be measured. A trained investigator will be seated outside the chamber to observe you at all times and a physician will be on site during the entire exposure session. During the exposure, your heart rhythm, blood pressure, and the amount of oxygen in your blood will be monitored. If it appears you are experiencing significant heart rhythm or breathing problems, or have headache, nausea or vomiting the exposure will be terminated immediately. In addition, you may elect to terminate the exposure at any time.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We may obtain another Endo-PAT measurement.
- Have blood drawn (about 50 ml or about 3 1/3 tablespoons).
- Have about 30 ml of urine sample.
- Fill out a symptom score questionnaire.
- Have another arm artery ultrasound at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You might have another Endo-PAT measurement.
- Have blood drawn (about 50 ml or about 3 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

Diesel exhaust exposure: Exposure to air pollution particles can cause cough, shortness of breath, chest discomfort, eye irritation, and headache. These symptoms typically last no more than a few hours, but could last longer if you are especially sensitive. You will wear protective goggles during the exposure time to reduce the eye irritation. There is a chance that exposure to particles can increase the likelihood that you will be more likely to come down with a respiratory infection within several days of the exposure. Diesel exhaust, even when diluted in this study, may have an unpleasant odor. Exposure to the air pollution particle concentrations used in this study for short periods of time has never been found to cause permanent health effects. However, some studies suggest that older people, particularly those with underlying cardiovascular diseases, are at increased risk for getting sick and even dying during episodes of high air pollution. At this time, no one understands exactly how these particles might cause people to become sick or die. While we can not exclude the possibility that you may have an adverse reaction to breathing theses exhausts, you will only be exposed to them for 2 hours, and you will

Initial/Date _____

not be exposed to more particles than you would be exposed to if you visited a large city such Los Angeles, New York, or Mexico City on a smoggy day.

You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound (BAU) and Finger Endo-PAT: There are no significant risks associated with imaging of the finger arteries, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Some risks and discomforts may be unforeseeable. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?

Initial/Date _____

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?

You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be required. In spite of all precautions, you might develop medical complications from participating in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company.

Neither the University of North Carolina at Chapel Hill nor the U.S. EPA has set aside funds to pay you for any such reactions or injuries, or for the related medical care. If you believe that you have suffered a research-related injury, you have the right to pursue legal remedy if you believe that your injury justifies such action. The Federal Tort Claims Act, 28 U.S.C. S 2671 et seq., provides for money damages against the United States when property loss or personal injury results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research-related injury occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

Initial/Date _____

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximately $2053.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point. If you are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons you will be paid for the entire study, excluding completion bonus.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e, equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml) (genotyping)	$30
Pre-study qualification total =	$60
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~50ml, pre; 3@$ 30 each)	$90
Holter monitor (3@$ 100 each)	$300
Urine sampling (~30 ml, pre, 3@$ 10 each)	$30
Chamber exposure (2 hours; 3@$ 72 each)	$216
Venipuncture (~50ml, post; 3@$ 30 each)	$90

Initial/Date _____

Urine sampling (~30 ml, post, 3@$ 10 each)	$30
Finger Endo-PAT (6@$10 each)	$60
Brachial artery ultrasound (6@ $50 each)	$300
Time (3X8h @$12/h)	$288
On-time bonus (3@$25 each)	$75

Total for completion of exposure = $1479

18 hours after exposure (3 hour)

Venipuncture (~50ml; 3@$ 30 each)	$90
Time (3X3 hrs @$12/h)	$108
Finger Endo-PAT (3@$10 each)	$30

Day 3 total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each) $150

Approximate TOTAL for completion of the study = $2053

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason.. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

Initial/Date _____

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators:
James Samet, PhD 919-966-0665 Robert Devlin, PhD 919-966-6255

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

A Pilot Study to Identify the Optimum Diesel Exhaust Concentration to Investigate the Cardiovascular Effects in Healthy Older Subjects

Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

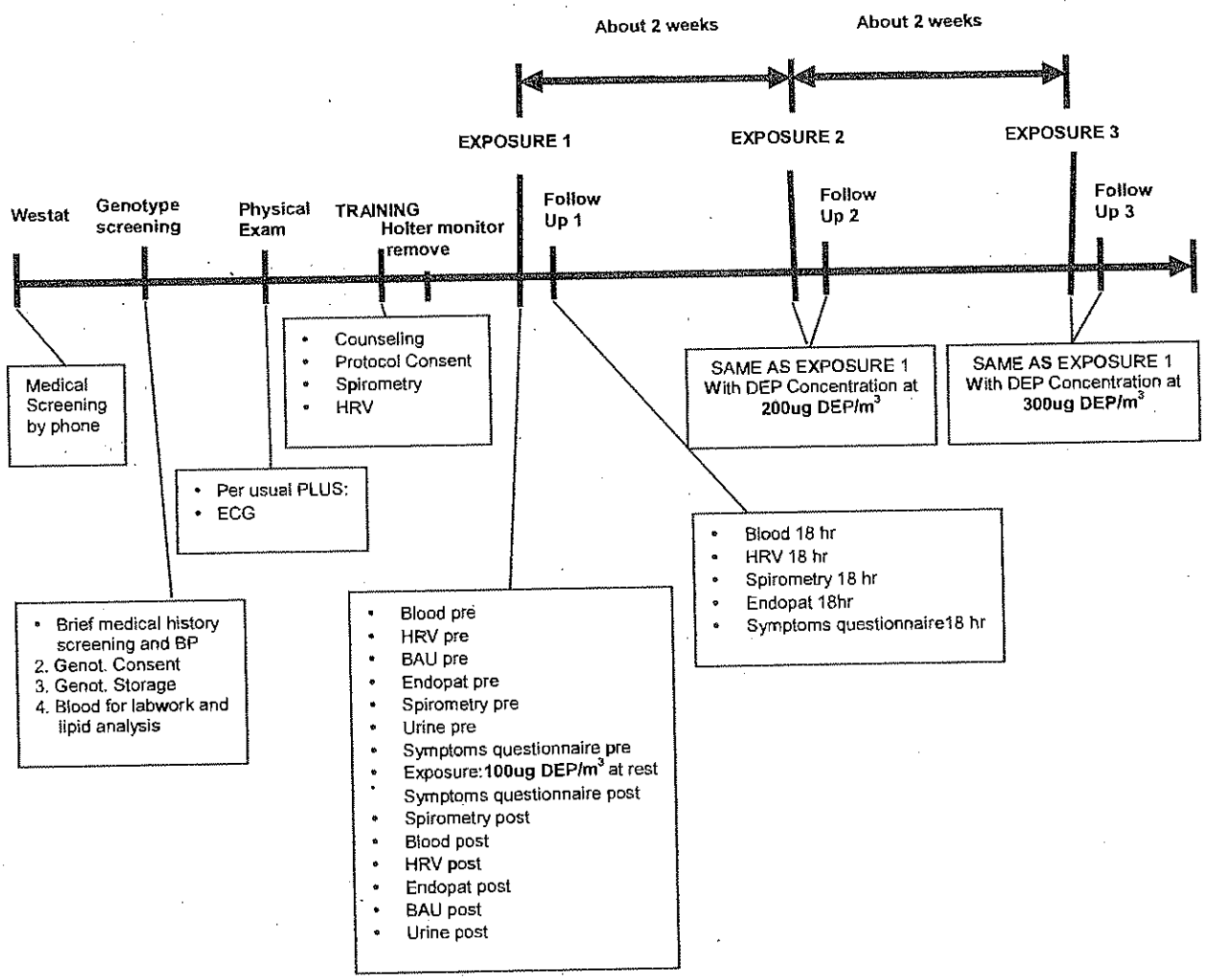
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



PILOT PHASE FLOW DIAGRAM

Initial/Date _____

First Review of IRB Submission Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Tracey Montilla, David Demarini, Martin Case, Wayne Cascio, Mary Bassett)

Receipt Date : 7/10/2008 **Expiration Date :** 2/09/2009 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☐ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Sonya H Bateman

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 7/11/08 HP)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☒ Hard copy sent

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

RECEIVED
7/8/08
CTRC

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

version 29-Apr-2008b

RECEIVED
JUL 10 2008
IRB UNC-CH

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

Modify the pilot exposure schedule in order to retain the subjects. The changes are highlighted in the pilot consent form.

The approved protocol specifies that all 6 subjects complete the second exposure before the third exposure is started. However, unexpected delay in the recruitment of the qualified number 6 subject now threaten the retention of the other 5 subjects who have finished their first and second exposures. No effects have been observed from these 5 subjects. Therefore, these subjects will now be scheduled for the third exposure while recruitment of number 6 continues.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: July 8, 2008**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James M Samet, PhD (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Hai Tong

Signature of Principal Investigator or designee

7/8/08

Date

Signature of Faculty Advisor (if applicable)

7-8-2008

Date

It sounds like they are planning to do this ~~[illegible]~~ ~~[illegible]~~ and they are letting us know does it read the same way to you?

This is fine. Herb

[illegible]

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 7/22/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: GCRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment dated 7/14/08 addresses the addition of modification of recruitment materials to be consistent with the changes to consent form approved 4/25/08

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (GCRC), Non-IRB Review Contact

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA PILOT STUDY**

DATE:

TIME:

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 7/22/08 AND 2/09/09
APPR
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[parking information provided here as available]

May be folded here for display

OMEGA Pilot Study Guidelines
For All Study Visits

Illness. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores.

No active allergies. If you have questions about allergies, please call the EPA medical station at 966-6232.

Medications

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before the exposure.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- No caffeine for 12 hours prior to the exposure and post-exposure visits.

Food

- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No omega-3 fatty acids or having more than one 4-6 oz. serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before the exposure.

Exercise. Clothing

- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the training session and follow up visit and between the exposure and follow-up visits.

If you are unable to keep your appointment, please call Recruitment. Please be on time!

Thank You! Westat EPA Support Services

Response to Minor Stipulations Modification

Receipt Date : 7/17/2008 Expiration Date : 2/09/2009 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Coordinator : Cynthia Wright

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Full Board Review ☑ Not Full Board Review

Study Description :

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description :

This amendment dated 7/14/08 addresses the addition of modification of recruitment materials to be consistent with the changes to consent form approved 4/25/08

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter: 7/22/08 for
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☑ Approved 7/22/08 for
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to Full IRB
- ☐ Return to sender
- ☐ Termination

First Review of IRB Submission
Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Tracey Montilla, David Demarini, Martin Case, Wayne Cascio, Mary Bassett)

Receipt Date : 7/17/2008 **Expiration Date :** 2/09/2009 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Wyomie Milliken

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
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  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☐ Approved
- ☒ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

To: IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

RECEIVED
JUL 22 2008
IRB UNC-CH

From: Haiyan Tong
Environment Protection Agency
CB# 7315
E-mail: tong.haiyan@epa.gov
Phone number: 966-4993

July 22, 2008

Study #: 07-0190
Other #: GCRC: 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Dear IRB review committee,

This memo is to respond the contingency following IRB review on July 17, 2008. Enclose please find the documentation of the completion of the CITI online human research ethics training certificate of Wayne Cascio.

Thank you!

Haiyan Tong

Haiyan Tong

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Date: 7/17/2008

RE: Contingencies to be addressed following IRB Review
Submission Type: Modification
Study #: 07-0190
Other #: GCRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been reviewed by the above IRB. This is not an IRB approval. You may not implement the research activities described in your submission until you have received a memo indicating final IRB approval. The IRB determined that this submission MAY BE APPROVED, pending stipulated change(s) and/or clarification(s) as detailed below.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the item(s) listed. **When you respond, please be sure to cite the IRB study number noted above, and indicate that you are responding to this memo as dated above; failure to link your response to this study, in general, and this memo, in particular, may result in delays.** Any additional changes (including new materials) must also be listed and discussed in the memo. If the application or any study materials must be revised, please submit 2 copies of all revised materials, one in which all changes are underlined and the other a "clean" copy with no underlining.

Study Specific Details:

- Please submit documentation of the completion of the CITI online human research ethics training course for [Wayne Cascio] or other IRB-approved training. The CITI course may be accessed at http://ohre.unc.edu/required education.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (GCRC), Non-IRB Review Contact

To: IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

RECEIVED
JUL 17 2008
IRB UNC-CH

RECEIVED
7/15/08
CTRC

From: Haiyan Tong
Environment Protection Agency
CB# 7315
E-mail: tong.haiyan@epa.gov
Phone number: 966-4993

July 14, 2008

Study #: 07-0190
Other #: GCRC: 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Dear IRB review committee,

This submission is to add the modification of recruitment material in consistent with the changes in the consent form which was approved by IRB on April 25, 2008.

The addition is now underlined in the IRB modification form.

Please let me know if you have any questions.

Thank you!

Haiyan Tong
Haiyan Tong

IRB

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 19-Feb-2008b

RECEIVED
JUL 17 2008
IRB UNC-CH

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change: (all the changes are highlighted)

I. Harmonization of exclusion criteria in the Pilot and Main Studies.

In order to control potential confounding effects of dietary supplementation use in the pilot phase, we are requesting an amendment to harmonize restrictions in the pilot phase with those already approved in the main study.

1. Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before the exposure. Accordingly, we have added the omega-3 fatty acids in the list of exclusion criteria on page 2 in the consent form.

2. Subjects will be required to avoid taking antioxidant supplements (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before the exposure. Accordingly, we have added this requirement in the list of exclusion criteria on page 3 in the consent form.

3. We have added these requirements in the recruitment material.

II. Steps to avoid eye discomfort during diesel exposures.

Two subjects have reported "moderate" eye irritation following diesel exhaust exposure. The symptom score was 3 to 4. In both instances, eye irritation reportedly resolved within in a few hours after the exposure. In order to prevent or minimize subject discomfort during the exposure and the period following exposure, subjects will be provided with chemical safety goggles to be worn during all diesel exposures. Goggles with indirect venting have been selected for maximum comfort for eyeglass wearers. Each subject will receive his/her own pair to wear. The modification has been added on page 7.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes _X__no

3. Do any of the proposed changes increase risk? ___yes _X__no If yes, explain.

IRB study #: 07-0190_____ GCRC #: 2579 **Date:** July 14, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James M Samet, PhD, MPH (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Haiyan Tong 7/14/08

Signature of Principal Investigator or designee Date

7-14-2008

Signature of Faculty Advisor (if applicable) Date

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
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https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 8/26/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: GCRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment dated 8/18/08 addresses:

1. exposure substance changed from diesel exhaust to concentrated fine & ultrafine air pollution particles
2. number of subjects reduced from 60 to 40
3. GSTM1 genotyping not selection criteria is not the selection criterion for the study
4. total payment for study is ~ $1225

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, EPA; Robert Truckner, (EPA), Wanda Simmons, (GCRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 8.26.08 AND 2.9.09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

3

Biomedical Form

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: August 14, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: James M Samet, PhD, MPH; Robert Devlin, PhD;; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Faculty Advisor: Divid Diaz-Sanchez, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Initial/Date _____

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).

Initial/Date _____

- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits and during the study. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Use olive oil for cooking, dressings, and sauces during the study.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 40 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 4-5 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) two-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a 15-day fish oil or olive oil supplementation and the exposure session. You will be given a 15-day supply of either fish oil or olive oil tablets. Please take 2 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next two weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this 15-day of supplementation period. We will give you another 15-day supply of either fish oil or olive oil tablets and you will need to restart the pills again.

We will perform a baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 24 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

Initial/Date _____

Exposure day
We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we do today).
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- We will bring you back to the HSF where we may also measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT) at HSF and we will compare the findings with ultrasound measurement of the artery in your arm. We will place two small PAT probes to both of your index fingers. The procedure is similar to that of ultrasound measurement and we will only place a blood pressure cuff on one of your arm. We will obtain recordings for 5 minutes at rest, 5 minutes during the blood pressure cuff inflated, and 5 minutes after the blood pressure cuff released.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to concentrated Chapel Hill air particles; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. Due to weather conditions, some chamber exposures will either be cancelled or terminated and you will be asked to re-schedule. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation. We will check your resting breathing rate during exposure.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will obtain another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to

Initial/Date _____

respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induces an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Finger Endo-PAT: Similar to the measurement by brachial artery ultrasound, there are no significant risks associated with index fingers arteries measurement.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this

Initial/Date _____

consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1225.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100

Initial/Date _____

Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Brachial artery ultrasound (2@ $50 each)	$100
Finger Endo-PAT	$40
Time (8h @$12/h)	$96
Food record (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $843

18 hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3 hrs @$12/h)	$36
Finger Endo-PAT	$20

Day 3 total for completion of post-exposure = $86

Protocol Completion Bonus	$100

Approximate TOTAL for completion of the study = $1225

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening

Initial/Date _____

history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

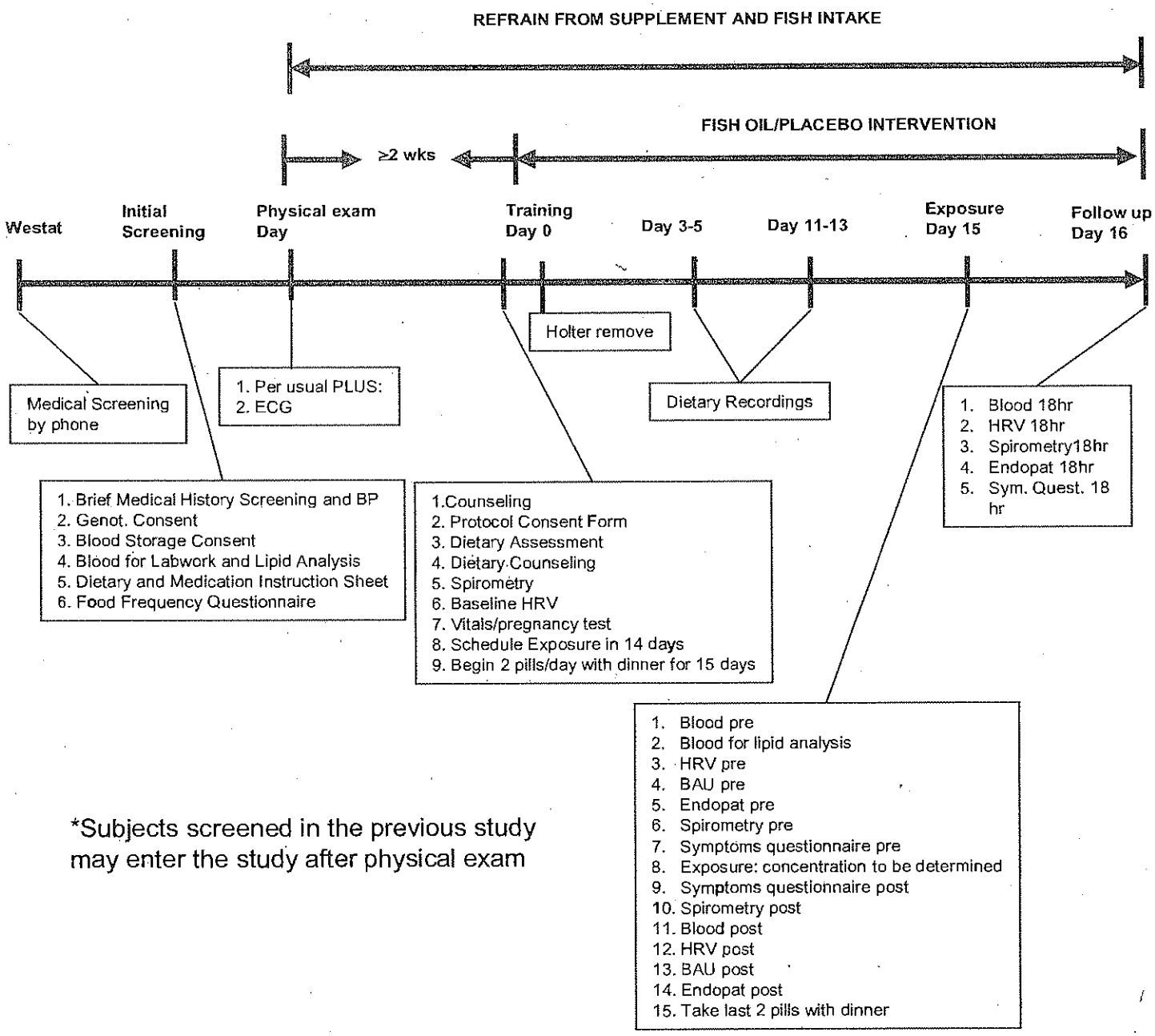
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



*Subjects screened in the previous study may enter the study after physical exam

STUDY FLOW DIAGRAM

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 8-26-08 AND 2-9-09 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotyping <u>With</u> Identifying Information 1

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: August 14, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: James M Samet, PhD; Robert Devlin, PhD;; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: David Diaz-Sanchez, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

<u>What are some general things you should know about this research study?</u>
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to air pollution . The purpose of the genotyping is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 40 participants in this study.

How long will your participation last?
Participation in this genetic session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

At the end, we will give you a copy of the Medical History Form and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and you will be instructed to mail the FFQ. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?

- You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and

Initial/Date _____

uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol). You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $30 for completing this procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate.

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

Initial/Date _____

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Initial/Date _____

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?

All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 8-26-08 AND 2-9-09
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Storing Biological Specimens With Identifying Information 2

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: August 14, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: James M Samet, PhD; Robert Devlin, PhD;; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; BA; Dave Peden, MD, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Faculty Advisor: David Diaz-Sanchez, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the

Initial/Date _____

researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
Air pollution exposure has been found to cause a range of lung and systemic changes in normal subjects. We will be collecting blood samples which will help us to further study this condition. In order to do so, we will need to collect and store blood. Blood samples will be collected in order to look for but not limited to indicators of inflammation due to the Air pollution exposure. We may further isolate RNA from blood for genetic analysis. RNA may be isolated for genetic analysis to see if there is a difference between pre- and post Air pollution exposures.

All samples will be stored where only project members will have access to the samples. There is a need to store samples in such a repository because this will be an ongoing study where samples from subjects will be collected over an extended period of time. Storing of samples allows for all samples to be processed at the same time and also allows our scientist the opportunity to further study these samples with as yet unknown questions and techniques.

How will the specimen be collected?
The description of the samples to be collected and the manner in which this will be done have been described in the main study consent.

What will happen to the specimen?
Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the Air pollution exposure. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens (or data) for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens (or data) will be provided without identifiers to these other researchers by employing a data use agreement.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding the cardiovascular effects of Air pollution exposure in older population.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:

1. Blood sampling will be performed by medical personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise.

Initial/Date _____

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you for storage of the specimens?
There will be no cost to you for the storage and use of the specimens for research purposes.

Will you receive anything for being in this study?
You will not receive anything for taking part in this study with regard to storage of excess specimens. Reimbursement for participation in the main study is addressed in the detailed consent for that study.

Who owns the specimens?
Any blood, body fluids, or tissue specimens obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Initial/Date _____

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the specimens from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Initial/Date _____

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

APPROVED - IRB UNC-CH
AUG 2 6 2008

OMEGA STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1225.

Procedures:

Volunteers initially undergo a blood test and genotype screening, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genotyping visit and $30 for the additional medical screening. If you are eligible for the study your participation will last approximately 4 to 5 weeks.

Participation will begin with a training visit that will last about 3 hours and a return visit the following morning. Following training you will be scheduled for two-weeks of fish oil or olive oil treatments (taken as capsules) prior to the exposure session. During each week that you take the supplements you will be asked to record your food intake twice for 3 days each. The exposure day lasts 8 hours and the follow up day lasts about 3.

Procedures involved in this study include breathing tests, heart rate monitoring, including wearing overnight monitors after training and the exposure, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be able refrain from certain foods and medications for at least four weeks and to take the supplements for 15 days prior to the exposure. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-0518, previously 95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Genotyping Visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (1 hour)

Physical Exam, days and times TBD (2 hours)

Training (Day 0): (3 hours)

Dieatry Supplementation (Days 0-14)

Training Follow-up Visit Day 1 (to return the Holter Monitor, 30 min)

Dietary Recordings, Days 3-5 and Days 11-13

Exposure Day 15: 8:00 AM (8 hours)

Follow-up Day 16: 18 hours after Exposure Day (3 hours)

Web Site Advertising: www.epastudies.org

OMEGA: Elder Diet and Air Pollution Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as PM, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

Participation in this study will include two-weeks of fish oil or olive oil treatments and one exposure to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. Study procedures include breathing tests, heart rate monitoring, blood tests, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. Total time commitment after screening is about 15 hours, over 4-5 weeks. Compensation is provided.

APPROVED - IRB, UNC-CH
AUG 26 2008

The US Environmental Protection Agency is seeking

ADULT VOLUNTEERS

Ages 50 to 75 for Research

This is a research study about genetics, diet supplementation and exposure to air pollution.

We are looking for healthy older adults to study diet supplementation and the effects of air pollution exposure on heart and lung function.

Total time commitment after screening is about 14 hours over 4 to 5 weeks. You will receive payment for screening, the study, and out of town travel. Parking is provided.

1-888-279-9353 or
919-966-0604
www.epastudies.org

The Human Studies Facility is located on the UNC-CH campus

APPROVED - IRB UNC-CH

AUG 26 2008

OMEGA STUDY
E-mail Announcement

SUBJECT: INFORMATIONAL: Research study for Healthy Older Adults

The main purpose of this research study is to determine if a component of air pollution, particulate matter (PM) elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Volunteers ages 50 to 75 will be tested to determine the type of a particular gene they carry, one of several genes responsible for protecting the body against oxidants such as diesel exhaust. Participation will include two-weeks of fish oil or olive oil treatments and one exposure to a controlled amount of PM. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Committee on the Protection of the Rights of Human Subjects Biomedical Institutional Review Board. IRB # 07-0190 (GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

APPROVED - IRB UNC-CH
AUG 26 2008

TO:

APPROVED ... UNC-CH

RE: **Your appointment at the EPA Human Studies Facility**

AUG 2 6 2008

STUDY: **OMEGA STUDY – GENOTYPING VISIT**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA STUDY SCREENING GUIDELINES
For Genotyping Visit

Illness. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores.

No active allergies. If you have questions about allergies, please call the EPA medical station at 966-6232.

Please do not **EAT or DRINK** anything except water after midnight the day of your visit.

If you are unable to keep your appointment, please call Recruitment. Please be on time!

Thank You!
Westat EPA Support Services

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA STUDY – EXPOSURE VISITS**

DATE:

TIME:

APPROVED - IRB UNC-CH
AUG 2 6 2008

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA Study Instructions
For Exposure and Follow-up Visits

Illness and Allergies. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores. If you have questions about allergies, please call the EPA medical station at 966-6232.

Medications

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits. No red wine throughout the study.
- No caffeine for 12 hours prior to all study visits.

Food and Food Supplements

- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for 2 weeks before and during the study.
- No antioxidants (beta-carotene, selenium, vitamin C, vitamin E, zinc) for 2 weeks before and during the study.
- Use olive oil only for cooking, dressings, and sauces during the study.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.

Exercise, Clothing

- No strenuous exercise for 24 hours prior to and after all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the exposure and follow-up visits.

If you are unable to keep your appointment, please call Recruitment. Please be on time!
Thank You!
Westat EPA Support Services

APPROVED - IRB UNC-CH

AUG 2 6 2008

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA STUDY – TRAINING VISIT**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA Study Instructions
For the Training Visit

Illness and Allergies. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores. If you have questions about allergies, please call the EPA medical station at 966-6232.

Medications

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to the training visit. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before training.
- Avoid drinking alcohol 24 hours before training. No red wine throughout the study.
- No caffeine for 12 hours prior to training.

Food and Food Supplements

- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for 2 weeks before TRAINING and during the study.
- No antioxidants (beta-carotene, selenium, vitamin C, vitamin E, zinc) for 2 weeks before TRAINING and during the study.
- Use olive oil only for cooking, dressings, and sauces during the study.
- You must begin your daily dietary supplements on _____ [day], _____[date].

Exercise, Clothing

- No strenuous exercise for 24 hours prior to and after TRAINING and all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the training and follow-up visit.

If you are unable to keep your appointment, please call Recruitment. Please be on time!
Thank You!
Westat EPA Support Services

First Review of IRB Submission Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Tracey Montilla, David Demarini, Martin Case, Wayne Cascio, Mary Bassett)

Receipt Date : 8/21/2008 **Expiration Date :** 2/09/2009 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Expedited Reviewer 2:

Entered by: Laura O Curry

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter: CRW 8/26/08
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 8/26/08)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☒ Hard copy sent

Consent forms attached: 3

Other attachments: 7

FINAL ACTIONS:

- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

07-0190

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

RECEIVED
AUG 21 2008
IRB UNC-CH

RECEIVED
8/21/08
CTRC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	I highlighted 1 clean
x	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1) We have changed the exposure substance from diesel exhaust to concentrated fine and ultrafine air pollution particles.
2) We have reduced the number of subjects from 60 to 40. Twenty subjects in each group randomly receive either omega-3 or placebo supplementation followed by an exposure.
3) We have modified the genetic screening session prior to the physical exam. We will still perform genotyping of GSTM1 gene to determine whether GSTM1 genotype contributes to the susceptibility to air pollution. However, the GSTM1 genotyping is not the selection criterion for the study.
4) The total payment of the study will be adjusted to about $1225.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

We are requesting to change the source exposure because the PM exposure may be more relevant to real world situation. Since we are exposing the subjects to a PM exposure burden they would likely encounter if visiting a large city, we feel the risk posed to subjects is exceedingly small and we expect that the effects from PM exposure will be resolved within few hours after exposure.

IRB study #: 07-0190 GCRC #: 2579 **Date: August 14, 2008**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: David Diaz-Sanchez, PhD (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Haiyan Tong — 8/15/08

Signature of Principal Investigator or designee — Date

8/18/08

Signature of Faculty Advisor (if applicable) — Date

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

07-0190
RECEIVED
AUG 21 2008
IRB UNC-CH

Part A.1. Contact Information, Agreements, and Signatures

Date: ~~July 29~~August 14, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to ~~Diesel Exhaust~~Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID:
Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _x_ postdoc __ resident __ other

Name of faculty advisor: David Diaz-Sanchez, PhD
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Diaz-Sanchez.david @epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department:
Mailing address/CB #:
Phone #:
Fax #:
Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** James M Samet, PhD, MPH; Robert Devlin, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; ~~Debbie Levin, BSN;~~ Margaret Herbst, RN, MSN; ~~Sally Ivins, BA;~~ Howard Kehrl, MD; Tracey Montilla, RN; ~~Lynne Newlin-Clapp, BA;~~ Dave Peden, MD, MS; ~~Carole Robinette, MS;~~ Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):

__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
x	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
x	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
☐	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
☐	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

[signature] 8/18/08

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

[signature] 8/20/08

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee Date

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*

	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for* ***Consent for Stored Samples***.	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. ~~The first part of this protocol is a pilot study to identify the optimum diesel exhaust concentration to investigate the cardiovascular effects in healthy older subjects. The main part of t~~This proposal is to examine the health effects of fine and ultrafine ~~diesel exhaust~~ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM~~diesel exhaust~~.

Participants Forty healthy 50-75 year-old male and female subjects ~~(30 subjects are GSTM1 positive genotype and 30 subjects are GSTM1 null)~~ will be involved in~~for~~ the ~~main~~ study.

Procedures (methods): ~~In the pilot study, subjects will have 3 sequential exposures to the diesel exhausts at concentrations approximately 100 μg/m³, 200 μg/m³, and 300 μg/m³ for 2 hours with a about 2 weeks of interval between exposures. In the main study, GSTM1 positive and GSTM1 null genotype subjects will be~~ The subject will be exposed to concentrated ambient fine and ultrafine PM ~~the optimum concentration (will be determined based on the pilot study) of diesel exhaust~~ for 2 hours after being randomly supplemented with 15 days of omega-3 fatty acid or olive oil. ~~15 days of omega-3 fatty acid fish oil or olive oil supplementations.~~

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from

cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13).

~~A recent epidemiological study involving eight European cities suggested that the primary effect of PM air pollution on cardiac admissions was likely attributable to diesel exhaust inhalation (14). Diesel exhaust is a component of air pollution in urban settings, and contributes both PM and gaseous phase compounds to the atmosphere. Diesel exhaust fumes are made up largely of fine and ultrafine carbonaceous particulates generated by incomplete combustion of fuel. The U.S. EPA and the National Research Council (NRC) PM Research Priorities currently include identification of components of PM (including PM size and chemical composition) that contribute to the reported increased morbidity and mortality (NRC, 1998; EPA, 1997). In addition, both the EPA and NRC have stressed the importance of examining gaseous co-pollutants that may also affect PM toxicity. Diesel exhaust (i.e., gas phase constituents and diesel exhaust particles (DEP)) is ideal for examining the role of these factors in inducing human health effects for the following reasons: (1) Because of the relatively high content of organic compounds on DEP (usually 10-30% by weight), DEP are ideal for examining the role of organics in inducing health effects; (2) The exhaust is a mixture of PM and gaseous pollutants including some (e.g., aldehydes, NOx, carbon monoxide) that are known to produce human toxicity in high enough concentrations that may potentially augment PM-induced effects; (3) the size of the DEP vary depending on engine type and other factors, allowing assessment of PM size on the induction of human health effects. Few controlled human exposures to diesel exhaust have been performed. One hour exposure to 300 μg/m^3 diesel exhaust with intermittent exercise in healthy human volunteers resulted in marked systemic and redox-sensitive pulmonary inflammatory responses (15, 16), and vascular dysfunction and impaired endogenous fibrinolysis that links diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (17).~~ Despite a decade of intensive studies, much about the PM health effects ~~problem~~, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ~~diesel exhaust~~ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of ~~diesel exhaust~~PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (~~18~~14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due

to its anti-arrhythmic effect (~~19~~15-~~21~~17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (~~22~~18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (~~23~~19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (~~24~~20). ~~Another enzyme called GSTP1 is also associated with anti-oxidants effects and we will determine the optimal concentration of diesel exhaust in the pilot study in the most vulnerable subgroup with GDTM1 null and 3 GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val).~~ Therefore, the role of GSTs in mediating the cardiovascular effects following acute ~~diesel exhaust~~PM exposure will also be studied in the ~~main~~ protocol in GSTM1 null and GSTM1 positive healthy older adults.

U.S. EPA regulations concerning ~~diesel emissions~~ambient PM, mandated by the Clean Air Act, have resulted in dramatic decreases in emissions from diesel engines since 1988, with the last set of significant engine modifications occurring in 1995 (EPA, 1998). The respiratory effects of ~~diesel exhaust~~PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to ~~diesel exhaust~~PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to ~~diesel exhaust~~PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.

11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
~~14. Le Tertre, A., Medina, S., Samoli, E., Forsberg, B., Michelozzi, P., Boumghar, A., Vonk, J.M., Bellini, A., Atkinson, R., Ayres, J.G., Sunyer, J., Schwartz, J. and Katsouyanni, K. (2002). Short-term effects of particulate air pollution on cardiovascular diseases in eight European cities. J Epidemiol Community Health 56:773-779.~~
~~15. Salvi, S., Blomberg, A., Rudell, B., Kelly, F., Sandstrom, T., Holgate, S.T. and Frew, A. (1999). Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. American journal of respiratory and critical care medicine 159:702-709.~~
~~16. Behndig, A.F., Mudway, I.S., Brown, J.L., Stenfors, N., Helleday, R., Duggan, S.T., Wilson, S.J., Boman, C., Cassee, F.R., Frew, A.J., Kelly, F.J., Sandstrom, T. and Blomberg, A. (2006). Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Respir J 27:359-365.~~
~~17. Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstrom, T. and Newby, D.E. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112:3930-3936.~~
1~~8~~4. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
1~~9~~5. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
~~20~~16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
~~21~~17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
~~22~~18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
~~23~~19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
~~24~~20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.
~~25. Lauer, M.S., Pothier, C.E., Chernyak, Y.B., Brunken, R., Lieber, M., Apperson-Hansen, C. and Starobin, J.M. (2006). Exercise-induced QT/R-R interval hysteresis as a predictor of myocardial ischemia. Journal of electrocardiology 39:315-323.~~

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal ~~includes~~ is approximately ~~6 subjects with both GSTM1 null and GSTP1 codon 105 (Ile/Ile, Ile/Val, and Val/Val; n=2 each) genotypes for the pilot study and 6~~40 subjects ~~(30 subjects~~

~~GSTM1 positive and 30 subjects GSTM1 null)~~ for the ~~main~~ study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

~~**Exclusion criteria (pilot study):**~~

- ~~A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.~~
- ~~Cardiac pacemaker.~~
- ~~Uncontrolled hypertension (> 150 systolic, > 90 diastolic).~~
- ~~Neurodegenerative diseases such as Parkinson's and Alzheimer disease.~~
- ~~A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.~~
- ~~History of bleeding diathesis.~~
- ~~Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin.~~
- ~~Currently taking β-blockers to control hypertension and/or arrhythmias.~~
- ~~Currently taking ACE inhibitors.~~
- ~~Use of oral anticoagulants.~~
- ~~Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.~~
- ~~Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).~~
- ~~Subject is pregnant, attempting to become pregnant or breastfeeding.~~
- ~~No exposure will be conducted within 4 weeks of a respiratory tract infection.~~
- ~~Have an allergy to latex.~~

Exclusion criteria ~~(main study)~~:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant ~~known cardiovascular disease,~~ chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.

- ~~Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin.~~
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- ~~Currently taking ACE inhibitors.~~
- Use of oral anticoagulants.
-
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- ~~Female subjects currently taking estrogen replacement therapy.~~
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine ~~prohibited from drinking red wine~~ during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within ~~4~~ 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

~~***Pilot study***: Approximately 6 subjects will undergo 3 exposures to air containing approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts sequentially for 2 hours in an exposure chamber. There will be a 2-week interval between exposures. We anticipate that there will be 6 subjects at each concentration of diesel exhaust exposure during the course of this pilot study. However, circumstances beyond our control may arise (i.e. subject/subjects might drop off due to illness and/or other reasons during the study) which may prevent finishing the proposed 3 exposures, and then we will have to add new subject/subjects at that point. Each new subject will have 3 exposures at approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts. Subjects who withdraw from the study will be compensated for all procedures and time completed to that point (See the pilot study design flow chart).~~

~~The purpose of the pilot study is to identify safe and measurable cardiovascular effects of diesel concentration. Our primary endpoints in the pilot study include HRV, pulmonary function, and biomarkers such as neutrophils, platelets, fibrinogen, c-reactive protein, lactate, ferretin, plasminogen activator inhibitor, and α1-antitrypsin. We will also assess the endothelial cell function by brachial artery ultrasound and finger artery Endo-Pat. We will analyze these endpoints after finishing each concentration of diesel exposure and determine whether we will continue the next higher concentration exposure. For example, if we find effects at approximately 100 $\mu g/m^3$ of diesel concentration then we will use that concentration for our main study. We may not need to test subjects at the next higher proposed concentrations. We will carefully monitor the symptoms that subjects may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, dyspnea, pallor, ataxia, and significant cardiac arrhythmias. In addition, analyses of blood samples taken after exposure will be scrutinized for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. Symptoms and/or changes detected in the blood samples that are considered clinically significant could prompt the study physicians to stop the study. Therefore, we will select the diesel concentration based solely on safety considerations.~~

Main study: This will be a randomized, double-blinded study. ~~Sixty~~ Forty study subjects ~~(30 subjects GSTM1 positive and 30 subjects GSTM1 null)~~ will be randomly given fish oil or olive oil supplements for 15 days.~~, in a double-blind fashion~~, The subjects will be given a~~a~~ container filled with 15 days supply of enteric-coated either 2g/day of fish oil ~~or~~ or 2g/day of olive oil capsules ~~for 15 days~~. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 25-35% of eicosapentaenoic acid (EPA) and 30-65% of docosahexaenoic acid (DHA). Each 1000 mg of ~~these olive oil capsule~~this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. ~~There will be four groups of subjects in this study with 15 subjects in each group: the first group is fish oil supplement in GSTM1 positive subjects, the second group is fish oil supplement in GSTM1 null subjects, the third group is olive oil supplement in GSTM1 positive subjects, and the fourth group is olive oil supplement in GSTM1 null subjects. In the exposure study, e~~Each subject will be exposed to air containing ~~approximately optimum diesel exhaust~~fine and ultrafine PM (PM<2.5) for 2 hours in an exposure chamber after the supplementation. (See the ~~main~~ study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet

the criteria will be scheduled for an ~~genetic screening~~ appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotype~~ screen~~ing ~~informed~~ consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 26, methods of recruiting) will not need genotype~~ screen~~ing. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have ~~approximately 50 ml of blood collected in the pilot study and~~ approximately 80 ml of blood collected ~~in the main study~~. A portion of the blood will be used for ~~genetic screening~~genotyping of GSTM1 ~~and/or GSTP1 Ile105 Val polymorphism~~ and another portion of the blood will be used for the cholesterol levels, phospholipid~~fatty acid analysis,~~ and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back ~~after they are selected on the genotype~~to the medical station.

- ***GSTM1/~~GSTP1~~ genotype~~ screening~~***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1 ~~and GSTP1~~. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene ~~and GSTP1 Ile105Val polymorphism~~. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null. ~~Since the pilot study only seeks people that are GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotypes, and since the joint GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotype is present in about 8%, 17%, and 14% respectively of the population, it is likely that about 80-90 % of the subjects will be excluded in this pilot study but they will be informed to participate in our future study which will enroll both GSTM1 positive and null genotypes. Those subjects with GSTM1 positive will not be asked to continue with the study and will be compensated for their time. Since the main study seeks to enroll equal numbers of people that are GSTM1 positive and null, it is likely that as the study nears completion, we will still be seeking people with the null allele but not with the sufficient allele. If that is the case, those subjects will not be asked to continue with the study and will be compensated for their time.~~
-
- ***Food frequency questionnaire in the ~~main~~ study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the ~~genetic~~ initial screening will be scheduled for a physical examination in the HSF. ~~Those subjects who are genetically screened but are not eligible for the pilot study, may be included in the main study if they are interested in participating.~~ During this visit, subjects will sign an informed consent for a physical ~~and the medical history form completed that contains information on general personal and family medical history~~. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam ~~and genetic screening~~ will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Baseline HRV measurement***: S~~In the pilot and main study,~~ subjects will have their baseline 24-hour heart rate viability (HRV) measured by Holter monitor on the training day. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Subjects will be released while wearing the Holter monitor and return to the HSF the next day to get the Holter monitor removed. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.

- ***Fish oil or olive oil pretreatment and dietary assessment in the ~~main~~ study:*** Subjects will be randomly given an approximately 15 days supply of either fish oil or olive oil supplements ~~randomly assigned~~. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of energy and nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record per week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 15 day of supplementation period. We will give the subjects another 15 days supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure

on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (~~blood pressure~~vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU) and ~~possibly by~~ Endo-PAT, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** ~~***in both pilot and main studies***~~ will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained.
- ***Brachial artery ultrasound*** ~~***in both the pilot and main studies:***~~ Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Peripheral endothelial function by Endo-PAT*** ~~***in both pilot and main studies***~~: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be induced by sudden cuff deflation. The PWA recordings will be measured continuously during suprasystolic occlusion and deflation reactive hyperemia. PAT measurement will be displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).
- ***Pulmonary function*** ~~***in both pilot and main studies***~~ will be measured by spirometry.
- ***Blood sampling*** ~~***in both pilot and main studies***~~: ~~approximately 50 ml of blood samples will then be collected in the pilot study and a~~approximately 80 ml of blood samples will ~~then~~ be collected ~~in the main study~~.
- ~~***Urine sampling in both the pilot and main studies:*** approximately 30 ml of urine samples will be collected.~~
- ***Symptoms questionnaire*** before exposure will be collected.

~~In the pilot study: Subjects will then enter the diesel exposure chamber for 3 sequentially exposures at concentrations approximately 100 μg/m³, 200 μg/m³, and 300 μg/m³ for 2 hours each with a about 2-week interval between exposures. In the main study: Subjects will have only one exposure at an optimum concentration of diesel exhaust that will be identified from the pilot study.~~

Exposure:

The subjects will be exposed to concentrated ambient fine and ultrafine PM after the dietary supplementation.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. The exposure atmosphere will be at approximately 40 ± 10% RH and approximately 22 ± 2 °C. ~~The diesel exhaust will be generated from a 6-cylinder, 205 hp, 5.9 L displacement diesel engine (Cummins, Columbus IN) mounted on a vehicle located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean filtered and humidified air by approximately 1/30th to give a chamber concentration of approximately 100 μg/m³, 200 μg/m³, and 300 μg/m³. The monitoring analyzer registers a concentration greater than 100 ug/m³ above the target concentration as the exposure limit. Specifically, exposures will be terminated at values ≥200, 300 or 400 during runs in which the exposure targets are 100, 200 and 300 ug/m³, respectively. The running sum of the 2-minute average concentrations displayed by the monitoring analyzer during the 2 hr exposure period exceeds 18,000 ug/m³. This safety limit is specifically designed to address exposures for which the target concentration is 300 ug/m³. It will prevent an inhalational exposure greater than 600 ug, which assumes that the resting subject will~~

~~inhale 2 m^3 during the 120 min exposure. It is expected that 75 to 95% of the diesel exhaust PM from this type of engine will be centered around approximately 0.1-0.2 μm. Preliminary testing has shown the mean particle size to be approximately 0.14 μm. Levels of carbon monoxide, and oxides of nitrogen (NOx; mainly NO, and some NO2), will be kept under 10 ppm CO, 15 ppm NO, and 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) for these substances are: carbon monoxide=50 ppm; NO2=5 ppm; NO=25 ppm; The American Conference of Governmental Industrial Hygienists (ACGIH) 8-hr threshold limit values (TLVs) are: NO=25 ppm, NO2=3 ppm, and CO=25 ppm]. Diesel fuel used for the study will be purchased as a certified fuel (Chevron Phillips Chemical Co., Borger TX, 0.05 LS Certification Fuel, type II). This certified fuel represents diesel fuel composition that is typically available at gas stations for most regions of the country except for California. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured with a tapered element oscillating microbalance (TEOM) and/or a Data-RAM, and chamber PM concentrations during exposure further validated by weighing filters obtained from versatile air pollution samplers (VAPS) impacters. Particle size distribution will be determined by scanning mobility particle size (SMPS) not during the subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles.~~

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces approximately a 20 fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 200 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure. During the air exposure days, airflow into the chamber will be filtered to remove a minimum 99% of ambient air particles.

The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m3 with lower values during the rest of the year. A facial mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the chamber will range between levels of 50 to 300 ug/m3 and the concentration will be in the range of 50 to 600 ug/ m3 with facial mask. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the air entering the chamber will be monitored continuously by a DataRAM monitor (DR4000; Thermal Electron Corp) calibrated to filter obtained measurements of particle ug/m3. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The computer is programmed to initiate a shutdown procedure if particulate mass levels exceed 600 ug/m3. This procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. The resting minute ventilation

during exposure will be measured. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and ~~approximately 50 ml of blood and 30 ml of urine samples will be collected in the pilot study and~~ approximately 80 ml of blood samples will be collected ~~in the main study~~. ~~Approximately 30 ml of urine samples will be collected in both the pilot and main studies.~~

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, ~~and approximately 50 ml of blood will be collected in the pilot study~~ and approximately 80 ml of blood will be taken ~~in the main~~. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound) ~~**and finger arteries Endo-PAT).**~~ Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Finger arteries Endo-PAT. Changes in pulse wave amplitude of index fingers caused by reactive hyperemia will be expressed as a percent change relative to resting baselilne values.

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood ~~(about 50 ml in the pilot study about 80 ml in the main study)~~ will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In theis ~~main~~ study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to ~~a diesel exhaust~~PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of ~~diesel exhaust~~PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound and finger arteries Endo-PAT:** There are no known risks associated with imaging of the brachial artery and index finger arteries. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will range from 50 to 600 ug/m^3 and the exposure will be terminated if the level exceeds 600 ug/m^3. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or

dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

•**Diesel exposure** The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban areas. Diesel particulate concentrations proposed for the pilot study will be 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m3$ and the optimum concentration will be used for the main study. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. During exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have EKG leads attached during the exposure to monitor cardiac function during exercise. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for their participation up to that point. The investigators or duty physician will end the exposure if the subject is found to be suffering from any significant adverse effect. The small pilot study will allow us to monitor the adverse effects of diesel exhaust closely and we will determine whether we will go to the next higher concentration based on the analysis. We will be able to identify a safe concentration of diesel exhaust that induces a measurable change in our study endpoints. No major adverse health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but

~~because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.~~

~~A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.~~

~~Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.~~

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

~~To test our~~To test the hypothesis~~s~~ that ~~diesel exhaust~~PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to ~~diesel exhaust~~PM.

We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to ~~diesel~~ PM will also be measured (e.g., blood pressure, HRV, brachial arterial ~~blood flow rate~~diameter, change in blood inflammation and coagulation factors, ~~platelets, fibrinogen, c-reactive protein, lactate, plasminogen activator inhibitor, ferretin, and α1-antitrypsin~~). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 of either fish oil or olive oil

supplemented subjects was derived using a power of 0.8 and an α of 0.05. Therefore, we will recruit 20 subjects in each group in this study.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to ~~diesel exhaust~~PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of ~~diesel exhaust~~PM on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen, D-dimer, and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data.** Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no
Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the ~~pilot study will be approximately 3 months and the main~~ study will take approximately 18 months. Participant recruitment ~~and screening~~ is expected to be continuous throughout the study until the intended number of participants is reached.

~~If the subject is eligible for the pilot study they will have 9 visits to the HSF over approximately 18-20 weeks. Subjects will have about 2 weeks break between 2 exposures. If the subject is eligible for the main study of diesel exhaust exposure t~~The individual will have ~~5~~ 6 visits to the research facility over approximately ~~5~~4-~~6~~ 5 weeks. The pre-enrollment ~~genotyping~~ and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after ~~genotyping of~~ the blood ~~sample~~work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment ~~for the main study~~and baseline HRV measurement) will require approximately 3 hours. Exposure day will last approximately 8 hours ~~for the pilot and main studies~~. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours ~~for the pilot and the main study~~.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. ~~Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be paid for the entire study, excluding completion bonus.~~

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled~~, and will be paid 50% up to a total maximum of $100 for canceled procedures~~. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study.

~~1. If following genotyping of the blood samples on screening day, it is determined that the individual's genotype is not needed for completion of the study, then that subject may be informed that his/her participation in this study is no longer necessary. The subject will be notified by phone and the total compensation for this study will be approximately $60.~~
~~2. If the subject is qualified and finished the entire pilot study, the total compensation for completion of this study will be approximate $2053.~~
~~3.~~ If the subject is qualified and finished the entire ~~main~~ study, the total compensation for completion of this study will be approximate $~~119~~512255~~.~~

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~~~50ml of pilot and~~ ~80ml ~~of main study; genotyping~~)	$30

Pre-study qualification total = $60

~~Subjects based on their genotype assessment may not be asked to further participate in the study.~~

Training day (3 hours)

Time (3h @$12/h)	$36
Holter monitor	$100

~~**Pilot study:**~~
~~Exposure (8 hours)~~

~~Venipuncture (~50ml, pre; 3@$30 each)~~	~~$90~~
~~Holter monitor (3 @$100 each)~~	~~$300~~
~~Chamber exposure (3 @$72 each)~~	~~$216~~
~~Venipuncture (~50ml, post; 3@$30 each)~~	~~$90~~
~~Time (3X8h @$12/h)~~	~~$228~~
~~Finger artery Endo-PAT (6 @$10 each)~~	~~$60~~
~~Brachial artery ultrasound (6@$50 each)~~	~~$300~~
~~On-time bonus (3@$25 each)~~	~~$75~~
~~Urine samplings (6 @$10 each)~~	~~$60~~

~~Total for completion of exposure = $1579~~

~~Eighteen hours after exposure (3 hour)~~

~~Venipuncture (~50ml; 3@$30 each)~~	~~$90~~
~~Time (3X3h @$12/h)~~	~~$108~~
~~Finger artery Endo-PAT (3 @$10 each)~~	~~$30~~

~~Total for completion of post-exposure = $228~~

~~Protocol Completion Bonus (3@$50 each) $150~~

~~Approximate TOTAL for completion of pilot study = $2053~~

~~Main study:~~

Exposure (8 hours)

Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Finger artery Endo-PAT (2 @$~~10~~ 20 each)	$~~20~~40
Food records (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $~~823~~843

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Finger artery Endo-PAT	$~~10~~20

Total for completion of post-exposure = $~~76~~86

Protocol Completion Bonus $100

Approximate TOTAL for completion of ~~main~~ study = $~~1195~~1225

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

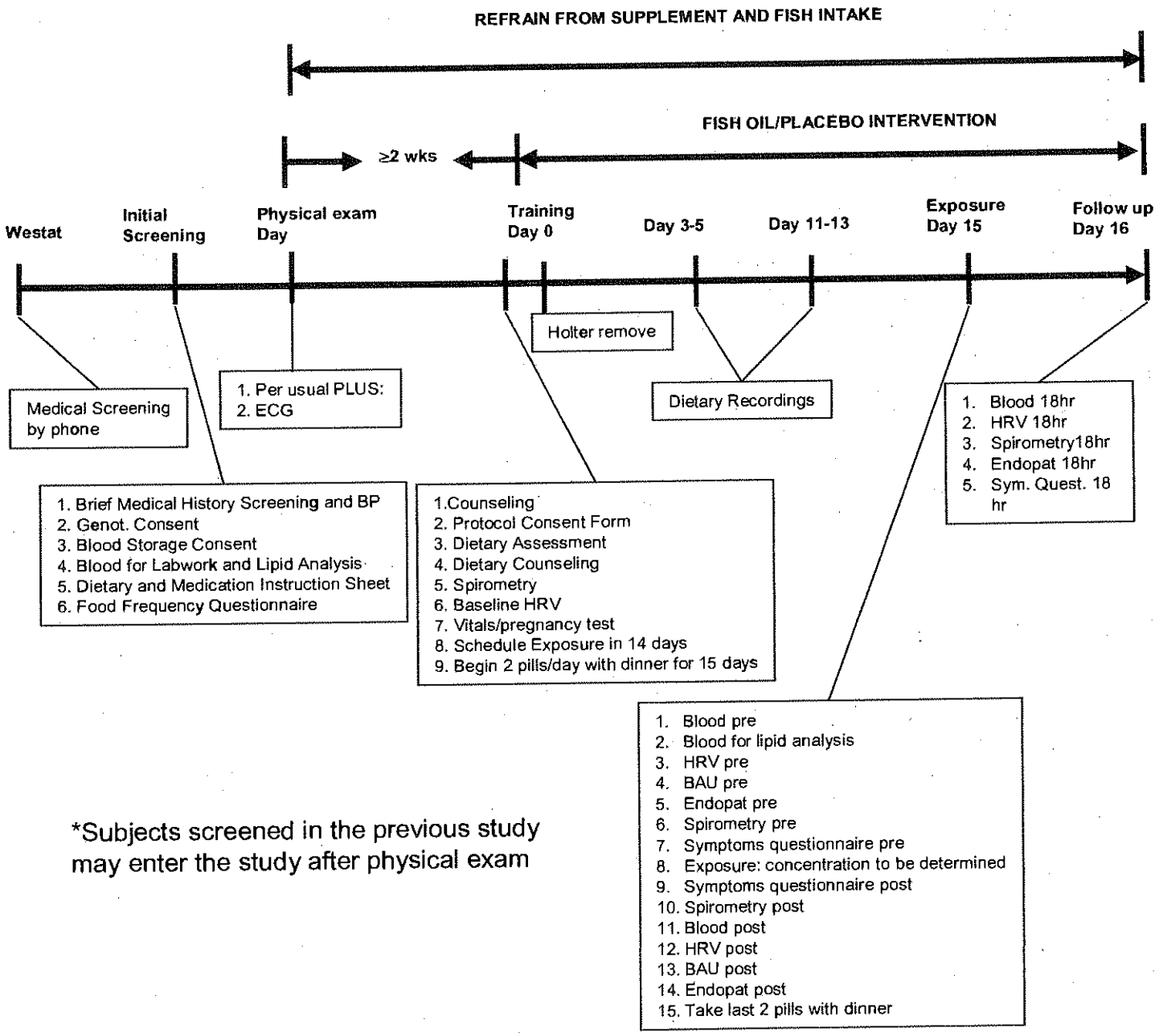
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



*Subjects screened in the previous study may enter the study after physical exam

STUDY FLOW DIAGRAM

RECEIVED
AUG 21 2008
IRB UNC-CH

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
Office of the Vice Chancellor for Research and Economic Development

Campus Box 4100, 201 Bynum Hall
Chapel Hill, NC 27599-4100
(919) 962-7757 FAX: (919) 962-6709
http://research.unc.edu

August 20, 2008

To Whom It May Concern:

In accordance with the National Institutes of Health (NIH) notices OD-00-039 of August 25, 2000 and OD-01-061 of September 5, 2001, The University of North Carolina at Chapel Hill submits the following information concerning the education in the protection of human research participants undertaken by the key personnel involved with this project. The following persons have been trained in the ethical and regulatory requirements for protection of human research participants in compliance with NIH requirements and The University of North Carolina at Chapel Hill "Policy on Education and Certification of Investigators Involved in Human Subjects Research." http://ohre.unc.edu/irbtraining/

Name	Dept	Training
David Diaz-Sanchez		CITI- Biomedical

Should any additional persons come to work on this project and meet the definition of key personnel, they will be trained similarly; their names and certification of their training in the protection of human research participants will be forwarded.

Sincerely,

John Gallagher
Interim Director
Office of Sponsored Research

07-0190
RECEIVED
Version: 7/13/2006
AUG 21 2008
IRB UNC-CH

GCRC ADDENDUM
to
IRB APPLICATION

(5 paper copies and one electronic copy (Word or PDF) of IRB application and this addendum are required for new submissions)

GCRC #: 2579

Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to ~~Diesel Exhaust~~Air Pollution Particles

Date: ~~5 June 2007~~ August 14, 2008

If you have had previous GCRC protocols, as PI or Co-PI, please list publications related to these protocols, from the last 3 years, and state whether or not they cited GCRC grant (RR00046).

Publications	Cited GCRC Yes	Cited GCRC No
	☐	☐
	☐	☐
	☐	☐
	☐	☐
	☐	☐
	☐	☐

Continue on an additional blank page, if necessary

USE OF THE GENERAL CLINICAL RESEARCH CENTER:

SERVICE (check all that apply)	YES	NO
Clinical Trial If so, what phase?	☐	☒
Outpatient rooms	☐	☒
Inpatient rooms	☐	☒
Blood drawing	☐	☒
Subjects seen at locations other than the GCRC	☒	☐
Subjects seen at the Dental Research GO Health Center	☐	☒
Use of GCRC investigator lab space for specimen processing	☐	☒
Temporary storage of samples in GCRC freezers	☐	☒
Bioanalytical Core (BAC) Lab Assays	☐	☒
Medication administration	☒	☐
Meals (Research diets, regular meals/nourishments for inpatients or for extended outpatient visits)	☒	☐
Research Nutritionist services (nutrition assessment, dietary intake data, diet instruction)	☐	☒
Body composition lab (DXA, Indirect calorimetry, pQCT, Erythromycin Breath Test, Activity Monitors, BIA, Physical Activity Questionnaires)	☐	☒
Database creation or other data management help	☒	☐
Other requests- specify:	☐	☒

Investigator: Haiyan Tong, MD, PhD; David Diaz-Sanchez, PhD; James M Samet, PhD, MPH; and Robert Devlin, PhD.	**Phone:** 966-4993 **Pager:**	**Email:** Tong.haiyan@epa.gov
Coordinator: Maryann Bassett	**Phone:** 966-6234 **Pager:**	**Email:** Bassett.maryann@epa.gov

A. SAFETY-MONITORING PLAN

All GCRC supported studies must have a written Safety Monitoring Plan that addresses the questions below. This is an NIH requirement and this Safety Monitoring Plan must be available for review by the NIH. For more information, contact David Weber, **dweber@unch.unc.edu**, phone 843-0725 OR Marie Zeldin, **mzeldin@med.unc.edu**, phone 966-6844. (*note: you can copy the email addresses in the preceding text boxes and paste into your email program.)*

If your study is not a clinical trial and does not involve a research intervention (administering a substance, exposure to experimental conditions or doing a procedure for research purposes) you may answer "N.A." to questions that do not apply.

1. **What potential risks are inherent in the study?** This should correspond with the risks that are described in the protocol and consent form.

Subjects in this study will be in GCRC only for brachial artery ultrasound measurements (flow-mediated dilatation, or FMD). This is a non-invasive test which involves minimal risk. A highly skilled investigator or technician will obtain the measurements. Occluding blood flow to the forearm may cause mild discomfort and unusual sensations such as tingling and numbness. There is a small risk (<1%) of developing petechiae in the arm which is examined. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.

General risks that may be encountered outside the GCRC are described below:

Ambient particulate matter (PM) is not expected to produce any permanent adverse health effects in this study. Dyspnea is the only predictable effect that may be encountered in the short term. All endpoints measurements, except for venipuncture, are non-invasive and involve only minimal risk.

The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will range from 50 to 300 ug/m^3 and the exposure will be terminated if the level exceeds 400 ug/m^3. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

~~The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban areas. Diesel particulate concentrations proposed for the pilot study will be 100 µg/m^3, 200 µg/m^3, and 300 µg/m3 and an optimum concentration will be used for the main study. These concentrations are the occupational levels for some truck drivers (generally about 100-300 µg/m^3) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had DEP levels >20 µg/m^3, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 µg/m^3 DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City.~~

Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.

There is minimal risk associated with non-invasive ~~e~~Endo-PAT measurement, which is similar to the risk associated with brachial artery ultrasound measurement, except that there is no nitroglycerin administration in the ~~e~~Endo-PAT measurement

2. Identify and justify the level of risk to subjects for this protocol. Refer to "GCRC Protocol Risk Assessment and Monitoring Guidelines click here at **http://gcrc.med.unc.edu/forms/Risk Assessment Guidelines.doc**. (*note: double click on the URL in the preceding text box and paste into your browser.)*

2. Select Risk Level
Justify Risk: Brachial artery ultrasound measurement at GCRC, Endo-PAT, ECG, and pulmonary function test will be rated at risk level 1. ~~Exposure sessions in the pilot study will be imposed a moderate risk and we will minimize the risk by continuously monitoring (see #3 below).~~ The exposure session in the ~~main~~ study will be rated at risk level 2.

3. How will risks be minimized? Describe the procedures that will be used to minimize risk to the subject.

3. Subjects will undergo a physical exam and 12-lead ECG to determine whether they can participate in this study. A physician will be onsite during the exposure. Heart rate, ECG, and pulse ~~goniometry~~ oximetry will be monitored continuously during the exposure. The subjects will be monitored for significant respiratory distress, chest pain, significant cardiac arrhythmias, pallor, and ataxia. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect which could be attributed to the exposure. The human exposure laboratory is also equipped with an emergency "crash cart" with standard emergency medications, IV fluids and a defibrillator in the unlikely event of a medical emergency during any exposure study. All subjects will return for assessment approximately 18 hours after the exposure as part of the study design. As standard procedure for all EPA studies, nursing personnel will make follow-up calls to the subjects in the days following the exposure to ensure that subjects are well and all subjects will be provided with a contact telephone number for access to a study physician who is on call 24 hours/day.

4. What tests, evaluations, or observations will be performed to monitor subject safety? List the specific tests, evaluations, examinations, observations, questionnaires, that will be used, and their frequency, to assess subject safety, to monitor for toxicity, or to detect adverse events. If there is a table or schedule of events, it should be reproduced here or referenced clearly.

1) On the physical exam day, subjects will undergo an ECG to screen for baseline cardiac arrhythmias. Subjects will have blood CBC, chemistries, and lipid tests to screen for hyperlipidemia, abnormal blood cell counts and differentials, and abnormal electrolytes.
2) A menstrual history will be collected on all female subjects on the physical exam day. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and the exposure day if it is more than 7 days since last pregnancy test it will be repeated.
3) Heart rate, heart rate viability (HRV), blood pressure, pulmonary function test, brachial artery ultrasound or eEndopat, blood sampling will be measured before exposure on the exposure day.
4) Heart rate, ECG, and pulse oxiimetry will be monitored continuously during the exposure. In addition, the subjects will be continuously monitored by a study investigator, duty physician, or nursing personnel.
5) Immediately after exposure, subjects will undergo blood pressure, heart rate, lung function, brachial artery ultrasound measurements and blood sampling.
6) Eighteen hours after exposure, subjects will undergo blood pressure, heart rate, lung function, brachial artery ultrasound measurements and blood sampling. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat.

5. Identify the classification scale that will be used to grade adverse events. Identify a scale that will be used to grade the severity of adverse events. If a protocol does not already have a specific grading scale, it is recommended that the Common Toxicity Criteria (CTC scale) be utilized. It can be found at **http://ctep.cancer.gov/**. For simple studies, a mild, moderate, severe scale may be utilized. (*note: double click on the URL in the preceding text box and paste into your browser.)*

5. A subjective adverse event classification will not be used in this study. If a subject experiences any event that he or she, or the investigators feel is sufficient to warrant stopping the exposure or other study procedure, that subjects' participation will immediately be stopped. Objective criteria which will lead the investigators to stop a study procedure will include:
1. Oxygen saturation < 91%.
2. Arrhythmia other than sinus tachycardia or sinus bradycardia.

6. List specific stopping rules that would lead to discontinuing a subject from the study or a change in the research intervention. If there is a specific number and grade of adverse events that will lead to subject discontinuation from the research intervention or if there are rules for dose escalation, please describe.

6. See #5 above. Additionally, if any subject experiences an event that requires significant medical attention the study will be stopped until a through review is completed by the investigators, the EPA National Health and Environmental Effects Research Laboratory (NHEERL) Human Research Official, and potentially the UNC IRB. The study will only be resumed after this review if all of these parties deem it appropriate.

7. For multicenter or high risk protocols, describe the Data Safety Monitoring Board or Safety Monitoring Committee that will monitor subject safety across all sites and describe study wide stopping rules. Provide details of the frequency of meetings, the membership, study wide stopping rules, and a copy of the DSMB charter. If a DSMB or SMC will not be utilized, explain why not. Confirm that the IRB will receive all reports of DSMB meetings and other safety reports.

7. N/A.

8. For single center studies or studies of moderate or lower risk, describe who is responsible for receiving and reviewing adverse events in aggregate across the entire study population. If this study is Phase I or II with no DSMB, please summarize how and at what intervals the safety data will be aggregated and reviewed, and who will be involved in these decisions.

8. There is no DSMB involved with this study, although adverse events will be monitored and noted by the investigators continually throughout the duration of the study. The UNC IRB will receive a report form the investigators regarding any adverse event that would cause a subject to be excluded from further participation in the study.

9. To whom and with what frequency will adverse events be reported? Discuss reporting to the IRB and GCRC as well as to sponsor, FDA and NIH if applicable, of expected and unexpected events.

9. See #8 above.

10. Describe who at UNC will monitor the safety of research subjects. Identify the individuals (Investigators, MDs, RNs, Research Associates, etc.) responsible *at this site* for monitoring the safety of research subjects. Describe the reporting mechanisms of the study staff to the P.I. and the frequency with which the P.I. reviews protocol data. Specify whether an outside monitor will review study charts and data.

10. All investigators having contact with the subjects during the course of the study will monitor the safety of the subjects. In addition, the health and well-being of the subjects during and for a time after the study will also be monitored by the EPA Human Studies Division nursing staff and physicians. As standard protocol for all EPA studies, the EPA nurses and physicians report any potential adverse events to the study PI.

B. Investigational Drug Service

The Investigational Drug Service is **not** a core service of the GCRC, however, all administration / dispensing of any agents (commercial or investigational) **must** go through the IDS for GCRC protocols. The GCRC addendum requires completion of the "Request For Investigational Drug Services" at **http://intranet.unchealthcare.org/site/w3/pharmacy** which is reviewed at the time of the GCRC protocol review. The IDS is located down the hall from the GCRC in Room 3001 of the main hospital. Please direct any questions to IDS by calling 966-3469. (*note: double click on the URL or email address in the preceding text boxes and paste into your browser or email application.)*

C. Biostatistics

All of the following questions should be answered in sufficient detail to allow useful biostatistical review. If you have already completely and adequately addressed these questions in previous sections of the IRB-GCRC application, then you may simply reference those sections rather than repeat the details in the boxes below. Alternatively, if you prefer to answer all the questions in the form of an attached word document, feel free to do that. (The protocol that was written for your study should have a complete statistical considerations section.) If your study is a multi-center trial with a master protocol that sufficently addresses all of these questions, you may paste all appropriate text in the first box below, OR reference the applicable page numbers of the master protocol. The master protocol must be submitted with this addendum.

For more information, contact Paul Stewart, **paul_stewart@unc.edu**, phone 966-7276.

Paste in page references to IRB Application or Master Protocol here:

1. What are the scientific aims and practical objectives of this study? (e.g., publication, get data for a grant proposal, pilot-test procedures/feasibility, estimate prevalence, test hypotheses, etc.)

This proposal is to examine the health effects of ~~diesel exhaust~~PM exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to ~~diesel exhaust~~PM.
The proposed study is designed to test the null hypothesis that there is no protective effect of omega-3 fatty acid supplements and presence of a functional GSTM1 gene on the effects of ~~diesel exhaust~~PM exposure.

2. What are the important features of the study design ?

~~In the pilot phase, three exposures to incremental increased levels of diesel exhaust with 2 weeks of interval to identify a safe dose of diesel exhaust that induces an alteration in heart rate viability (HRV) in healthy older subjects with GSTM1 null and GSTP1 Ile105Val polymorphism (Ile/Ile, Ile/Val, and Val/Val).~~
The ~~main~~ study will follow a randomized, double-blinded design to test the effects of dietary supplementation ~~and GSTM1 status~~ on the effect of ~~optimal level of diesel exhaust~~PM ~~identified from the pilot study~~. The study coordinator will provide subjects randomization assignments for dietary supplementation. The study investigators and subjects will be blinded for the dietary supplements ~~and GSTM1 genotype~~.

3. **Data used in statistical analyses**

3.1 For what variables will measurements be recorded? (e.g., age, blood pressures, treatment.)

Heart rate viability (HRV), flow-mediated dilation of the brachial artery, blood pressure, lung function, and blood biomarkers in responses to fish oil/olive oil, GSTM1 ~~and GSTP1~~ genotypes, and ~~different levels of diesel exhaust~~PM exposure will be recorded.
Safety monitoring will include continuous observation of heart rate, electrocardiogram, pulse

oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmia, pallor, and ataxia.

3.2. How often and on what occasions will these measurements be made?

Heart rate viability, flow-mediated dilation of the brachial artery, blood pressure, lung function, and blood biomarkers will be obtained at baseline before ~~diesel~~ PM exposure (0 min), immediately after exposure (2 hours), and 18 hours after exposure (20 hours).

3.3. As used in the analyses, what will be the units and scales of these variables?

HRV will be expressed as the standard deviation of normal-to-normal RR intervals (SDNN) and the square root of the mean of squared differences between adjacent RR intervals (r-MSSD), the unit of HRV is second. Flow-mediated dilation of the brachial artery will be expressed as % change to the baseline level. Blood pressure will be in mmHg. Blood markers will be expressed as ug/ml or units/ml. Lung function will be expressed as liter and liter/first second.

3.4. What is the role of each of these variables? (e.g., primary outcome of the study, secondary outcome, explanatory covariate, etc.)

The primary endpoint will be HRV and peripheral blood samples. Secondary endpoints will include flow-mediated dilation (FMD) of brachial artery and lung function will be collected. Explanatory variable will be "period", ~~"exposure level" in the pilot study, and~~ "treatment" and "GSTM1 ~~and GSTP1~~ genotypes" ~~in the main study~~.

4. **Statistical analysis of the data**

4.1 Who will perform the statistical computations and who will provide interpretation of the results? (The Biostatistics Core does not provide these efforts. Referrals may be available.)

Study investigators will perform the statistical analysis and interpret the results with the assistance of biostatistician.

4.2. What statistical methods will be used?

We will compare the different responses to ~~diesel~~ PM exposure between fish oil and olive oil groups and GSTM1 positive and GSTM1 null genotypes using ANOVA, and the differences between pre-exposure and post-exposure measurement using Student's paired t-test.

4.3. What are the details of the statistical models (if any) that will be fitted to the data? (e.g. ANOVA, linear regression, nonlinear PK models.)

ANOVA will be used for the ~~main~~ study to examine the differences between fish oil and olive oil treatment, GSTM1 null and positive genotypes; paired t-test will be used to examine the differences between pre- and post-exposures.

4.4. For what parameters (e.g., means, differences, etc.) will statistical estimates be obtained ?

Means will be obtained.

4.4.1. Are adequate levels of precision anticipated for these estimates? (e.g., results are not informative if confidence intervals are too wide.)

Yes.

4.4.2. What are your plans for computing confidence intervals for these estimates?

Supposed we will have a normal shape of viable from subjects and we will use 95% confidence intervals values for one-tailed test.

4.5. What hypothesis tests (if any) will be performed ?

Our hypothesis is that ~~diesel exhaust~~PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment will attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower HRV responses cardiovascular risk than the subjects with GSTM1 null genotype when exposed to ~~diesel exhaust~~PM.

4.5.1. What statistical testing procedures will be used for these tests?

ANOVA will be used for the ~~main~~ study to examine the differences between fish oil and olive oil treatment, GSTM1 null and positive genotypes; paired t-test will be used to examine the differences between pre- and post-exposures.

4.5.2. What is your strategy for controlling the probability of type I errors (false discoveries) ?

We control the probability of type I errors at 0.05. If we only have one comparison, then it means a p-value of 0.05 or less will be considered significant. If we have multiple comparisons, since the number of comparisons is not big, we will use Bonferroni adjustment. That is, we compare the p-value of each of the comparison to 0.05/number of comparisons. Then we can decide whether each individual comparison is significant or not.

4.5.3. What levels of power are anticipated for these tests ?

We anticipate power will be 0.8.

4.5.4. Will this study be a success even if none of the tests are statistically significant?

If the primary null hypothesis test is not statistically significant, then the test will be inconclusive.

4.6. What are the plans for coping with incomplete data (if any) ?

Every effort will be made to obtain complete data on subjects according to protocol—including those subjects who do not complete the protocol. It is preferable to include the data from subjects with incomplete response in the primary statistical analysis computations. All available data values will be used whenever possible.

Since we only have ~~15~~ 20 subjects and we do not expect missing data problem would be an issue. Furthermore, when deciding the sample size of ~~15~~20, we have already taken into account the retention. Even if we lose a couple of subjects in some endpoints, we can still get the targeted results.

Several plausible mechanisms have the potential to cause unexpected missing data in this study. It will be necessary to carefully examine the apparent cases of unexpected missing data and subject dropout. The occurrences of unexpectedly missing our mis-timed data values will be carefully examined and explanations will be sought for the causes. If indicated, auxiliary analyses designed for non-ignorable missing data will be performed to gauge implications.

5. What were the primary considerations in choosing the proposed sample size?

The sample size was determined in order to detect a difference of 10% change in HRV assuming

a standard deviation of 9%. Using GraphPad software, an N of 15 was derived using a power of 0.8 and an α of 0.05.

The proposed sample size of ~~15~~ 20 subjects was chosen based on considerations of feasibility, previous experience with levels of precision in similar studies, statistical methods to be used, and formal computations of statistical power for the proposed hypothesis tests. Toward achieving acceptable levels of *precision* and *power*, we have considered information available from previous studies about the standard deviations and correlations that can be anticipated in the proposed study. By making reasonable conjectures about the underlying distributions of key variables, and about enrollment and retention, power calculations useful for sample size selection have been made and have been presented in the discussion of the primary analysis plans. These considerations collectively indicate that the proposed sample sizes and study design are appropriate.

6. What are the details of the randomization procedure (if any) and who will perform the computations?

In the ~~main~~ study, each subject will be randomized to receive either fish oil or olive oil supplementation;. ~~however, we will try to balance the assignment by genotypes so that we have 15 subjects in each of the four study group (fish oil/GSTM1 positive, fish oil/GSTM1 null, olive oil/GSTM1 positive, and olive oil/GSTM1 null).~~ The randomization procedure will be implemented in two stages. In stage 1, the study coordinator will use a computer program to generate a sequence of ID numbers and assignment profiles. The study coordinator will securely store the assignment key in a blinded manner, but with provision of ready access in case of medical emergency. Stage 1 will be completed before enrollment begins. Stage 2, each of the eligible subjects will be assigned to one of the randomization ID numbers. The method of generating the assignment sequence in stage 1 will use a *permuted block* method.

Stage 2 will use the simplest approach—an "envelope system"—implemented as described below. This implementation can be a literal one using paper envelope. Upon completing stage 1, a set of sealed envelopes, each bearing a "randomization ID number" printed on the outside. These envelopes will be available for use one-by-one according to numbered sequence. The study coordinator will open the next envelop whenever she is notified that another eligible subject has be enrolled and needs to be randomized.

Other information / comments:

D. DATABASE MANAGEMENT AND CONFIDENTIALITY

If this is a multi-center trial with a master protocol that sufficently covers all the required details, you may paste all appropriate text in the first box below, OR reference the applicable page numbers of the master protocol. The master protocol must be submitted with this addendum. For more information, contact Clarence Potter, **cpotter@med.unc.edu**, phone 966-4802

1. How will you secure the data from unauthorized access or inadvertent alteration?

All electronic records generated at the EPA Human Studies Facility (HSF) will be kept on a computer-database that is password protected. Subject paper files will be maintained and kept in the EPA HSF nursing station. The EPA HSF building can only be entered by persons with EPA

identification or accompanied by persons with EPA identification. Flow-mediated dilatation data generated in the GCRC will be stored on a stand-alone CPU and access to this data will be limited to the investigators and the necessary GCRC personnel. Other data or patient information generated by the GCRC will be maintained either on the UNC CIS system or medical records system.

2. How is confidentiality maintained of the subject's protected health information (PHI)?

All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files which may contain the subject's name or screening number are secure in the EPA building which has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

3. What are the plans for research database management (RDM)? (i.e., system development, data entry, quality assurance, data querying and editing, data retrieval, data archival, etc.)

The study investigators will be responsible for ensuring data management quality. While the study protocol defined the scientific objectives and methods of the study, additional written guidelines and detailed procedure manuals will be needed for uniform adherence to the detailed intent of the protocol. In the end a written record of how the study was performed is essential. Preferably, this documentation is written prior to the execution of the study and is updated during the course of the study. The study investigators will have overall responsibility for the definition and production of the documents necessary to describe all aspects of the study in sufficient detail to insure the study can be conducted in a scientifically sound, standardized manner. The study investigators will be assisted by their data management staff in monitoring adherence to protocol.

4. How will you verify that the data has been entered into the database correctly from data collection forms or other sources?

Data collection will adhere to precise written instructions included in manual of operations. All data collected will be double-key verified on entry into the data management system. Data collected will be stored in the EPA HSF. This system is password protected and protected by daily archival to magnetic tape.

The error rate should be exceed one erroneous data value per 100,000 data values. For estimation purposes, a 5% random sample of data collection forms, stratified by type, will be compared against the database at six months and then annually during the years of data collection.

This study will involve a variety of data sources. The study investigators will be responsible for managing the data streams, reviewing data-monitoring reports, and investigating questions raised about remarkable data values.

5. Who will perform the computations for RDM?

Study investigators or the HSD QA manger will perform the computations for RDM.

6. What software will be used for database management ?

Database analysis will be carried out using lotus 123, Microsoft excel, and Prism 3 from GraphPad, Inc.

7. How will you validate formulas, functions, or macros to ensure that they will perform data manipulations correctly?

One study investigator will enter the formulas and the other investigator will confirm the entry is correct. And this will be validated by HSD QA manager.

8. What protections are there from lost data?

All paper files will be maintained and kept in the EPA HSF nursing station. All computer-database will be backed up regularly.

Other information / comments:

We do not anticipate using the Informatics Core for database management for out study.

E. NURSING INVOLVEMENT

For more information, contact Nancy Cozart, **ncozart@med.unc.edu**, phone 843-0560, pager 216-6991

Nursing Requirements	Yes	No
1. Do you require vital sign measurement? **If yes,** Indicate frequency: One measurement before diesel exposure and 1 immediately after and 1 at 18 hrs after diesel exposure (these measurements will be done by nurses at EPA).	☒	☐
2. Does your protocol require blood sampling? Hospital labs and/ or study kit: Lab? Blood sampling will occur at EPA medical station.	☒	☐
3. Will you be using the GCRC investigator lab for **specimen processing?**	☐	☒
4. Will you be using the GCRC investigator lab for **temporary (< 90 days) sample** storage?	☐	☒
5. Are you requesting GCRC staff to **process any samples**? GCRC can only be responsible for sample processing after 1pm each day. If yes, provide processing and storage instructions.	☐	☒
6. Does your protocol require IV placement, IVF administration, and monitoring? If yes, please provide details	☐	☒
7. Does the protocol involve **administration of chemotherapy, drugs** or study medication **infusions**? Length of infusion: Repeated (frequency):	☐	☒
8. Does the protocol require **other tests or procedures**, such as :	☒	☐

Nursing Requirements	Yes	No
EKG, PFT, etc?		

Procedure	Description / Who will perform procedure and where?
Brachial artery ultrasound (BAU)	BAU will be obtained by Dr. Alan Hillderliter or a research technician instructed by him at NCMH using a 12.5 MHz image probe interfaced with an ATL HDI 5000 ultrasound machine.
ECG	ECG will be obtained by physicians and nurses at EPA.
PFT	PFT will be performed by investigators in this study.
Endo-PAT	Endo-PAT will be performed by investigators in this study.

	Yes	No
9. Are you requesting any assistance to **perform study interventions?** i.e., exercise testing, patient education.	☐	☒
10. Will you have any **special patient care** needs, such as telemetry, chest PT, isolation, indwelling catheters? Please describe:	☐	☒
11. Does the protocol require an **inpatient stay**? (over midnight admission)	☐	☒
Will you be able to do advanced scheduling (at least one week before)?	☐	☐
12. Does the protocol require **outpatient visits**? Please estimate length of outpatient visits (30 minutes, 1 hour, etc.) 1 hr for 3 times.	☒	☐
13. Will your protocol require unscheduled (< 48 hours notice) visits?	☐	☒
14. Are you requesting that the GCRC provide any supplies or special equipment necessary to conduct the protocol? If yes, describe here and add to Cost Assignment Sheet. BAU will be measured at NCMH using a 12.5 MHz image probe interfaced with an ATL HDI 5000 ultrasound machine.	☒	☐
15. Describe any other requests of GCRC nursing services: None.		
16. Are there personnel working on the protocol that have not been oriented by GCRC staff? If so, please contact Nancy Cozart prior to study initiation to complete this orientation.	☐	☒

Intervention	Description

Nursing Requirements	Yes	No
Other information / comments: The GCRC nursing stuff will only be asked to check the subjects in and out as outpatients; there are no requests for data to be collected during this process. The endpoint to be measured is flow-mediated dilation as measured by brachial artery ultrasound which will be obtained by study investigator Dr. Alan Hillderliter or a research technician instructed by him.		

For extensive outpatient visits or inpatient admissions, a Time and Event schedule is required to outline the requirements of your protocol. Use the sample format found at: **http://gcrc.med.unc.edu/ftp/forms/time_event.dot** *(note: double click on the URL in the preceding text box and paste into your browser.)*

F. Nutrition, Body Composition, and Research Kitchen

For more information, contact Marjorie Busby, **mgbusb@med.unc.edu**, phone 966-4746, pager: 216-3469

Please define **ALL** of the specific procedures that you request the GCRC Nutrition Service to carry out during the course of the study:

Nutrition Requirements	Yes	No
Inpatient All inpatients require meal description. Describe meal below.	☐	☐
Outpatient	☒	☐
If yes to either inpatient or outpatient meals, please describe the type of diet, e.g., research, regular; or nourishment required: Please provide subjects with "research" lunch on the BAU measurement day. In the pilot study, this will be a low-fat lunch; in the main study, the meal should not contain a significant source of omega-3 fatty acids.		
Measurements by Research Nutritionist	Yes	No
Subject Screening for Research Diet Adherence	☒	☐
Nutrition Assessment / Diet History	☒	☐
Food Frequency Questionnaire	☒	☐
Food Records / Dietary Intake (# of days per patient)	☒	☐
Diet Instruction	☒	☐
Nutrient Intake Analysis of Research Diet	☒	☐
Other: These measurements will be obtained by Dr. Susan Steck, a nutritionist at the University of South Carolina.		
Body Composition Measurements	Yes	No
DXA (bone density, body composition) Which measurements? If so, fill out the DXA Radiation Safety form (**http://gcrc.med.unc.edu/forms/diet/radcommitteeapp-dexa.doc**). Send the completed Radiation Safety Form to the Radiation Safety Office. The	☐	☒

address is on the form. .		
peripheral QCT (bone structure) If so, fill out the pQCT Radiation Safety form **(http://gcrc.med.unc.edu/forms/diet/radcommitteeapp-pQCT.doc)**. Send the completed Radiation Safety Form to the Radiation Safety Office. The address is on the form.	☐	☒
Indirect Calorimetry (Resting Energy Expenditure, Respiratory Quotient)	☐	☒
Bioelectric Impedance Analysis	☐	☒
Anthropometric Measurements	☐	☒
Physical Activity Monitors	☐	☒
Physical Activity Questionnaires	☐	☒
Other information / comments:		

G. GCRC BIOANALYTICAL CORE (BAC) LABORATORY

For more information, contact David Barrow, **david_barrow@dentistry.unc.edu**, 962-0633

Here's the URL to review BAC Laboratory Policies is
(http://gcrc.med.unc.edu/investigators/corelab/BACLabPolicies.doc).

The URL for assay planning document is (**http://gcrc.med.unc.edu/forms/BACAssay.xls**). Identify the labs and quantities in the planning document, then email the document to David Barrow prior to submitting this addendum to the GCRC. After coordinating with David, attach the final version of the assay planning document, to this addendum. (*note: double click on the URL in the preceding text box and paste into your browser.)*

	Yes	No
Will the BAC lab store samples for the investigator? If so, for how long?	☐	☒

H. GCRC Specimen Processing Facilities

	Yes	No
1. If so, have the staff that will use these facilities completed the GCRC lab online training? If not please go to **http://gcrc.med.unc.edu/investigators/labtraining**	☐	☒
3. Do you need temporary (< 90 days) storage of samples in the GCRC freezers?	☐	☒

I. Cost Assignment Sheet

For more information, contact Mary Beth Lister, 966-4767, **marybeth_lister@med.unc.edu**

Cost Sheet.xls must be attached to this addendum before submitting to the GCRC. It was included in the zip file with this document. It can be downloaded from **http://gcrc.med.unc.edu/forms/cost%20sheet.xls**. *(note: double click on the URL in the preceding text box and paste into your browser.)*

What are the funding sources for this protocol? Check all that apply

☐ Industry-Funded

Must choose one: ☐ Industry-Initiated
☒ Investigator-Initiated

If Industry-Initiated, check if applicable: ☐ Rare Disease
☐ Therapeutic Disease Network

If you marked industry-funded and investigator-initiated, please provide a **justification** for the GCRC Clinical Research Advisory Committee to consider this a Category A protocol. Use the criteria found at **http://gcrc.med.unc.edu/investigators/admin/category.htm** :

Justification goes here

Check any other sources of funding:

☐ Department Funds

☐ NIH (Please provide Grant #) ___ ___

☒ Other (Please specify) _X__EPA intramural federal research ___

☐ No funding source outside GCRC resources

J. Data Sharing Plan

As of October 1, 2003, the NIH requires all investigators requesting $500,000 or more in direct costs in any single year to include a plan for data sharing or to state why data sharing is not possible. For more information on the NIH policy on data sharing, click on http://grants.nih.gov/grants/policy/data_sharing/. The General Clinical Research Center supports the concept of data sharing and agrees that data sharing is essential for expedited translation of research results into knowledge, products and procedures to improve human health.

	Yes	No
Do you have an NIH grant totaling $500,000 or more in direct cost funding per year?	☐	☒

If you checked yes, please select one of the following options:

☐ I agree to share data collected for this research study in accordance with NIH regulations upon publication of the main findings from the final data set in a timely manner (provide below a description of your data sharing plan).
☐ Research data will not be made available for sharing as explained below (state reason why data sharing is not possible).
Description of data sharing / Reason for not sharing:

Checklist for submission to the GCRC

Required sections for all applications:

☒ A. Safety Monitoring Plan
☒ C. Biostatistical considerations
☒ D. Database Management and Confidentiality of Research Data
☒ I. Cost Assignment Sheet
☒ J. Data Sharing Plan

Must be submitted if you are requesting use of these services:

☒ B. Investigational Drug Service
☐ E. Nursing
☐ F. Dietary/Nutrition Services
☐ G. GCRC Bioanalytical Core Laboratory

We continuously strive to improve this process, please send suggestions for improvements or corrections to this form to **gcrc-sys-mgr@med.unc.edu**.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 12/18/2008
Expiration Date of Approval: 2/09/2009

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment dated 12/08/09 addresses:

1. change in the description of the particle exposure session based on change in monitoring methods to ensure subject safety without any changes to other study methodology.
2. change in concentrations of omega-e fatty acide based on updated information from provider.
3. updated IRB application with these changes.

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, Environmentally Protection Agency; Robert Truckner, (EPA), Non-IRB Review Contact; Wanda Simmons, (CTRC), Non-IRB Review Contact

First Review of IRB Submission Modification

Training Not Met (Haiyan Tong, Michael Schmitt, David Demarini, Martin Case, Mary Bassett, Tracey Montilla)

Receipt Date : 12/08/2008 **Expiration Date :** 2/09/2009 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Sonya H Bateman

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

This amendment dated 12/08/09 addresses:

1. IRB application/protocol change in the description of the particle exposure session based on change in monitoring methods to ensure subject safety.

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter:
  - ☒ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 12/18/08)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☒ Hard copy sent

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☒ Approved 12/18/08
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

07-0190

RECEIVED
DEC 08 2008
IRB UNC-CH

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

RECEIVED
12/12/08
CTRC

back to IRB 12/16/08

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
□	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1) We have modified the description of particle exposure session in the IRB protocol (page14-15) without changing the air pollution type, concentration, concentration limit of exposure shutdown, and exposure duration. The changes came after the TRC engineer support group has changed the monitoring method to ensure the safety of subjects. The changes are as the following,

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure. During the

air exposure days, airflow into the chamber will be filtered to remove a minimum 99% of ambient air particles.

The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will range between levels of 50 to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously by a DataRAM monitor (DR4000; Thermo Electron Corp) calibrated to filter obtained measurements of particle ug/m^3. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The computer is programmed to initiate a shutdown procedure if chamber particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. The resting minute ventilation during exposure will be measured. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

2) We have changed the concentration of EPA and DHA in omega-3 fatty acid in the protocol (page11) based on the updated information from the provider.

EPA concentration will be 410 mg and DHA will be 274 mg to replace the 25-35% of EPA and 30-65% of DHA respectively.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: December 8, 2008**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD (if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

Haiyan Tong
Signature of Principal Investigator or designee — Date 12/8/08

Signature of Faculty Advisor (if applicable) — Date 12/8/08

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

07-0190

08-2075
RECEIVED
DEC 10 2008
IRB UNC-CH

Part A.1. Contact Information, Agreements, and Signatures

Date: December 8, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID:
Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _x_ postdoc __ resident __ other

Name of faculty advisor: James Samet, PhD
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department:
Mailing address/CB #:
Phone #:
Fax #:
Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH

__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
x	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
x	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
□	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
□	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*

	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for* ***Consent for Stored Samples***.	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.
Participants Forty healthy 50-75 year-old male and female subjects will be involved in the study.
Procedures (methods): The subject will be exposed to concentrated ambient fine and ultrafine PM for 2 hours after being randomly supplemented with 15 days of omega-3 fatty acid or olive oil.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal

studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (15-17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (20). Therefore, the role of GSTs in mediating the cardiovascular effects following acute PM exposure will also be studied in the protocol in GSTM1 null and GSTM1 positive healthy older adults.

The respiratory effects of PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.

7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
15. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal is approximately 40 subjects for the study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by

criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This will be a randomized, double-blinded study. Forty study subjects will be randomly given fish oil or olive oil supplements for 15 days., The subjects will be given a container filled with 15 days supply of enteric-coated either 2g/day of fish oil or 2g/day of olive oil capsules. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 410 mg of eicosapentaenoic acid (EPA) and 274 mg of docosahexaenoic acid (DHA). Each 1000 mg of this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. Each subject will be exposed to air containing fine and ultrafine PM (PM<2.5) for 2 hours in an exposure chamber after the supplementation. (See the study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotyping consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 25, methods of recruiting) will not need genotyping. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 80 ml of blood collected. A portion of the blood will be used for genotyping of GSTM1 and another portion of the blood will be used for the cholesterol levels, phospholipid and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back to the medical station.

- ***GSTM1 genotyping***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null.
- ***Food frequency questionnaire in the study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses.

Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Baseline HRV measurement***: Subjects will have their baseline 24-hour heart rate viability (HRV) measured by Holter monitor on the training day. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Subjects will be released while wearing the Holter monitor and return to the HSF the next day to get the Holter monitor removed. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.

- ***Fish oil or olive oil pretreatment and dietary assessment in the study:*** Subjects will be randomly given an approximately 15 days supply of either fish oil or olive oil supplements. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record per week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 15 day of supplementation period. We will give the subjects another 15 days supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU) and Endo-PAT, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained.
- ***Brachial artery ultrasound:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual

nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Peripheral endothelial function by Endo-PAT***: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be induced by sudden cuff deflation. The PWA recordings will be measured continuously during suprasystolic occlusion and deflation reactive hyperemia. PAT measurement will be displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).
- ***Pulmonary function*** will be measured by spirometry.
- ***Blood sampling***: approximately 80 ml of blood sample will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

Exposure:

The subjects will be exposed to concentrated ambient fine and ultrafine PM after the dietary supplementation.

Prior to exposure, the height of subject's mouth while seated will be measured so that the particle tubing height can be adjusted. All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. During the exposure, subject will be monitored continuously by visual camera monitor, continuous pulse oximetry, blood pressure every 15 minutes, and telemetry. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C.

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.

The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will range between levels of 50 to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously by a DataRAM monitor (DR4000; Thermo Electron Corp) calibrated to filter obtained measurements of particle ug/m^3. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The computer is programmed to initiate a shutdown procedure if chamber particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. The resting minute ventilation during exposure will be measured. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 80 ml of blood samples will be collected.

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 80 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound) Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual

nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Finger arteries Endo-PAT. Changes in pulse wave amplitude of index fingers caused by reactive hyperemia will be expressed as a percent change relative to resting baselilne values.

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In this study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he

feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.
- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound and finger arteries Endo-PAT:** There are no known risks associated with imaging of the brachial artery and index finger arteries. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will range from 50 to 600 ug/m^3 and

the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test the hypothesis that PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.

We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to PM will also be measured (e.g., blood pressure, HRV, brachial arterial diameter, change in blood vasoactive, inflammation and coagulation factors). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 of either fish oil or olive oil supplemented subjects was derived using a power of 0.8 and an α of 0.05. Therefore, we will recruit 20 subjects in each group in this study.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of PM on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen, D-dimer, and platelets, changes in IL 6 and 1L 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data**. Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no

Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no

Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the study will take approximately 18 months. Participant recruitment is expected to be continuous throughout the study until the intended number of participants is reached.

The individual will have 6 visits to the research facility over approximately 4-5 weeks. The pre-enrollment and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after the blood work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment and baseline HRV measurement) will require approximately 3 hours. Exposure day will last approximately 8 hours.. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study. If the subject is qualified and finished the entire study, the total compensation for completion of this study will be approximate $1225

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total =	$60
Training day (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Finger artery Endo-PAT (2 @$20 each)	$40
Food records (2 times)	$300
FFQ	$50
On-time bonus	$25
Total for completion of exposure =	$843
Eighteen hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Finger artery Endo-PAT	$20
Total for completion of post-exposure =	$86
Protocol Completion Bonus	$100

Approximate TOTAL for completion of study = $1225

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in

effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

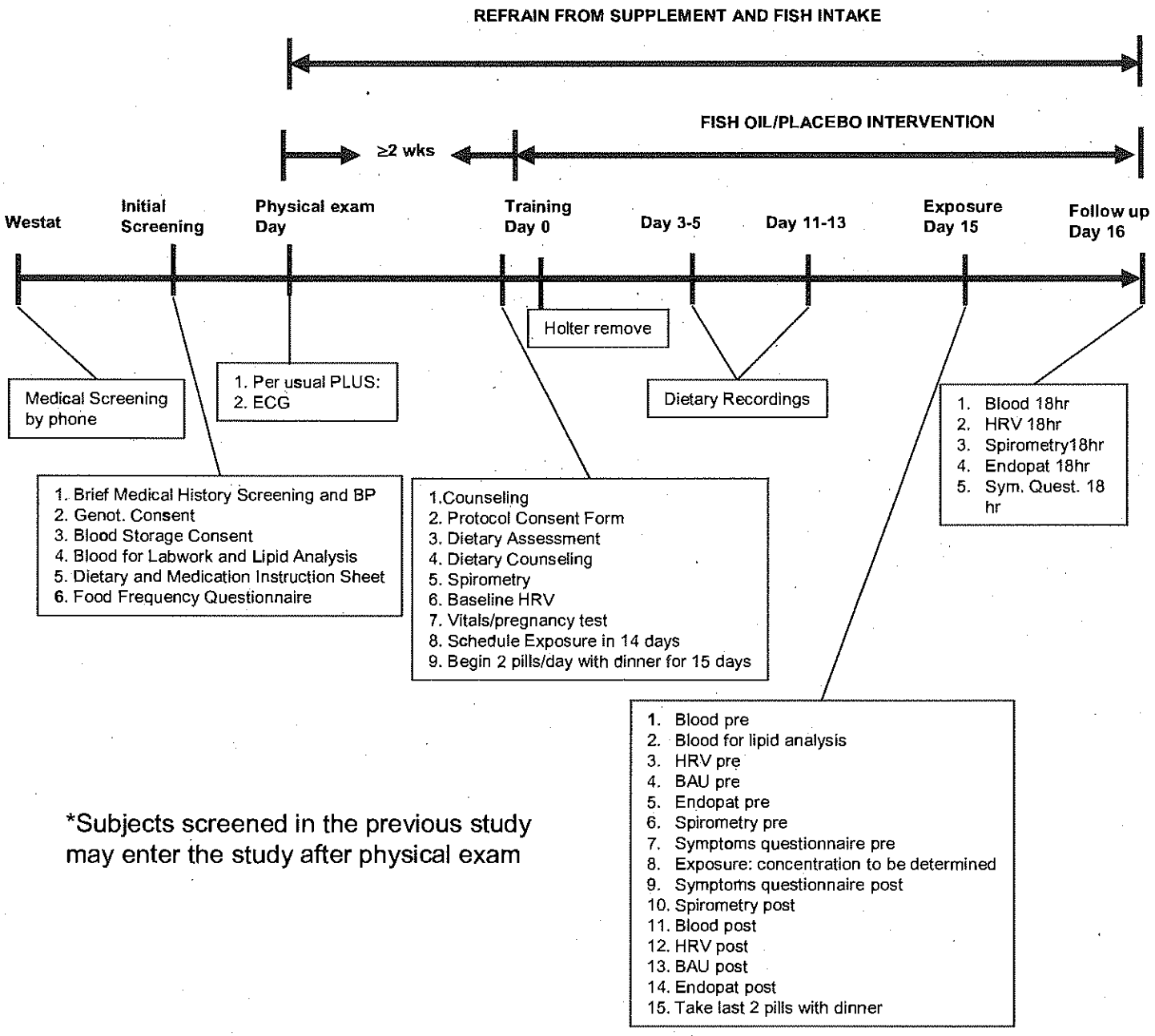
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	**NONE**	**TRACE**	**MILD**	**MODERATE**	**SEVERE**
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



*Subjects screened in the previous study may enter the study after physical exam

STUDY FLOW DIAGRAM

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

TO: Haiyan Tong
Environmentally Protection Agency
CB# 7315

FROM: Biomedical IRB

DATE: 12/10/2008

RE:
Study #: 07-0190
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

The UNC IRB Office received a modification form and application today for the above named study. However, this is a CTRC (formerly GCRC) study and should have been forwarded to them first for their approval. We are sending these forms to the CTRC (formerly GCRC). In the future, please send all CTRC studies to their office for approval before forwarding them to the IRB Office. Thank you.

UNC IRB Office

First Review of IRB Submission
Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 12/08/2008 **Expiration Date :** 2/09/2009 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☑ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Sonya H Bateman

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

This # no longer valid.

First Review of IRB Submission
Initial

Training Not Met (Haiyan Tong, Susan Steck, Michael Schmitt, Tracey Montilla, Fernando Holguin, Margaret Herbst, David Diaz-Sanchez, David Demarini, Martin Case, Mary Carraway, Mary Bassett)

Receipt Date : 12/10/2008 **Expiration Date :** **Previous Review Type:**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 08-2075 **IRB Coordinator :**

Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

☐ Not-HSR ☐ Exempt (Category:) ☐ Not Full IRB (Category:) ☒ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Wyomie Milliken

Study Description:

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GXTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. The PI will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM. Participants: Forty healthy 50-75 year-old male and female subjects will be involved in the study. Procedures (methods): The subject will be exposed to concentrated ambient fine and ultrafine PM for 2 hours after being randomly supplemented with 15 days of omega-3 fatty acid or olive oil.

PROCESSING STEPS (OFFICE USE ONLY):
- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached:
Other attachments:

FINAL ACTIONS:
- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB 2/6/09

Authorized signature on behalf of IRB

Approval Date: 2/06/2009
Expiration Date of Approval: 2/01/2010

RE: Notice of IRB Approval by Full Board Review
Submission Type: Renewal
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated.

Study Description:
The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:
With this renewal, an amendment dated 1/12/09 address:

1. change in the dose and duration of dietary supplementation.
2. updated IRB application
3. updated consents
4. updated recruitment materials

Regulatory and other findings:
Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Investigator's Responsibilities:
Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Should any adverse event or unanticipated problem involving risks to subjects or others occur it must be reported immediately to the IRB using the adverse event form at the same web site.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

NC TRACS INSTITUTE The North Carolina Translational and Clinical Sciences Institute

RECEIVED
FEB 12 2009
IRB UNC-CH

TO: Dr. Haiyan Tong

FROM: Dr. Michael W. Fried, Director

DATE: February 10, 2009

SUBJECT: Approval of Renewal for **GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust IRB Study #: 07-0190**

Both the Clinical and Translational Research Center Oversight Committee and the Committee on the Protection of the Rights of Human Subjects have reviewed this proposal and have approved it until **February 1, 2010.** Approximately two months prior to its expiration date, you will receive a renewal reminder.

REMEMBER:
This approval is contingent on your citing the CTSA grant number (UL1RR025747) and/or the GCRC grant number (M01RR00046) in any publications that might ensue from the work conducted on this NIH funded center.
For recommended language to use in your publications, please see http://gcrc.med.unc.edu/investigators/citation.htm

Inpatient admissions should be scheduled with Ms. Uli Blankenship (843-0560) and outpatient visits with Ms. Sherry McLaurin (6-1437). The approved consent form must be signed by each patient before any studies are done.

Should any significant side effects occur in any volunteer participating in this study, you are expected to inform the Director of the Clinical and Translational Research Center and the Chairman of the Human Rights Committee immediately.

Michael W. Fried, MD, Director

Enclosure

cc: Biomedical IRB

CLINICAL AND TRANSLATIONAL RESEARCH CENTER
UNC HOSPITAL ROOM 3005
CAMPUS BOX#7600 • CHAPEL HILL, NC • 27599-7600
PHONE: 919.966.1435 • FAX: 919.966-1576

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 2/6/09 AND 2/1/10
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Storing Biological Specimens With Identifying Information 2

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: January 8, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; BA; Dave Peden, MD, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Air pollution exposure has been found to cause a range of lung and systemic changes in normal subjects. We will be collecting blood samples which will help us to further study this condition. In order to do so, we will need to collect and store blood. Blood samples will be collected in order to look for but not limited to indicators of inflammation due to the Air pollution exposure. We may further isolate RNA from blood for genetic analysis. RNA may be isolated for genetic analysis to see if there is a difference between pre- and post Air pollution exposures.

All samples will be stored where only project members will have access to the samples. There is a need to store samples in such a repository because this will be an ongoing study where samples from subjects will be collected over an extended period of time. Storing of samples allows for all samples to be processed at the same time and also allows our scientist the opportunity to further study these samples with as yet unknown questions and techniques.

How will the specimen be collected?
The description of the samples to be collected and the manner in which this will be done have been described in the main study consent.

What will happen to the specimen?
Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the Air pollution exposure. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens (or data) for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens (or data) will be provided without identifiers to these other researchers by employing a data use agreement.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding the cardiovascular effects of Air pollution exposure in older population.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:

1. Blood sampling will be performed by medical personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be

Initial/Date _____

entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you for storage of the specimens?
There will be no cost to you for the storage and use of the specimens for research purposes.

Will you receive anything for being in this study?
You will not receive anything for taking part in this study with regard to storage of excess specimens. Reimbursement for participation in the main study is addressed in the detailed consent for that study.

Who owns the specimens?
Any blood, body fluids, or tissue specimens obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Initial/Date _____

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the specimens from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Initial/Date _____

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 2/6/09 AND 2/1/10 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

3

Biomedical Form

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: January 8, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits and during the study. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Use olive oil for cooking, dressings, and sauces during the study.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 40 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

We will perform a baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 24 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

Initial/Date _____

Exposure day
We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we do today).
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- We will bring you back to the HSF where we may also measure the pulse wave of your finger arteries by a non-invasive method Endo-PAT 2000 (called Endo-PAT) at HSF and we will compare the findings with ultrasound measurement of the artery in your arm. We will place two small PAT probes to both of your index fingers. The procedure is similar to that of ultrasound measurement and we will only place a blood pressure cuff on one of your arm. We will obtain recordings for 5 minutes at rest, 5 minutes during the blood pressure cuff inflated, and 5 minutes after the blood pressure cuff released.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to concentrated Chapel Hill air particles; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. Due to weather conditions, some chamber exposures will either be cancelled or terminated and you will be asked to re-schedule. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation. We will check your resting breathing rate during exposure.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- We will obtain another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another Endo-PAT measurement at HSF.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to

Initial/Date _____

respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induces an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Finger Endo-PAT: Similar to the measurement by brachial artery ultrasound, there are no significant risks associated with index fingers arteries measurement.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this

Initial/Date _____

consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1225.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total = $60	
Pre-exposure screening/training (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure sessions (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100

Initial/Date _____

Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Brachial artery ultrasound (2@ $50 each)	$100
Finger Endo-PAT	$40
Time (8h @$12/h)	$96
Food record (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $843

18 hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3 hrs @$12/h)	$36
Finger Endo-PAT	$20

Day 3 total for completion of post-exposure = $86

Protocol Completion Bonus	$100

Approximate TOTAL for completion of the study = $1225

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening

Initial/Date _____

history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

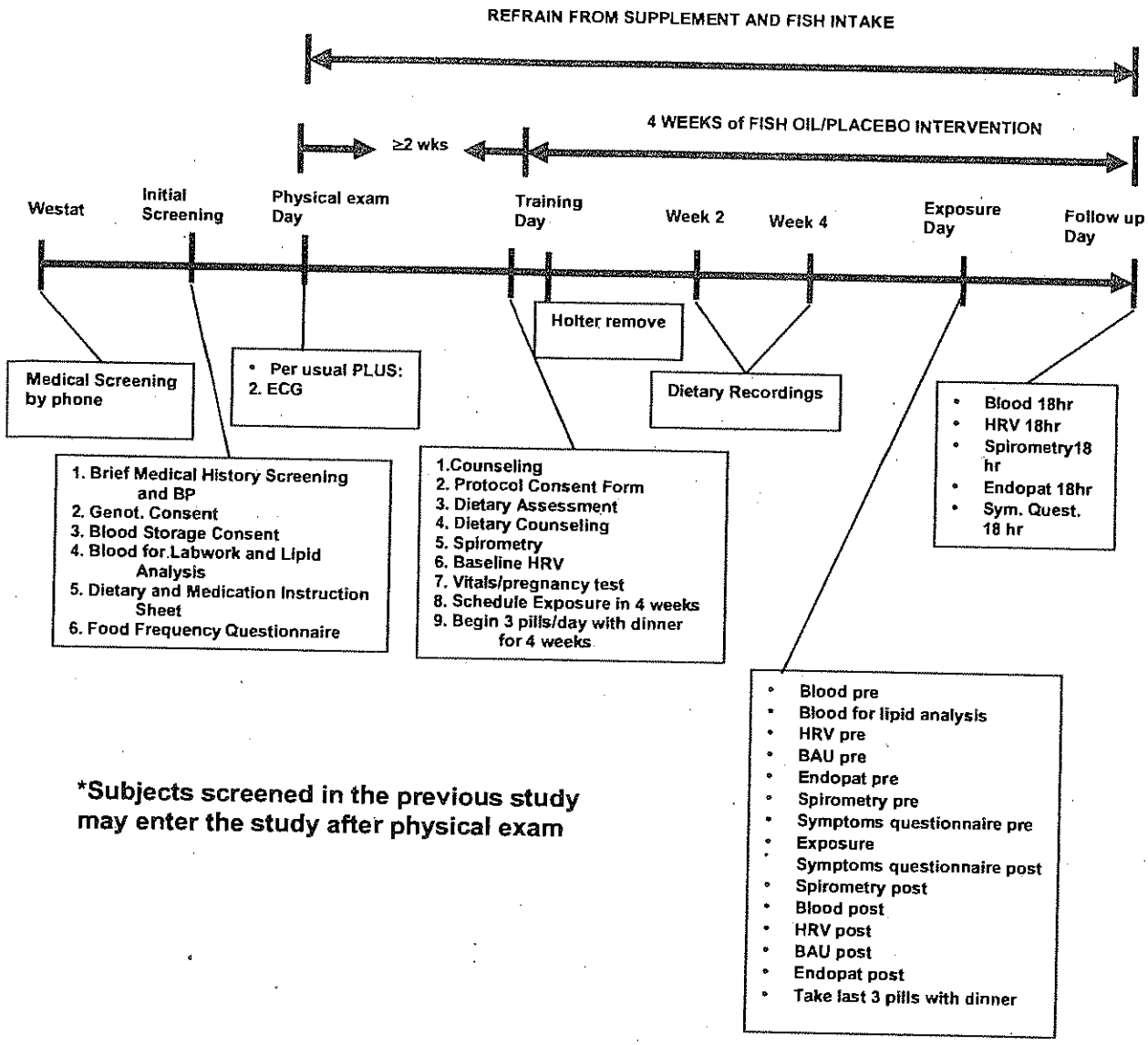
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 2/6/09 AND 2/1/10 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotyping With Identifying Information 1

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: January 8, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.
What is the purpose of this study?

Initial/Date _____

Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to air pollution. The purpose of the genotyping is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 40 participants in this study.

How long will your participation last?
Participation in this genetic session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

At the end, we will give you a copy of the Medical History Form and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and you will be instructed to mail the FFQ. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?

- You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do

Initial/Date _____

not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol). You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate.

Who owns the blood samples?

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?

Initial/Date _____

You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the

Initial/Date _____

researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

FEB 0 6 2009

OMEGA STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1225.

Procedures:

Volunteers initially undergo a blood test and genotype screening, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genotyping visit and $30 for the additional medical screening. If you are eligible for the study your participation will last approximately 6 to 7 weeks.

Participation will begin with a training visit that will last about 3 hours and a return visit the following morning. Following training you will be scheduled for four-weeks of fish oil or olive oil treatments (taken as capsules) prior to the exposure session. You will be asked to record your food intake twice for 3 days. The exposure day lasts 8 hours and the follow up day lasts about 3.

Procedures involved in this study include breathing tests, heart rate monitoring, including wearing overnight monitors after training and the exposure, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be able refrain from certain foods and medications for at least six weeks and to take the supplements for four weeks prior to the exposure. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-0518, previously 95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Genotyping Visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (1 hour)

Physical Exam: days and times TBD (2 hours)

Training (3 hours)

Dietary Supplementation (4 weeks)

Training Follow-up Visit Day 1 (to return the Holter Monitor, 30 min)

Dietary Recordings, week 2 and week 4 (3 days/week)

Exposure Day (end of 4 weeks supplementation): 8:00 AM (8 hours)

Follow-up Day: 18 hours after Exposure Day (3 hours)

Dietary restrictions

Participants are asked to eliminate use of nutritional supplements containing omega-3 fatty acids and antioxidants (e.g. beta-carotene, selenium, vitamin C, vitamin E, zinc) for **two weeks** prior to beginning the intervention and during the intervention.

During the intervention, participants are asked to refrain from consuming the following foods:

1. All types of fish and shellfish
2. Walnuts
3. Flaxseed and flaxseed oil
4. Rapeseed oil
5. Canola oil
6. Soybeans and soy products
7. Eggland's Best eggs
8. Alcoholic beverages

FEB 0 6 2009

Food Record Instructions

Thank you for participating in this important research study.

During the course of the study, we are asking you to complete food records for three days each week. Please record everything you consume (food, beverage, and/or supplements) on the notepad provided for three days each week, with two of the three days being weekdays, and one of the three days being a weekend day.

For each eating occasion, please write down the following:
1) date
2) time of day
3) location (if not at home, please provide name of restaurant or eating establishment).
Then list all items consumed in as much detail as possible. Next to each item, write down the estimated portion size. Use the portion size booklet provided to guide you in estimating the serving size of each food and beverage item. For mixed dishes, please list all ingredients and describe the preparation method (e.g., baked or fried entrée?; cooked or raw vegetables?). Remember to list all beverages, including water. Please write as clearly as possible. You may be contacted by phone by the study nutritionist for clarification if needed.

An **example** of breakfast, snack and lunch on one day follows:

January 5, 2009
6:30am
Home
2 cups coffee, black
1/3 cup Kashi Go Lean Crunch cereal
½ cup Cheerios, regular
½ cup skim milk
4 ounces cranberry-grape juice (100% juice)

FEB 0 6 2009

January 5, 2009
11:00am
Work
6 ounce container fat-free Stonyfield Farm vanilla yogurt

January 5, 2009
12:30pm
Work
1 pimento cheese sandwich, with 2 slices 100% whole wheat bread, ¼ cup pimento cheese (store-bought pimento cheese)
1 medium-size Gala red apple
8 ounces water

OMEGA STUDY
E-mail Announcement

SUBJECT: INFORMATIONAL: Research study for Healthy Older Adults

The main purpose of this research study is to determine if a component of air pollution, particulate matter (PM) elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Volunteers ages 50 to 75 will be tested to determine the type of a particular gene they carry, one of several genes responsible for protecting the body against oxidants such as diesel exhaust. Participation will include four-weeks of fish oil or olive oil treatments and one exposure to a controlled amount of diesel PM. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Office of Human Research Protection (OHRE) Biomedical Institutional Review Board. IRB # 07-0190 (GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

FEB 0 6 2009

The US Environmental Protection Agency is seeking

ADULT VOLUNTEERS

Ages 50 to 75 for Research

This is a research study about genetics, diet supplementation and exposure to air pollution.

We are looking for healthy older adults to study diet supplementation and the effects of air pollution exposure on heart and lung function.

Total time commitment after screening is about 15 hours over 6 to 7 weeks. You will receive payment for screening, the study, and out of town travel. Parking is provided.

1-888-279-9353 or

919-966-0604

www.epastudies.org

In care of the environment • WESTAT • EPA Recruiting

The Human Studies Facility is located on the UNC-CH campus

FEB 06 2009

Web Site Advertising: www.epastudies.org

OMEGA: Elder Diet and Air Pollution Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as PM, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

Participation in this study will include four -weeks of fish oil or olive oil treatments and one exposure to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. Study procedures include breathing tests, heart rate monitoring, blood tests, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. Total time commitment after screening is about 15 hours, over 6-7 weeks. Compensation is provided.

FEB 0 6 2009

Response to Minor Stipulations
Renewal

Receipt Date : 1/14/2009 **Expiration Date :** 2/09/2009 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :** Joy Bryde

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☑ Full Board Review ☐ Not Full Board Review

Study Description :

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description :

With this renewal, an amendment dated 1/12/09 address:

1. change in the dose and duration of dietary supplementation.
2. updated IRB application
3. updated consents
4. updated recruitment materials

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter:
  - ☑ ~~Draft~~ letter prepared JNB 02/05/09
  - ☑ Approved by chair as attached (Initials/Date: 2/6/09)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 3

Other attachments: ~~4~~ 6

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to Full IRB
- ☐ Return to sender
- ☐ Termination

RECEIVED
FEB 05 2009
IRB UNC-CH

To: IRB,
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

From: Haiyan Tong
Environment Protection Agency
CB# 7315
E-mail: tong.haiyan@epa.gov
Phone number: 966-4993

RECEIVED
2/5/09
CTRC

February 5, 2009

Study #: 07-0190
Other #: GCRC: 2579
Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Dear IRB review committee,

Thanks for the Board Review for the above study! Enclosed are the revised sbumission in response to the "Contingencies Approval on 2/3/09".

To clearify the study specific details:

On the renewal form, question 2d, "number to be included in upcoming year", the number of subjects has been changed to 40 in the revised IRB renewal form.

Please let me know if you have any questions regarding this revision.

Sincerely,

Haiyan Tong

Haiyan Tong

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
REQUEST FOR RENEWAL OF IRB APPROVAL OR STUDY CLOSURE
version 8-Sep-2008

RECEIVED
FEB 05 2009
IRB UNC-CH

If the research is continuing:

- Check the relevant items.
- Include two collated sets of copies (sorted in the order listed) of checked items.

→ **Submissions will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This form (renewal or closure).	2
x	2. Any items specifically requested in questions # 4 through 9 (in that order).	2
x	3. The most recent application submitted for IRB approval. This application should be updated to include any modifications since the study was initially approved or last renewed. **If there are any new modifications included with this renewal, highlight the proposed modifications by underlining.**	2
x	4. Clean copies of all consent document(s) to be used in the upcoming approval period, for stamping.	2
x	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

IRB study #: 07-0190 **Date:** February 5, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor** (if applicable): James Samet, PhD

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

1. In a few sentences, describe the past year's work, and describe what you plan for the upcoming year, including data analysis, if relevant.

We have finished the pilot phase of the study. We plan to start the main phase of the study in the upcoming months and we expect the study will last about 11/2 to 2 years. We have modified the type of air pollution and reduced the number of subjects in the main study which was approved in August of 2008.

2. Number of subjects involved through direct contact or use of their data (for multi-site studies, include only subjects covered by this IRB) (Note: **b+d** should not be larger than **a**)
   a. Total projected number as approved by IRB: 6 subjects in the pilot phase and 40 in the main phase.
   b. Total number of subjects involved to date: 8 subjects in the pilot phase (2 subjects were disqualified before the exposure).
   c. Number of subjects added since last renewal: 4 subjects in the pilot phase.
   d. Number to be included in upcoming year: 40 subjects in the main phase.

Answer the following questions based on information since initial approval or last renewal. Only include subjects covered by this IRB.

3. Have there been any modifications approved since the last review? If research is continuing, be sure all changes are reflected in the attached revised application for IRB approval.	_x yes __ no
4. Have any subjects withdrawn voluntarily or been withdrawn from the study? **If yes, explain**; give number and reasons for withdrawals.	__ yes _x no
5. Have there been any findings (e.g., publications) that alter the risk/benefit ratio or otherwise impact the study? **If yes, explain.**	__ yes x_ no
6. Does this study have a Data and Safety Monitoring Committee (DSMC or DSMB)? **If yes, provide a report** from the DSMC.	__ yes x_ no
7. Have there been unanticipated problems or serious adverse events since the last renewal? **If yes, include all copies** of local Adverse Event reports with this submission.	__ yes x_ no
8. Has this study been audited by external sponsor or monitor since approved or last renewed? **If yes, include a copy** of the audit report.	__ yes _x no
9. Are you requesting any changes? **If yes, include the form** requesting *Modification of Approved Human Subjects Research* and underline the proposed change in the updated application.	_x yes __ no
10. Will you be enrolling, consenting or re-consenting subjects in the upcoming approval period? **If yes, include clean copies** of consents/assents/fact sheets to receive a new stamp. **Include any new recruitment materials** to be used with subjects.	_x yes __ no

Action requested by Principal Investigator (choose only one):

Renew approval:

__ Study has always involved *only* analysis of existing data or specimens. Continue as approved.

Study involves(ed) direct interaction/intervention or contact with subjects:

_x_ Continue as approved: Enrollment of new subjects continues.

__ Enrollment of new subjects closed; interaction/intervention with previously enrolled subjects continues.

__ Direct interaction with subjects completed but subsequent monitoring or follow up continues.

__ Subjects' involvement completed but renewal is requested for data analysis.

Closure of Study:

__ Research completed: Identifiable data or human biological specimens are stored according to plan already approved by the IRB.

__ Research completed: All data or human biological specimens are deidentified.

__ Lack of funding or other *(specify)*:

Signature of Principal Investigator — Date: 2/5/09

Signature of Faculty Advisor (if applicable) — Date

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB 2/4/09

Date: 2/03/2009

RE: Minor Contingencies to be addressed following Full Board IRB Review on 2/02/2009
Submission Type: Renewal
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

This submission has been reviewed by the above IRB at a convened meeting. This is not an IRB approval. You may not implement the research activities described in your submission until you have received a memo indicating final IRB approval. The IRB determined that this submission MAY BE APPROVED, pending stipulated change(s) and/or clarification(s) as detailed below.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the item(s) listed. **When you respond, please be sure to cite the IRB study number noted above, and indicate that you are responding to this memo as dated above; failure to link your response to this study, in general, and this memo, in particular, may result in delays.** Any additional changes (including new materials) must also be listed and discussed in the memo. If the application or any study materials must be revised, please submit 2 copies of all revised materials, one in which all changes are underlined and the other a "clean" copy with no underlining.

Study Specific Details:

1. On the renewal form, question 2, it is indicated that 30 subjects are to be included in the main phase yet elsewhere in the IRB application and in the consents it states 40. Please reconcile.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

Full Committee Review
Renewal

Receipt Date : 1/14/2009 **Expiration Date :** 2/09/2009 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Agenda Date 2/02/2009 Reviewer 1: **Lea, James** Reviewer 2: **Weber, David J.** (chair)

Additional Findings and/or Special Populations: ☐ Children ☐ Prisoners ☐ Pregnant Women

☐ Sensitive Info ☑ IND ☐ IDE ☐ DNA/Gene transfer ☑ Genetics ☑ GCRC ☐ ORC ☐ LAR

Meeting Notes and Required Findings : w/MOD

CONSENTS: GENOTYPING
SPEC
ADULT

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☑ Minor Stipulation letter:
  - ☑ Draft letter prepared — 02/03/09
  - ☑ Approved by chair as attached (Initials/Date: 2/4/09)
  - ☑ Approved by chair, see edits (Initials/Date: 2/3/09)
  - ☑ Email copy sent
  - ☑ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Consent forms attached:
- Other attachments:

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Deferred
- ☐ Disapproved
- ☐ Return to sender
- ☐ Termination

L9

Full Committee Review
Renewal

Receipt Date : 1/14/2009 Expiration Date : 2/09/2009 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Coordinator :

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Agenda Date 2/02/2009 Reviewer 1: **Lea, James** Reviewer 2: **Weber, David J.** (chair)

Additional Findings and/or Special Populations: ☐ Children ☐ Prisoners ☐ Pregnant Women

☐ Sensitive Info ☑ IND ☐ IDE ☐ DNA/Gene transfer ☑ Genetics ☑ GCRC ☐ ORC ☐ LAR

Meeting Notes and Required Findings : w/MOD

CONSENTS: GENOTYPING
SPEC
ADULT

J page 9 - 40 should be 30

Minor stip ~~Approve~~ Y - 11 N - 0 Ab - 0

PROCESSING STEPS (OFFICE USE ONLY):
- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached:
Other attachments:

FINAL ACTIONS:
- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Deferred
- ☐ Disapproved
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
REQUEST FOR RENEWAL OF IRB APPROVAL OR STUDY CLOSURE
version 8-Sep-2008

renewal & Mod

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JAN 14 2009
IRB UNC-CH

If the research is continuing:

- Check the relevant items.
- Include two collated sets of copies (sorted in the order listed) of checked items.

→ **Submissions will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This form (renewal or closure).	2
x	2. Any items specifically requested in questions # 4 through 9 (in that order).	2
x	3. The most recent application submitted for IRB approval. This application should be updated to include any modifications since the study was initially approved or last renewed. **If there are any new modifications included with this renewal, highlight the proposed modifications by underlining.**	2
x	4. Clean copies of all consent document(s) to be used in the upcoming approval period, for stamping.	2
x	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

IRB study #: 07-0190 **Date:** January 8, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor** (if applicable): James Samet, PhD

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

1. In a few sentences, describe the past year's work, and describe what you plan for the upcoming year, including data analysis, if relevant.

We have finished the pilot phase of the study. We plan to start the main phase of the study in the upcoming months and we expect the study will last about 11/2 to 2 years. We have modified the type of air pollution and reduced the number of subjects in the main study which was approved in August of 2008.

2. Number of subjects involved through direct contact or use of their data (for multi-site studies, include only subjects covered by this IRB) (Note: **b+d** should not be larger than **a**)
   a. Total projected number as approved by IRB: 6 subjects in the pilot phase and 40 in the main phase.
   b. Total number of subjects involved to date: 8 subjects in the pilot phase (2 subjects were disqualified before the exposure).
   c. Number of subjects added since last renewal: 4 subjects in the pilot phase.
   d. Number to be included in upcoming year: 30 subjects in the main phase.

Answer the following questions based on information since initial approval or last renewal. Only include subjects covered by this IRB.

3. Have there been any modifications approved since the last review? If research is continuing, be sure all changes are reflected in the attached revised application for IRB approval.	_x yes __ no
4. Have any subjects withdrawn voluntarily or been withdrawn from the study? **If yes, explain**; give number and reasons for withdrawals.	__ yes _x no
5. Have there been any findings (e.g., publications) that alter the risk/benefit ratio or otherwise impact the study? **If yes, explain.**	__ yes x_ no
6. Does this study have a Data and Safety Monitoring Committee (DSMC or DSMB)? **If yes, provide a report** from the DSMC.	__ yes x_ no
7. Have there been unanticipated problems or serious adverse events since the last renewal? **If yes, include all copies** of local Adverse Event reports with this submission.	__ yes x_ no
8. Has this study been audited by external sponsor or monitor since approved or last renewed? **If yes, include a copy** of the audit report.	__ yes _x no
9. Are you requesting any changes? **If yes, include the form** requesting *Modification of Approved Human Subjects Research* and underline the proposed change in the updated application.	_x yes __ no
10. Will you be enrolling, consenting or re-consenting subjects in the upcoming approval period? **If yes, include clean copies** of consents/assents/fact sheets to receive a new stamp. **Include any new recruitment materials** to be used with subjects.	_x yes __ no

Action requested by Principal Investigator (choose only one):

Renew approval:

__ Study has always involved *only* analysis of existing data or specimens. Continue as approved.

Study involves(ed) direct interaction/intervention or contact with subjects:

_x_ Continue as approved: Enrollment of new subjects continues.

__ Enrollment of new subjects closed; interaction/intervention with previously enrolled subjects continues.

__ Direct interaction with subjects completed but subsequent monitoring or follow up continues.

__ Subjects' involvement completed but renewal is requested for data analysis.

Closure of Study:

__ Research completed: Identifiable data or human biological specimens are stored according to plan already approved by the IRB.

__ Research completed: All data or human biological specimens are deidentified.

__ Lack of funding or other *(specify)*:

Signature of Principal Investigator — Date: 1-12-09

Signature of Faculty Advisor (if applicable) — Date: 1/12/09

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

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JAN 14 2009
IRB UNC-CH

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

We have changed the dose and duration of dietary supplementation. The dose will be increased from 2 pills/day to 3 pills/day and the duration will be increased from two weeks to four weeks. These changes are made based on a published study (Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clin Chem. 2006 Dec;52(12):2265-72) showing that the omega-3 concentration increases during short intervals in response to supplementation at rates related to the amount of supplementation. Therefore we extend the supplementation time to four weeks with increased dose in order to produce a protective effect.

We have made such changes in the protocol, consent forms, and recruitment materials. Please see the highlighted changes.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: January 8, 2009**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD
(if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

Signature of Principal Investigator or designee — Date: 1-12-09

Signature of Faculty Advisor (if applicable) — Date: 1/12/09

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

RECEIVED
JAN 14 2009
IRB UNC-CH

Part A.1. Contact Information, Agreements, and Signatures

Date: January 8, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _x_ postdoc __ resident __ other

Name of faculty advisor: James Samet, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH

__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
x	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
x	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
☐	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
☐	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for* ***Consent for Stored Samples***.	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.
Participants Forty healthy 50-75 year-old male and female subjects will be involved in the study.
Procedures (methods): The subject will be exposed to concentrated ambient fine and ultrafine PM for 2 hours after being randomly supplemented with four weeks of omega-3 fatty acid or olive oil.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal

studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (15-17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (20). Therefore, the role of GSTs in mediating the cardiovascular effects following acute PM exposure will also be studied in the protocol in GSTM1 null and GSTM1 positive healthy older adults.

The respiratory effects of PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.

7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
15. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal is approximately 40 subjects for the study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by

criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This will be a randomized, double-blinded study. Forty study subjects will be randomly given fish oil or olive oil supplements for 4 weeks., The subjects will be given a container filled with 4 weeks supply of enteric-coated either 3g/day of fish oil or 3g/day of olive oil capsules. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 410 mg of eicosapentaenoic acid (EPA) and 274 mg of docosahexaenoic acid (DHA). Each 1000 mg of this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. Each subject will be exposed to air containing fine and ultrafine PM (PM<2.5) for 2 hours in an exposure chamber after the supplementation. (See the study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotyping consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 25, methods of recruiting) will not need genotyping. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 80 ml of blood collected. A portion of the blood will be used for genotyping of GSTM1 and another portion of the blood will be used for the cholesterol levels, phospholipid and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back to the medical station.

- ***GSTM1 genotyping***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null.
- ***Food frequency questionnaire in the study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses.

Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Baseline HRV measurement***: Subjects will have their baseline 24-hour heart rate viability (HRV) measured by Holter monitor on the training day. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Subjects will be released while wearing the Holter monitor and return to the HSF the next day to get the Holter monitor removed. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.

- ***Fish oil or olive oil pretreatment and dietary assessment in the study:*** Subjects will be randomly given an approximately 4 weeks supply of either fish oil or olive oil supplements. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record every other week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 4 weeks of supplementation period. We will give the subjects another 4 weeks supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU) and Endo-PAT, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained.
- ***Brachial artery ultrasound:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual

nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Peripheral endothelial function by Endo-PAT***: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be induced by sudden cuff deflation. The PWA recordings will be measured continuously during suprasystolic occlusion and deflation reactive hyperemia. PAT measurement will be displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).
- ***Pulmonary function*** will be measured by spirometry.
- ***Blood sampling***: approximately 80 ml of blood sample will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

Exposure:

The subjects will be exposed to concentrated ambient fine and ultrafine PM after the dietary supplementation.

Prior to exposure, the height of subject's mouth while seated will be measured so that the particle tubing height can be adjusted. All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. During the exposure, subject will be monitored continuously by visual camera monitor, continuous pulse oximetry, blood pressure every 15 minutes, and telemetry. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C.

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.

The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will range between levels of 50 to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously by a DataRAM monitor (DR4000; Thermo Electron Corp) calibrated to filter obtained measurements of particle ug/m^3. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The computer is programmed to initiate a shutdown procedure if chamber particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. The resting minute ventilation during exposure will be measured. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 80 ml of blood samples will be collected.

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 80 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound) Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual

nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Finger arteries Endo-PAT. Changes in pulse wave amplitude of index fingers caused by reactive hyperemia will be expressed as a percent change relative to resting baselilne values.

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In this study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he

feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.
- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound and finger arteries Endo-PAT:** There are no known risks associated with imaging of the brachial artery and index finger arteries. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 3g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will range from 50 to 600 ug/m^3 and

the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test the hypothesis that PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.

We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to PM will also be measured (e.g., blood pressure, HRV, brachial arterial diameter, change in blood vasoactive, inflammation and coagulation factors). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTMI positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 of either fish oil or olive oil supplemented subjects was derived using a power of 0.8 and an α of 0.05. Therefore, we will recruit 20 subjects in each group in this study.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of PM on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen, D-dimer, and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data.** Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no

Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no

Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the study will take approximately 18 months. Participant recruitment is expected to be continuous throughout the study until the intended number of participants is reached.

The individual will have 6 visits to the research facility over approximately 6-7 weeks. The pre-enrollment and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after the blood work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment and baseline HRV measurement) will require approximately 3 hours. Exposure day will last approximately 8 hours. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study. If the subject is qualified and finished the entire study, the total compensation for completion of this study will be approximate $1225

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total =	$60
Training day (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
Exposure (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Finger artery Endo-PAT (2 @$20 each)	$40
Food records (2 times)	$300
FFQ	$50
On-time bonus	$25
Total for completion of exposure =	$843
Eighteen hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Finger artery Endo-PAT	$20
Total for completion of post-exposure =	$86
Protocol Completion Bonus	$100

Approximate TOTAL for completion of study = $1225

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in

effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

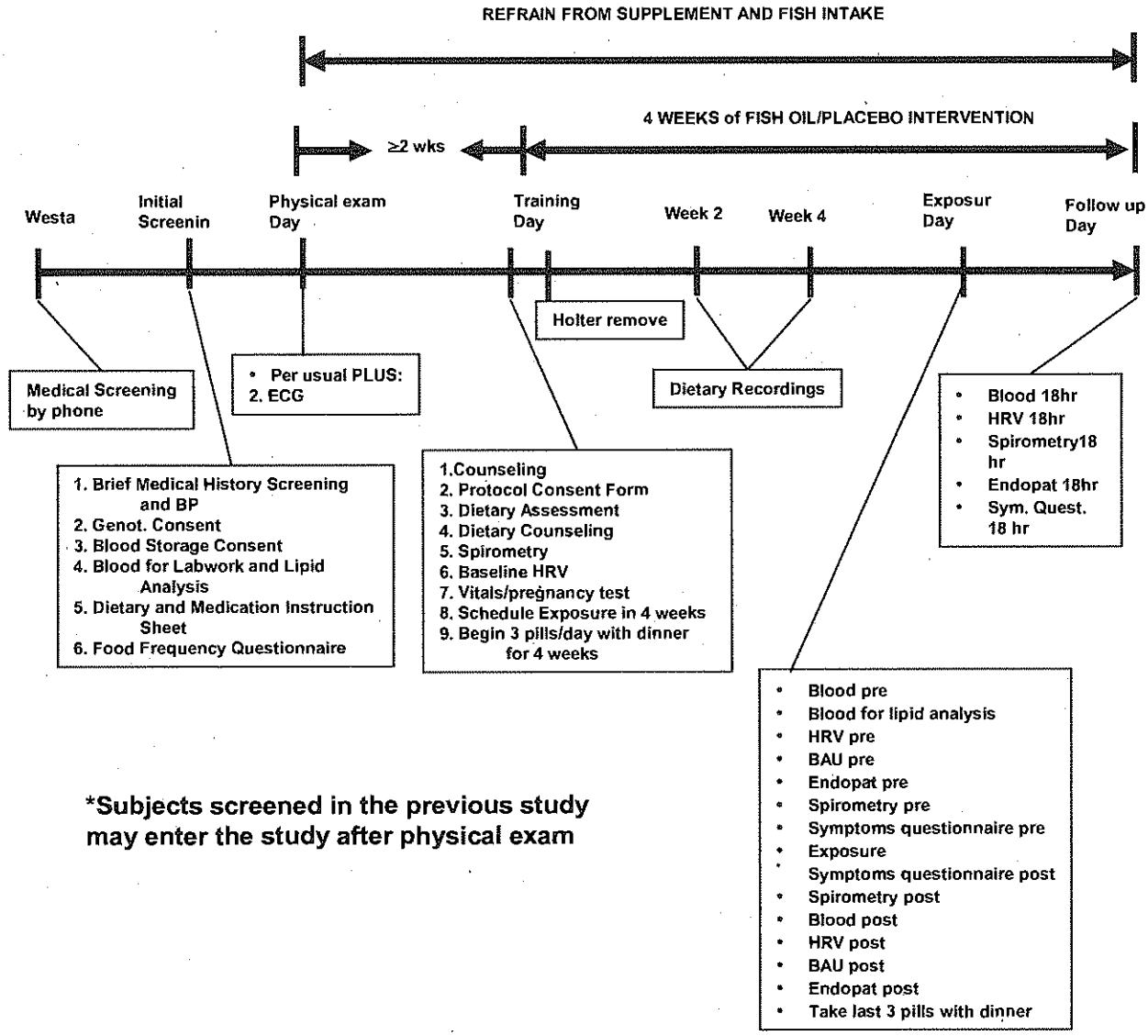
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	**NONE**	**TRACE**	**MILD**	**MODERATE**	**SEVERE**
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



*Subjects screened in the previous study may enter the study after physical exam

STUDY FLOW DIAGRAM

CTRC RENEWAL

CTRC Project **GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust**
IRB Study #: 07-0190

P.I.: **Dr. Haiyan Tong**

Original Approval: 2/25/07

Project Expiration: **2/9/09**

Date Submitted: **1/14/09**

RECEIVED
JAN 14 2009
IRB UNC-CH

RECOMMENDATION OF REVIEWER:

✓ Approval.

_____ Returned for re-submission. (state reason):

_____ Disapproval.

DURATION OF APPROVAL

12 months. (usually 12 months, never longer)

_____ Contingent on review or progress after:

Marie Rape RN BSN — 1/14/09

Regulatory Core (Marie Rape,RN, David Weber, MD, Diane Towle, RN) (Date)

Michael Fried, MD (Date)

CLINICAL AND TRANSLATIONAL RESEARCH CENTER
UNC HOSPITAL ROOM 3005
CAMPUS BOX #7600 • CHAPEL HILL, NC • 27599-7600
PHONE: 919.966.1435 • FAX: 919.966.1576

UNIVERSITY OF SOUTH CAROLINA

OFFICE OF RESEARCH COMPLIANCE

June 2, 2006

To Whom It May Concern

Dear Colleague:

This is to certify that the person listed below has been educated on the protection of human research participants:

Key Personnel: **Susan Steck**

Date Trained: 1/18/2006

Education Program Description:
Web-Based Instruction on Conducting Human Research: An interactive web-based program that provides information on conducting human research and the informed consent process. The program, organized into modules, includes detailed information, examples and exercises related to basic principles, history, the informed consent process, and after approval requirements such as continuing review and adverse events.

Sincerely,

Thomas A. Coggins (am)
Thomas A. Coggins
Director

BYRNES BUILDING • 901 SUMTER STREET • COLUMBIA, SOUTH CAROLINA 29208
803/777-7095 • FAX: 803/576-5589

AN AFFIRMATIVE ACTION / EQUAL OPPORTUNITY INSTITUTION

CITI Course in The Protection of Human Research Subjects

Friday, April 29, 2005

CITI Course Completion Record for Wayne Cascio

To whom it may concern:

On *4/29/2005*, *Wayne Cascio* (username=casciow; Employee Number=) completed all *CITI Program* requirements for the *Basic CITI* Course in The Protection of Human Research Subjects.

Learner Institution: *East Carolina University*

Learner Group: *Group 1*,

Learner Group Description: *All Biomedical Investigators and Key Personnel*

Contact Information:

Gender: Male

Department: Medicine

Which course do you plan to take?: Biomedical Investigator Course Only

Role in human subjects research: Clinical Reseacher

May we re-contact you to complete a course survey?: Yes

Mailing Address:

Brody School of Medicine at ECU
PCMH 378-TA
Greenville
NC
27834
USA

Email: casciow@mail.ecu.edu
Office Phone: 252-744-0083
Home Phone: 252-3212-0847

The Required Modules for *Group 1*. are:	Date completed
Introduction	04/29/05
History and Ethical Principles	04/29/05
Basic Institutional Review Board (IRB) Regulations and Review Process	04/29/05

Informed Consent	04/29/05
Social and Behavioral Research for Biomedical Researchers	04/29/05
Records-Based Research	04/29/05
Genetic Research in Human Populations	04/29/05
Research With Protected Populations - Vulnerable Subjects: An Overview	04/29/05
Vulnerable Subjects- Research With Prisoners	04/29/05
Vulnerable Subjects- Research Involving Minors	04/29/05
Vulnerable Subjects- Research Involving Pregnant Women and Fetuses in Utero	04/29/05
Group Harms:Research With Culturally or Medically Vulnerable Groups	04/29/05
FDA-Regulated Research	04/29/05
HIPAA and Human Subjects Research	04/29/05
Workers as Research Subjects-A Vulnerable Population	04/29/05
Hot Topics	04/29/05
Conflicts of Interest in Research Involving Human Subjects	04/29/05

Additional optional modules completed:	**Date completed**

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI participating institution. Falsified information and unauthorized use of the CITI course site is unethical, and may be considered scientific misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Course Coordinator

CITI Course in The Protection of Human Research Subjects

Monday, November 20, 2006

CITI Course Completion Record # 225240
for Fernando Holguin

To whom it may concern:

On *3/17/2006*, *Fernando Holguin* (username=fholgui; Emory ID Number=0014081Employee Number=0014081) completed all *CITI Program* requirements for the *Basic CITI* Course in The Protection of Human Research Subjects.

Learner Institution: *Emory University*

Learner Group: *Group 1. Biomedical Focus*

Learner Group Description: *This course is suitable for investigators and staff conducting BIOMEDICAL RESEARCH with human subjects. The IRB can assist you in determining whether these additional modules are necessary.*

Contact Information:

Gender: Male
Department: Medicine
Which course do you plan to take?: Biomedical Investigator Course Only
Role in human subjects research: Principal Investigator
Mailing Address:
NCEH
1600 Clifton RD NE MS E-17
Atlanta
GA
30307
USA
Email: fch5@cdc.gov
Office Phone: 404-498-1027
Home Phone: 404-377-5672

The Required Modules for *Group 1. Biomedical Focus* are:	Date completed
Introduction	01/27/06
History and Ethical Principles	03/17/06

Basic Institutional Review Board (IRB) Regulations and Review Process	03/17/06
Informed Consent	03/17/06
Research With Protected Populations - Vulnerable Subjects: An Overview	03/17/06
FDA-Regulated Research	03/17/06
HIPAA and Human Subjects Research	03/17/06
Conflicts of Interest in Research Involving Human Subjects	03/17/06
Emory University	03/17/06

Additional optional modules completed:	**Date completed**

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI participating institution. Falsified information and unauthorized use of the CITI course site is unethical, and may be considered scientific misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Course Coordinator

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 3/02/2009
Expiration Date of Approval: 2/01/2010
RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment, dated 02/26/09, addresses:

1. addition of Ana Rappold
2. changes to the protocol: deletion of Endo-PAT measurement and addition of BAU measurement; changed limits of PM mass concentration from "50-600 ug/m3" to "up to 600 ug/m3"; modified payment
3. updated protocol with these changes
4. update consent form with appropriate changes
5. updated recruitment materials

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 3/2/09 AND 2/1/10
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form

3

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: February 19, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 40 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and one exposure session which last approximately 8 hours, and one session 18 hours after exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. You will tour the exposure chamber and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

We will perform a baseline heart rate viability (HRV) measurement. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 24 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

Initial/Date _____

Exposure day
We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your HRV measured by a Holter monitor (the same test we do today).
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to concentrated Chapel Hill air particles; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative

Initial/Date _____

humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently. We will check your resting breathing rate during exposure. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return the next morning, for your safety you should not shower or bathe until after the monitor is removed.

Eighteen hour follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the

Initial/Date _____

samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be

Initial/Date _____

in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

Initial/Date _____

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1090.

Initial/Date _____

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total = $60	
Training day (3 hours)	
Time (3h @$12/h)	$36
Holter monitor	$100
FFQ	$25
Training day total = $161	
Exposure (8 hours)	
Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus	$25
Total for completion of exposure =$653	

Eighteen hours after exposure (3 hour)

Initial/Date _____

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Total for completion of post-exposure =	$116
Protocol Completion Bonus	$100

Approximate TOTAL for completion of study =$1090

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

Initial/Date _____

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

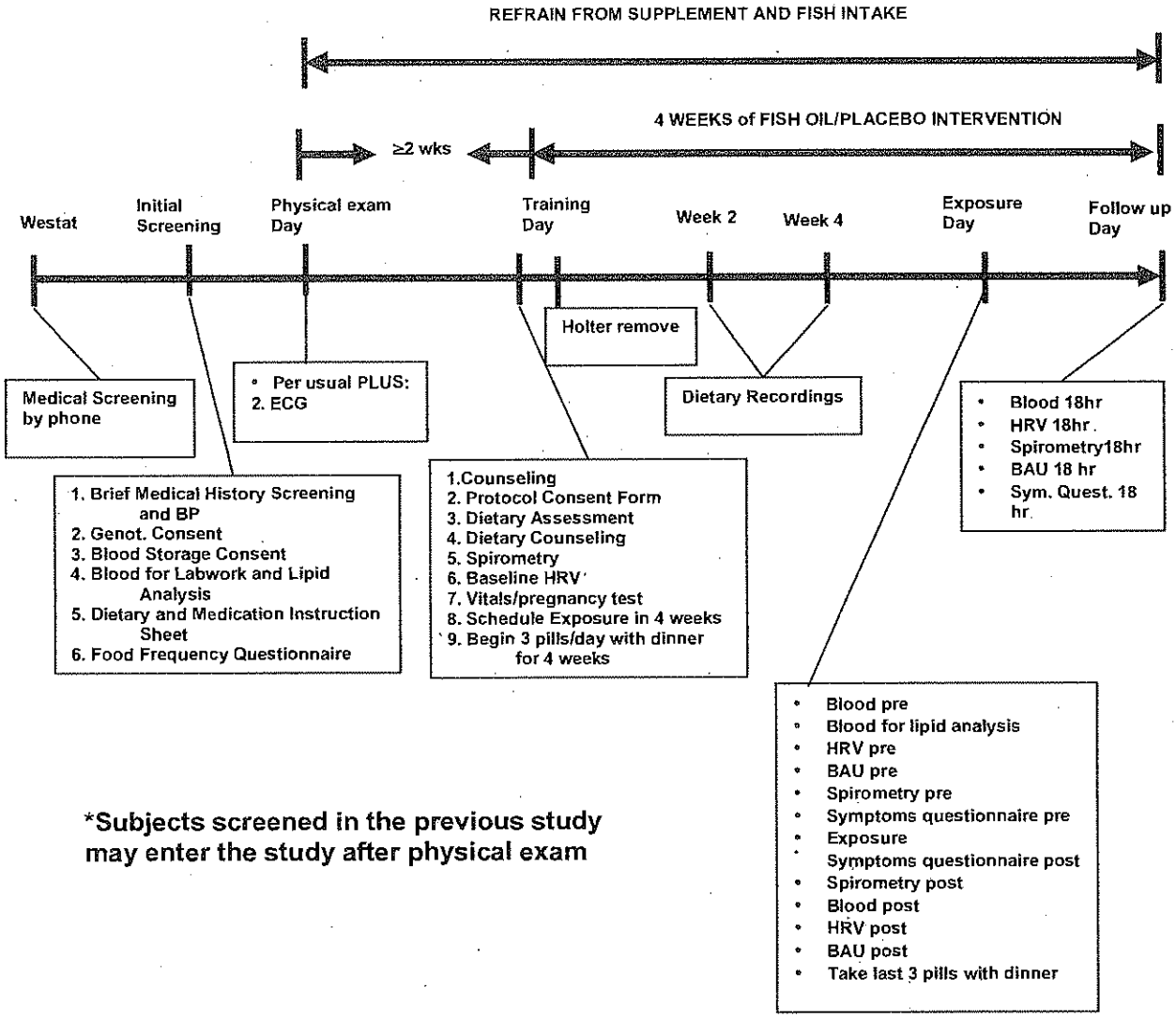
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

MAR 0 2 2009

OMEGA CON STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1090.

Procedures:

Volunteers initially undergo a blood test and genotype screening, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genotyping visit and $30 for the additional medical screening. If you are eligible for the study your participation will last approximately 6 to 7 weeks.

Participation will require 6 weeks of dietary restrictions and four weeks of fish oil or olive oil treatment. The study will begin with a training visit that will last about 3 hours and a return visit the following morning. Following training you will begin the treatment (taken as capsules) for 4weeks prior to your exposure session. You will be asked to record your food intake twice for 3 days. The exposure day lasts 8 hours and the follow up day lasts about 3.

Procedures involved in this study include breathing tests, heart rate monitoring, including wearing overnight monitors after training and the exposure, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be able refrain from certain foods and medications for at least six weeks and to take the supplements for four weeks prior to the exposure. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-0518, previously 95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Genotyping Visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (1 hour)

Physical Exam: days and times TBD (2 hours)

Training (3 hours)

Dietary Supplementation (4 weeks)

Training Follow-up Visit Day 1 (to return the Holter Monitor, 30 min)

Dietary Recordings, week 2 and week 4 (3 days/week)

Exposure Day (end of 4 weeks supplementation): 8:00 AM (8 hours)

Follow-up Day: 18 hours after Exposure Day (3 hours)

OMEGA CON STUDY
E-mail Announcement

SUBJECT: INFORMATIONAL: Research study for Healthy Older Adults

The main purpose of this research study is to determine if a component of air pollution, particulate matter (PM) elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Volunteers ages 50 to 75 will be tested to determine the type of a particular gene they carry, one of several genes responsible for protecting the body against oxidants. Participation will include four-weeks of fish oil or olive oil treatments and one exposure to a controlled amount of PM. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Office of Human Research Protection (OHRE) Biomedical Institutional Review Board. IRB # 07-0190 (GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

MAR 0 2 2009

Web Site Advertising: www.epastudies.org

OMEGA CON: Elder Diet and Air Pollution Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as PM, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

Participation in this study will require six weeks of dietary restrictions and four weeks of fish oil or olive oil treatments, as well as one exposure to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. Study procedures include breathing tests, heart rate monitoring, blood tests, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. Total time commitment after screening is about 15 hours, over 6-7 weeks. Compensation up to $1090 is provided.

MAR 02 2009

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA CON STUDY – GENOTYPING VISIT**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA CON STUDY SCREENING INSTRUCTIONS
For Genotyping Visit

Illness. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores.

No active allergies. If you have questions about allergies, please call the EPA medical station at 966-6232.

Please do not **EAT or DRINK** anything except water after midnight the day of your visit.

If you are unable to keep your appointment, please call Recruitment. Please be on time!

Thank You!
Westat EPA Support Services

MAR 0 2 2009

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA CON – BEGIN DIETARY RESTRICTIONS!**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA CON DIETARY RESTRICTIONS

Please begin your dietary restrictions on _____ [day], _____]date].

Food and Food Supplements

Follow the diet plan discussed during your Screening Visit. This includes:

- No omega-3 fatty acids or more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for 2 weeks before training and throughout the study.
- No antioxidants (beta-carotene, selenium, vitamin C, vitamin E, zinc) for 2 weeks before training and throughout the study.
- Use olive oil only for cooking, dressings, and sauces for two weeks before training and throughout the study.

If you have any questions about your diet, please call the EPA Medical Station at 919-966-6232.

Thank You!
Westat EPA Support Services

MAR 0 3 2009

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA CON STUDY – TRAINING VISIT**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA CON INSTRUCTIONS
For the Training Visit

Illness and Allergies. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores. If you have questions about allergies, please call the EPA medical station at 966-6232.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before training.
- Avoid drinking alcohol 24 hours before training. No red wine throughout the study.
- No caffeine for 12 hours prior to training.

MAR 0 2 2009

Food and Food Supplements

- Continue your dietary restrictions as instructed.

Exercise, Clothing

- No strenuous exercise for 24 hours prior to and after training and all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the training and follow-up visit.

If you are unable to keep your appointment, please call Recruitment. Please be on time!

Thank You!
Westat EPA Support Services

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA CON STUDY – EXPOSURE VISITS**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA CON STUDY INSTRUCTIONS
For Exposure Visits

Illness and Allergies. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores. If you have questions about allergies, please call the EPA medical station at 966-6232.

Medications
- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to all study visits. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

Smoke, Alcohol and Caffeine
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits. No red wine throughout the study.
- No caffeine for 12 hours prior to all study visits.

MAR 05 2009

Food and Food Supplements
- Continue your dietary restrictions as instructed.
- On the exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to the exposure day.

Exercise, Clothing
- No strenuous exercise for 24 hours prior to and after all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the exposure and follow-up visits.

If you are unable to keep your appointment, please call Recruitment. Please be on time!
Thank You!
Westat EPA Support Services

Dietary restrictions

Participants are asked to eliminate use of nutritional supplements containing omega-3 fatty acids and antioxidants (e.g. beta-carotene, selenium, vitamin C, vitamin E, zinc) for **two weeks** prior to beginning the intervention and during the intervention.

During the intervention, participants are asked to refrain from consuming the following foods:

1. All types of fish and shellfish
2. Walnuts
3. Flaxseed and flaxseed oil
4. Rapeseed oil
5. Canola oil
6. Soybeans and soy products
7. Eggland's Best eggs
8. Red wine

MAR 0 9 2009

First Review of IRB Submission Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 2/26/2009 Expiration Date : 2/01/2010 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Coordinator :

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) 7 ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Wyomie Milliken

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter:
  - ☒ Draft letter prepared JMB 03/02/09
  - ☒ Approved by chair as attached (Initials/Date: 3/2/09 HB)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☐ Hard copy sent

Consent forms attached: 1

Other attachments: 8

FINAL ACTIONS:

- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

RECEIVED
FEB 26 2009
IRB UNC-CH

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

RECEIVED 2/26/09 CTRL

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
x	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
☐	4. The sponsor's document describing the amendment, if any.	1
☐	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1). We have added Biostatistician Dr. Ana Rappold in the co-PI list.
2). In the pilot study there was no meaningful results from the Endo-PAT measurement, so we have dropped off the Endo-PAT measurement and added BAU measurement on the 18 hr post-exposure day visit. See page 13-15 in the protocol.
3). We have changed the lower limit of PM mass concentration during the exposure from "50-600 ug/m3"to "up to 600 ug/m3" in order to ease the exposure scheduling difficulty. See page 15 in the protocol.
4). We have modified the payment section and the new total payment will be $1090. See page 27-28 in the protocol.

We have made such changes in the protocol, consent forms, and recruitment materials. Please see the highlighted changes.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: February 20, 2009**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Haiyan Tong — 2-26-09

Signature of Principal Investigator or designee — Date

James M. Samet — 2/26/09

Signature of Faculty Advisor (if applicable) — Date

RECEIVED
FEB 26 2009
IRB UNC-CH
07-0190

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

Part A.1. Contact Information, Agreements, and Signatures

Date: February 19, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _x_ postdoc __ resident __ other

Name of faculty advisor: James Samet, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):

__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
x	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
x	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
☐	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
☐	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator — Date: 2/26/09

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor — Date: 2/26/09

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee — Date

Print Name of Department Chair or designee — Department

Part A.2. Summary Checklist *Are the following involved?*

	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for* ***Consent for Stored Samples***.	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept nltimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procednres.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.
Participants Forty healthy 50-75 year-old male and female subjects will be involved in the study.
Procedures (methods): The subject will be exposed to concentrated ambient fine and ultrafine PM for 2 hours after being randomly supplemented with four weeks of omega-3 fatty acid or olive oil.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal

studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (15-17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (20). Therefore, the role of GSTs in mediating the cardiovascular effects following acute PM exposure will also be studied in the protocol in GSTM1 null and GSTM1 positive healthy older adults.

The respiratory effects of PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.

7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
15. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal is approximately 40 subjects for the study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by

criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This will be a randomized, double-blinded study. Forty study subjects will be randomly given fish oil or olive oil supplements for 4 weeks., The subjects will be given a container filled with 4 weeks supply of enteric-coated either 3g/day of fish oil or 3g/day of olive oil capsules. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 410 mg of eicosapentaenoic acid (EPA) and 274 mg of docosahexaenoic acid (DHA). Each 1000 mg of this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. Each subject will be exposed to air containing fine and ultrafine PM (PM<2.5) for 2 hours in an exposure chamber after the supplementation. (See the study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotyping consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 25, methods of recruiting) will not need genotyping. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 80 ml of blood collected. A portion of the blood will be used for genotyping of GSTM1 and another portion of the blood will be used for the cholesterol levels, phospholipid and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back to the medical station.

- ***GSTM1 genotyping***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null.
- ***Food frequency questionnaire in the study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses.

Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. The height of subject's mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Baseline HRV measurement***: Subjects will have their baseline 24-hour heart rate viability (HRV) measured by Holter monitor on the training day. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Subjects will be released while wearing the Holter monitor and return to the HSF the next day to get the Holter monitor removed. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.

- ***Fish oil or olive oil pretreatment and dietary assessment in the study:*** Subjects will be randomly given an approximately 4 weeks supply of either fish oil or olive oil supplements. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record every other week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC

immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 4 weeks of supplementation period. We will give the subjects another 4 weeks supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU) ~~and Endo-PAT~~, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained.
- ***Brachial artery ultrasound:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a

pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ~~***Peripheral endothelial function by Endo-PAT***: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be induced by sudden cuff deflation. The PWA recordings will be measured continuously during suprasystolic occlusion and deflation reactive hyperemia. PAT measurement will be displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).~~
- ***Pulmonary function*** will be measured by spirometry.
- ***Blood sampling***: approximately 80 ml of blood sample will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

Exposure:

The subjects will be exposed to concentrated ambient fine and ultrafine PM after the dietary supplementation.

Prior to exposure, the height of subject's mouth while seated will be measured so that the particle tubing height can be adjusted. All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. During the exposure, subject will be monitored continuously by visual camera monitor, continuous pulse oximetry, blood pressure every 15 minutes, and telemetry. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. ~~The exposure atmosphere will be at approximately 40 ± 10% RH and approximately 22 ± 2 °C.~~

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a

face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.

The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will be up ~~range between levels of 50~~ to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously by a DataRAM monitor (DR4000; Thermo Electron Corp) calibrated to filter obtained measurements of particle ug/m^3. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The computer is programmed to initiate a shutdown procedure if chamber particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. The resting minute ventilation during exposure will be measured at about one hr time point. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 80 ml of blood samples will be collected.

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function (BAU) measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 80 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound) Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

~~**Finger arteries Endo-PAT.** Changes in pulse wave amplitude of index fingers caused by reactive hyperemia will be expressed as a percent change relative to resting baselilne values.~~

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In this study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.
- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound ~~and finger arteries Endo-PAT~~:** There are no known risks associated with imaging of the brachial artery ~~and index finger arteries~~. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 3g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.

- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will be up ~~range from 50~~ to 600 ug/m^3 and the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test the hypothesis that PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.

We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to PM will also be measured (e.g., blood pressure, HRV, brachial arterial diameter, change in blood vasoactive, inflammation and coagulation factors). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of

fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 of either fish oil or olive oil supplemented subjects was derived using a power of 0.8 and an α of 0.05. Therefore, we will recruit 20 subjects in each group in this study.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of PM on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen, D-dimer, and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data.** Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no

Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no

Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the study will take approximately 18 months. Participant recruitment is expected to be continuous throughout the study until the intended number of participants is reached.

The individual will have 6 visits to the research facility over approximately 6-7 weeks. The pre-enrollment and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after the blood work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment and baseline HRV measurement) will require approximately 3 hours. Exposure day will last approximately 8 hours. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study. If the subject is qualified and finished the entire study, the total compensation for completion of this study will be approximate ~~$1225~~ $1090.

Pre-study qualifications		
Recruitment screening		$15
Physical exam		$15
Venipuncture (~80ml)		$30
	Pre-study qualification total =	$60
Training day (3 hours)		
Time (3h @$12/h)		$36
Holter monitor		$100
FFQ	~~$50~~	$25
	Training day total =	$161
Exposure (8 hours)		
Venipuncture (~80ml, pre)		$30
Holter monitor		$100
Chamber exposure (2 hours)		$72
Venipuncture (~80ml, post)		$30
Time (8h @$12/h)		$96
Brachial artery ultrasound (2 @$50 each)		$100
~~Finger artery Endo-PAT (2 @$20 each)~~		~~$40~~
Food records (2 times)	~~$300~~	$150
~~FFQ~~		~~$50~~
Dietary supplementation completion bonus		$50
On-time bonus		$25
	Total for completion of exposure = ~~$843~~	$653
Eighteen hours after exposure (3 hour)		
Venipuncture (~80ml)		$30
Time (3h @$12/h)		$36
~~Finger artery Endo-PAT~~		~~$20~~
Brachial artery ultrasound		$50
	Total for completion of post-exposure = ~~$86~~	$116
	Protocol Completion Bonus	$100
	Approximate TOTAL for completion of study = ~~$1225~~	$1090

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

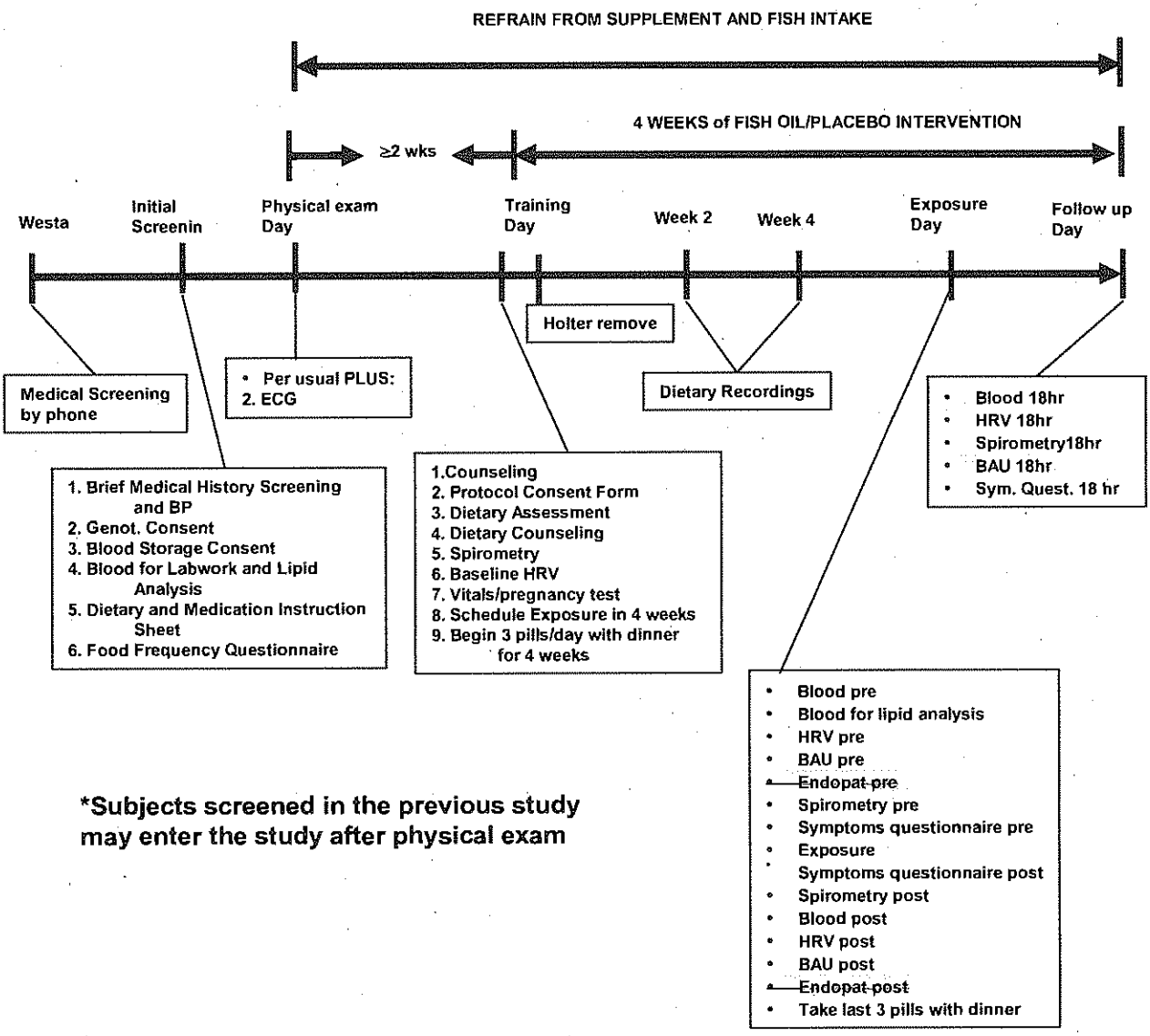
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	**NONE**	**TRACE**	**MILD**	**MODERATE**	**SEVERE**
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



STUDY FLOW DIAGRAM

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmental Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 3/19/2009
Expiration Date of Approval: 2/01/2010

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment dated 3/16/09 addresses:

1. study design changed to add in a clean air exposure on the 1st day followed by the original PM exposure on the 2nd day (this enables enrollment of fewer subjects & shortens study duration)
2. decrease in total number of subjects from 40 to 30
3. baseline HRV measurement by Holter monitor replaces by the measurement on the air exposure day
4. payment change to total $1481
5. instructions added for the Holter monitor

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, Environmental Protection Agency; Robert Truckner, (EPA), Non-IRB Review Contact; anda Simmons, (CTRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 3-19-09 AND 2-1-10
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotyping <u>With</u> Identifying Information 1

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: March 9, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

<u>What are some general things you should know about this research study?</u>
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to air pollution. The purpose of the genotyping is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 30 participants in this study.

How long will your participation last?
Participation in this genetic session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

At the end, we will give you a copy of the Medical History Form and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and you will be instructed to mail the FFQ. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?

- You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and

Initial/Date _____

uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol). You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $30 for completing this procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate.

Who owns the blood samples?
Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

Initial/Date _____

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Initial/Date _____

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 3-19-09 AND 2-1-10
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form

3

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: March 9, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
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What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 30 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and two exposure sessions which last approximately 8 hours each, and one session 18 hours after air pollution exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. You will tour the exposure chamber and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

Exposure days

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive

Initial/Date _____

deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 48 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to clean air on the first day then to concentrated Chapel Hill air particles on the second day; the amount of particles you will be exposed to is less than what you

Initial/Date _____

would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently. We will check your resting breathing rate during exposure. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.
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Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return on the follow-up day, for your safety you should not shower or bathe until after the monitor is removed. However, you will be given an instruction to follow if you need to have a shower or bath.

Eighteen hours after PM exposure follow up visit (about 3 hours)
You will return to the HSF the next morning (approximately 18 hours after PM exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.

Initial/Date _____

- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?
You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first

Initial/Date _____

evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

<u>What if we learn about new findings or information during the study?</u>

Initial/Date _____

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

Initial/Date _____

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1418.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total = $60	
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
Training day total =$61	
Exposures (2 x 8 hours)	
Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Time (16h @$12/h)	$192
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus	$25
Total for completion of 2 exposures=$1081	

Initial/Date _____

Eighteen hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50

Total for completion of post-exposure =$116
Protocol Completion Bonus $100

Approximate TOTAL for completion of study=$1418

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered

Initial/Date _____

or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

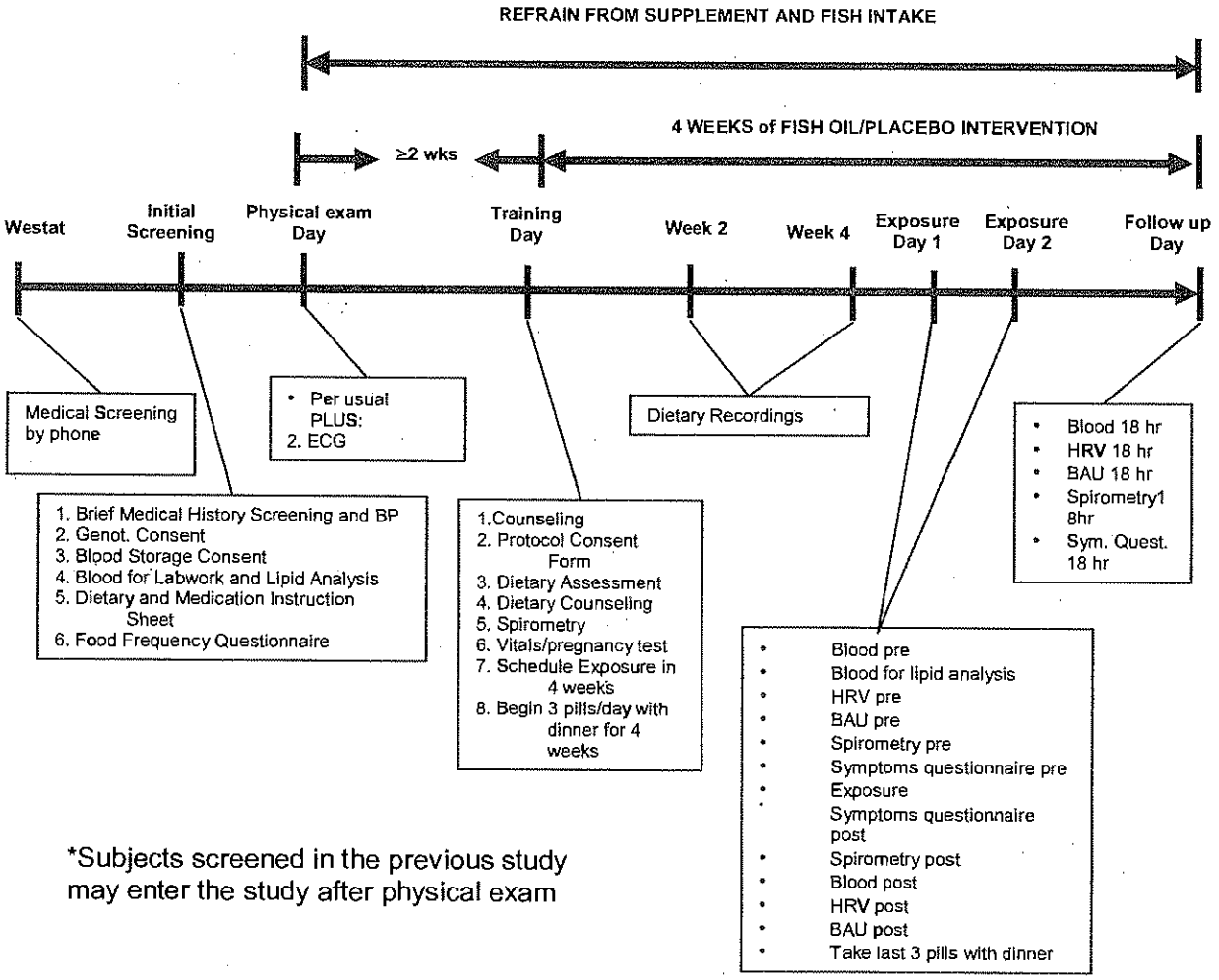
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

APPROVED - IRB, UNC-CH
MAR 19 2009

OMEGA CON STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1418.

Procedures:

Volunteers initially undergo a blood test and genotype screening, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genotyping visit and $30 for the additional medical screening. If you are eligible for the study your participation will last approximately 6 to 7 weeks. Participation will require 6 weeks of dietary restrictions and four weeks of fish oil or olive oil treatment.

The study will begin with a training visit that will last about 3 hours and two exposure sessions which last about 8 hours each, with one 3-hour follow up session 18 hours after Exposure 2. Following training you will begin the treatment (taken as capsules) for 4weeks prior to your exposure sessions. You will be asked to record your food intake twice for 3 days each.

Procedures involved in this study include breathing tests, heart rate monitoring, including wearing overnight monitors after training and the exposure, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be able refrain from certain foods and medications for at least six weeks and to take the supplements for four weeks prior to the exposure. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-0518, previously 95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

APPROVED - IRB, UNC-CH
MAR 19 2009

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposures. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Genotyping Visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (1 hour)

Physical Exam: days and times TBD (2 hours)

Training (3 hours)

Dietary Supplementation (4 weeks)

Dietary Recordings, week 2 and week 4 (3 days/week)

Two Exposure Days (end of 4 weeks supplementation): 8:00 AM (8 hours each)

Follow-up Day: 18 hours after Exposure Day 2 (3 hours)

OMEGA CON STUDY
E-mail Announcement

SUBJECT: INFORMATIONAL: Research study for Healthy Older Adults

The main purpose of this research study is to determine if a component of air pollution, particulate matter (PM) elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Volunteers ages 50 to 75 will be tested to determine the type of a particular gene they carry, one of several genes responsible for protecting the body against oxidants. Participation will include four-weeks of fish oil or olive oil treatments and two exposures, one to clean air and the other to a controlled amount of PM. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Office of Human Research Protection (OHRE) Biomedical Institutional Review Board. IRB # 07-0190 (GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

APPROVED - IRB, UNC-CH
MAR 19 2009

APPROVED - IRB, UNC-CH
MAR 19 2009

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA CON STUDY – EXPOSURE VISITS**

DATES:

TIMES:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA CON STUDY INSTRUCTIONS
For Exposure Visits

Illness and Allergies. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores. If you have questions about allergies, please call the EPA medical station at 966-6232.

Medications

- No over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposures. Low-dose aspirin and Tylenol (acetaminophen) are permitted.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits. No red wine throughout the study.
- No caffeine for 12 hours prior to all study visits.

Food and Food Supplements

- Continue your dietary restrictions as instructed.
- On each exposure day, eat a light breakfast.
- Do not eat pan fried and/or grilled foods after midnight prior to each exposure day.

Exercise, Clothing

- No strenuous exercise for 24 hours prior to and after all visits.
- Do not apply lotions, powders or perfumes to your chest.
- Wear comfortable washable clothing (button front shirt), socks and bring a light jacket or sweat shirt.
- Do not bathe or shower between the exposure visits and follow-up visit.

If you are unable to keep your appointment, please call Recruitment. Please be on time!
Thank You!
Westat EPA Support Services

APPROVED - IRB, UNC-CH
MAR 19 2009

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA CON STUDY – TRAINING VISIT**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA CON INSTRUCTIONS
For the Training Visit

Illness and Allergies. If you are sick or have been sick or injured in the last 4 weeks, please call Recruitment at 966-0604 or 888-279-9353. This includes sore throats, coughs, colds, and cold sores. If you have questions about allergies, please call the EPA medical station at 966-6232.

Smoke, Alcohol and Caffeine

- Avoid smoke and fumes for 24 hours before training.
- Avoid drinking alcohol 24 hours before training. No red wine throughout the study.
- No caffeine for 12 hours prior to training.

Food and Food Supplements

- Continue your dietary restrictions as instructed.

If you are unable to keep your appointment, please call Recruitment. Please be on time!

Thank You!
Westat EPA Support Services

APPROVED - IRB. UNC-CH
MAR 19 2009

TO:

RE: **Your appointment at the EPA Human Studies Facility**

STUDY: **OMEGA CON – BEGIN DIETARY RESTRICTIONS!**

DATE:

TIME:

SPECIAL NOTICE
If you are a UNC Student or are UNC staff or faculty:
you MUST print this appointment slip and display it on the dashboard in your car.
If you do not display it, you will be ticketed.

[Parking information to be provided here as available.]

May be folded here for display

OMEGA CON DIETARY RESTRICTIONS

Please begin your dietary restrictions on _____ [day], _____]date].

Your training session is scheduled for _____.

Food and Food Supplements

Follow the diet plan discussed during your Screening Visit. This includes:

- No omega-3 fatty acids or more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for 2 weeks before training and throughout the study.
- No antioxidants (beta-carotene, selenium, vitamin C, vitamin E, zinc) for 2 weeks before training and throughout the study.
- Use olive oil only for cooking, dressings, and sauces for two weeks before training and throughout the study.

Medications

Also, please remember that you must not take over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 week prior to exposure. Low dose aspirin and Tylenol (acetaminophen) are permitted.

If you have any questions about your diet or medications, please call the EPA Medical Station at 919-966-6232.

Thank You!
Westat EPA Support Services

APPROVED - IRB, UNC-CH
MAR 19 2009

Web Site Advertising: www.epastudies.org

OMEGA CON: Elder Diet and Air Pollution Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as PM, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

Participation in this study will require six weeks of dietary restrictions and four weeks of fish oil or olive oil treatments, as well as one exposure to clean air and one to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. Study procedures include breathing tests, heart rate monitoring, blood tests, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. Total time commitment after screening is about 22 hours, over 6-7 weeks. Compensation up to $1418 is provided.

APPROVED - IRB, UNC-CH
MAR 19 2009

HOLTER MONITOR INSTRUCTIONS

1. Do not remove battery cover, battery or card as this turns off the monitor.

2. The monitor, wires, and electrodes should remain attached to each other and you at all times.

3. Try not to allow the electrodes or wires to be pulled on.

4. **DO NOT DO CAR MAINTENANCE DURING THE TIME OF THE STUDY.**

5. Avoid all electrical equipment including electric blankets, heating pads, shop tools waterbed heater, etc. Computers appear to be okay.

6. **DO NOT GET THE HOLTER WET.**

   - Keep the wires and electrodes dry as well.
   - Do not carry damp or wet objects close to monitor.

7. **SHOWERING/REMOVING THE MONITOR**
   - Remove the monitor in another room besides the bathroom
   - Press any arrow to mark the event.
   - Detach wires one by one but leave the electrodes on your skin
   - Shower but do not scrub around the electrodes.
   - Dry off.
   - Reattach the wires observing the lettering on the electrodes matching the lettering on the wires.
   - Press any arrow to mark the start of monitoring again.

8. If an electrode loosens you may apply pressure around the edges for about 30 seconds to try and make it stick again. You should not push on the very center of the electrode. If the electrode falls off or doesn't have good contact, you can replace it with a new one.

9. Keep the monitor next to you in the bed while sleeping. Do not place it down outside of your bed.

10. If you experience any severe itching, redness, or swelling of your skin around the electrode sites, remove electrodes.

First Review of IRB Submission
Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 3/16/2009 **Expiration Date :** 2/01/2010 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☑ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Sonya H Bateman

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):
- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date: JDM 3/19/09)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached: 2

Other attachments: ~~6~~ 7

FINAL ACTIONS: JDM 3/19/09
- ☑ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

RECEIVED
MAR 16 2009
IRB UNC-CH

RECEIVED
3/16/09
CTRC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
x	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
☐	4. The sponsor's document describing the amendment, if any.	1
☐	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1). In consideration of the recruitment difficulty in the pilot phase of the study and the statistical power of the data analysis, we have changed the study design by adding a clean air exposure on the first day followed by the original PM exposure on the second day. The new study design will enable us to enroll less study subjects and shorten the study duration.

2). Total of 30 subjects will be needed for the study in stead of 40.

3). The baseline HRV measurement by Holter monitor will be replaced by the measurement on the air exposure day.

4). The payment has been changed and the total payment for the study will be $1481.

5). Add an instruction for the Holter monitor.

We have made such changes in the protocol, consent forms, and recruitment materials. Please see the highlighted changes.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

IRB study #: 07-0190 GCRC #: 2579 **Date: March 10, 2009**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Signature of Principal Investigator or designee — Date: 3/12/09

Signature of Faculty Advisor (if applicable) — Date: 3-12-2009

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

RECEIVED
MAR 16 2009
IRB UNC-CH

Part A.1. Contact Information, Agreements, and Signatures

Date: March 9, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID:
Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _x_ postdoc __ resident __ other

Name of faculty advisor: James Samet, PhD
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department:
Mailing address/CB #:
Phone #:
Fax #:
Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):

__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
x	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
x	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
☐	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
☐	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*

	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? *If yes*, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the *Addendum for Multi-site Studies*.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide IND #)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide IDE #)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for Consent for Stored Samples.*	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the UNC-CH Radiation Safety Committee is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the UNC-CH Institutional Biosafety Committee is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the Oncology Protocol Review Committee.*	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.
Participants ~~Forty~~ Thirty healthy 50-75 year-old male and female subjects will be involved in the study.
Procedures (methods): The subjects will be exposed to clean air for 2 hours on the first day then concentrated ambient fine and ultrafine PM for 2 hours on the second day after being randomly supplemented with four weeks of omega-3 fatty acid or olive oil.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic

nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (15-17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (20). Therefore, the role of GSTs in mediating the cardiovascular effects following acute PM exposure will also be studied in the protocol in GSTM1 null and GSTM1 positive healthy older adults.

The respiratory effects of PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.

5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
15. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal is approximately ~~40~~ 30 subjects for the study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4.. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This will be a randomized, double-blinded study. ~~Forty~~ Thirty study subjects will be randomly given fish oil (15 subjects) or olive oil (15 subjects) supplements for 4 weeks. The subjects will be given a container filled with 4 weeks supply of enteric-coated either 3g/day of fish oil or 3g/day of olive oil capsules. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 410 mg of eicosapentaenoic acid (EPA) and 274 mg of docosahexaenoic acid (DHA). Each 1000 mg of this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. Each subject will be exposed to clean air for 2 hour on the first day then air containing fine and ultrafine PM (PM<2.5) for 2 hours on the second day in an exposure chamber after the supplementation. (See the study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotyping consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 25, methods of recruiting) will not need genotyping. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 80 ml of blood collected. A portion of the blood will be used for genotyping of GSTM1 and another portion of the blood will be used for the cholesterol levels, phospholipid and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back to the medical station.

- ***GSTM1 genotyping***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null.
- ***Food frequency questionnaire in the study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency

Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. The height of subject's mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ~~***Baseline HRV measurement:*** Subjects will have their baseline 24-hour heart rate viability (HRV) measured by Holter monitor on the training day. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Subjects will be released while wearing the Holter monitor and return to the HSF the next day to get the Holter monitor removed. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.~~

- ***Fish oil or olive oil pretreatment and dietary assessment in the study:*** Subjects will be randomly given an approximately 4 weeks supply of either fish oil or olive oil supplements. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record every other week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification

of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 4 weeks of supplementation period. We will give the subjects another 4 weeks supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the exposure, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU), pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately ~~24~~ 48 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.
- ***Brachial artery ultrasound:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right

upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Pulmonary function*** will be measured by spirometry.
- ***Blood sampling***: approximately 80 ml of blood sample will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

Exposure:

The subjects will be exposed to clean air on the first day then concentrated ambient fine and ultrafine PM on the second day after the dietary supplementation.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. During the exposure, subject will be monitored continuously by visual camera monitor, continuous pulse oximetry, blood pressure every 15 minutes, and telemetry. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours.

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.

The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will be up to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously by a DataRAM monitor (DR4000; Thermo Electron Corp) calibrated to filter obtained measurements of particle ug/m^3. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The computer is programmed to initiate a shutdown procedure if

chamber particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. The resting minute ventilation during exposure will be measured at about one hr time point. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 80 ml of blood samples will be collected.

Eighteen Hours Post-PM exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function (BAU) measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 80 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for two times of 24 hours duration using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound) Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before and immediately after (within 1 hr after exposure ends) each exposure, and approximately 18 hrs after PM exposure, blood will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In this study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound:** There are no known risks associated with imaging of the brachial artery. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 3g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will be up to 600 ug/m^3 and the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like

pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test the hypothesis that PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.

We will measure a number of endpoints (e.g., neutrophils, inflammation markers, and lung function). Several markers of the cardiovascular response to PM will also be measured (e.g., blood pressure, HRV, brachial arterial diameter, change in blood vasoactivators, and coagulation factors). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect 0.13 units of change in BAU diameter as observed in the pilot study and estimated that 15 subjects per group are needed to achieve type I error rate 0.05 at power of 80%. We used estimates of variance in BAU diameter (0.27) and correlation among the repeated measurements (0.97) from the OMEGA pilot study. We used the equation for comparing two groups (placebo and treatment) with a single explanatory variables (PM concentration).

~~The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 of either fish oil or olive oil supplemented subjects was derived using a power of 0.8 and an α of 0.05. Therefore, we will recruit 20 subjects in each group in this study.~~

Other Exploratory Observations:

We hypothesize that GSTM1 null subjects will have increased responses to PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of PM on changes in: heart rate variability in both time and frequency domains, blood CBC, fibrinogen, D-dimer, and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data.** Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects.** If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent.** *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no
Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**

If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the study will take approximately ~~18~~ 12 months. Participant recruitment is expected to be continuous throughout the study until the intended number of participants is reached.

The individual will have 6 visits to the research facility over approximately 6-7 weeks. The pre-enrollment and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after the blood work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment ~~and baseline HRV measurement~~) will require approximately 3 hours. Each exposure day will last approximately 8 hours. Eighteen hours after the PM exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study. If the subject is qualified and finished the entire study, the total compensation for completion of this study will be approximate ~~$1090~~ $1418.

Pre-study qualifications

Recruitment screening		$15
Physical exam		$15
Venipuncture (~80ml)		$30
Pre-study qualification total =		$60

Training day (3 hours)

Time (3h @$12/h)		$36
~~Holter monitor~~		~~$100~~
FFQ		$25
Training day total =	~~$161~~	$61

Exposures (2 x 8 hours)

Venipuncture (~80ml, pre, 2@$30 each)	~~$30~~	$60
Holter monitor(2@$100 each)	~~$100~~	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	~~$72~~	$144
Venipuncture (~80ml, post, 2@$30 each)	~~$30~~	$60
Time (~~8~~-16h @$12/h)	~~$96~~	$192
Brachial artery ultrasound (~~2~~ 4@$50 each)	~~$100~~	$200
Food records (2 times)		$150
Dietary supplementation completion bonus		$50
On-time bonus		$25
Total for completion of 2 exposures =	~~$653~~	$1081

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Total for completion of post-exposure =	$116
Protocol Completion Bonus	$100
Approximate TOTAL for completion of study = ~~$1090~~	$1418

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

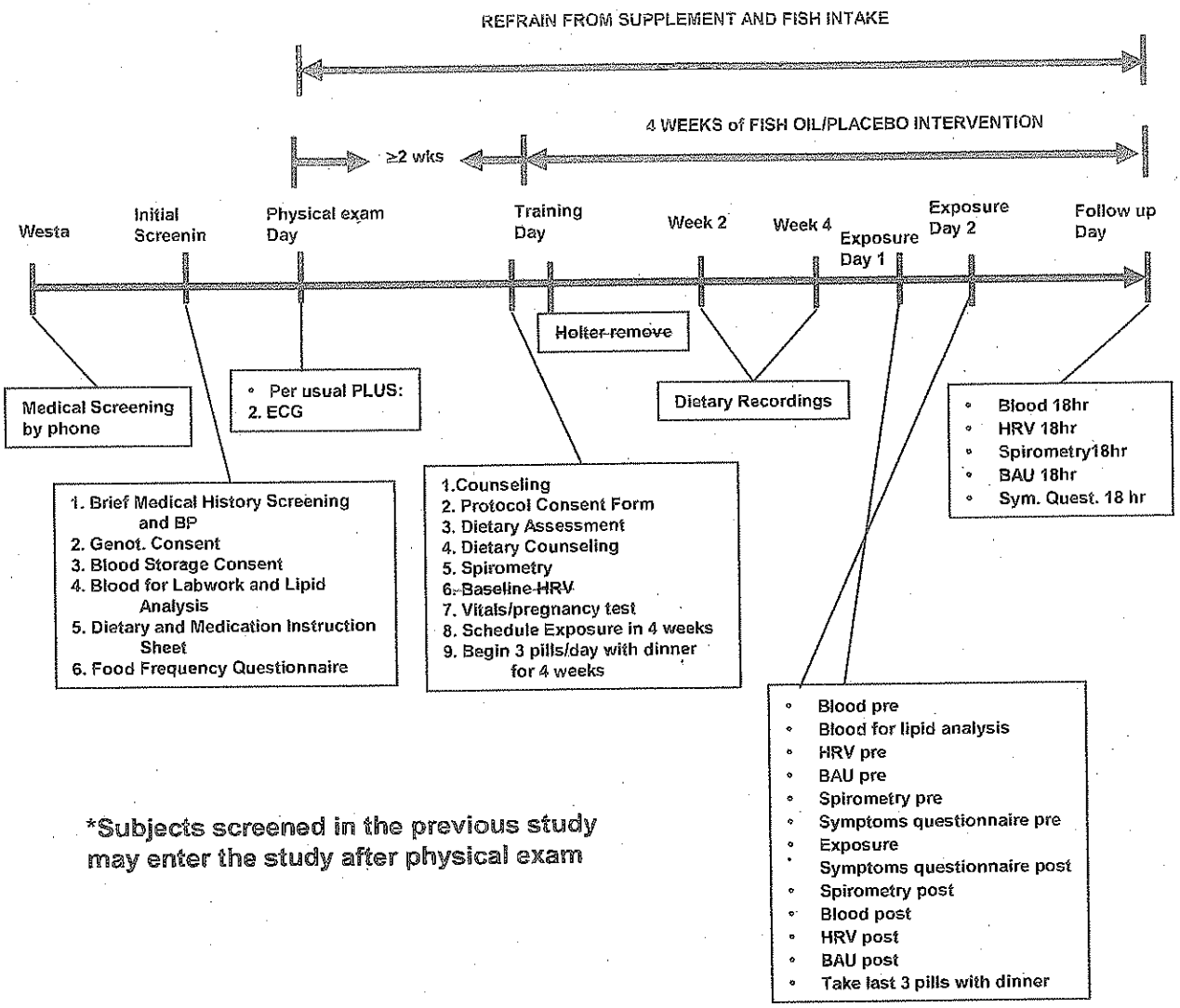
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



STUDY FLOW DIAGRAM

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 4/09/2009
Expiration Date of Approval: 2/01/2010
RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment dated 4/6/09 addresses:

1. changes to consents to enroll subjects who have been genotyped previously
2. changes to consent so minute ventilation measurement during exposures moved up to before the face mask is put on
3. changes to consent so blood pressure & heart rate measurements by a BP monitor added on the exposure days

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, Robert Truckner, (EPA); Non-IRB Review Contact; Wanda Simmons, (CTRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 4-9-09 AND 2-1-10 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotyping With Identifying Information 1

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: April 6, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to air pollution. The purpose of the genotyping is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 30 participants in this study.

How long will your participation last?
Participation in this genetic session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

If you have been genotyped before, there will be no genotyping this time. You will be given the information below.

At the end, we will give you a copy of the Medical History Form and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and bring back the FFQ on the physical exam day. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?

Initial/Date _____

- You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol). You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate.

Who owns the blood samples?

Initial/Date _____

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board,

Initial/Date _____

University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 4-9-09 AND 2-1-10
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form

3

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: April 6, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 30 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and two exposure sessions which last approximately 8 hours each, and one session 18 hours after air pollution exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. You will tour the exposure chamber and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

Exposure days

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and shave the areas of your chest where these leads will be placed. Excessive

Initial/Date _____

deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 48 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor. You will wear a pressure cuff and a monitor which is about the size of the Holter monitor. We will remove the monitor at the end of the exposure days.
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will then enter the exposure chamber and be exposed to PM.

Initial/Date _____

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to clean air on the first day then to concentrated Chapel Hill air particles on the second day; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently. We will check your resting breathing rate before you put on a face mask.

If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return on the follow-up day, for your safety you should not shower or bathe until after the monitor is removed. However, you will be given an instruction to follow if you need to have a shower or bath.

Eighteen hours after PM exposure follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after PM exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Initial/Date _____

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Initial/Date _____

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?

Initial/Date _____

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1418.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total = $60	
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
Training day total =$61	
Exposures (2 x 8 hours)	
Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Time (16h @$12/h)	$192
Brachial artery ultrasound (4@$50 each)	$200

Initial/Date _____

Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus	$25

Total for completion of 2 exposures=$1081

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50

Total for completion of post-exposure =$116

Protocol Completion Bonus $100

Approximate TOTAL for completion of study=$1418

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Will it cost you anything to be in this study?

There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

Initial/Date _____

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?

All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

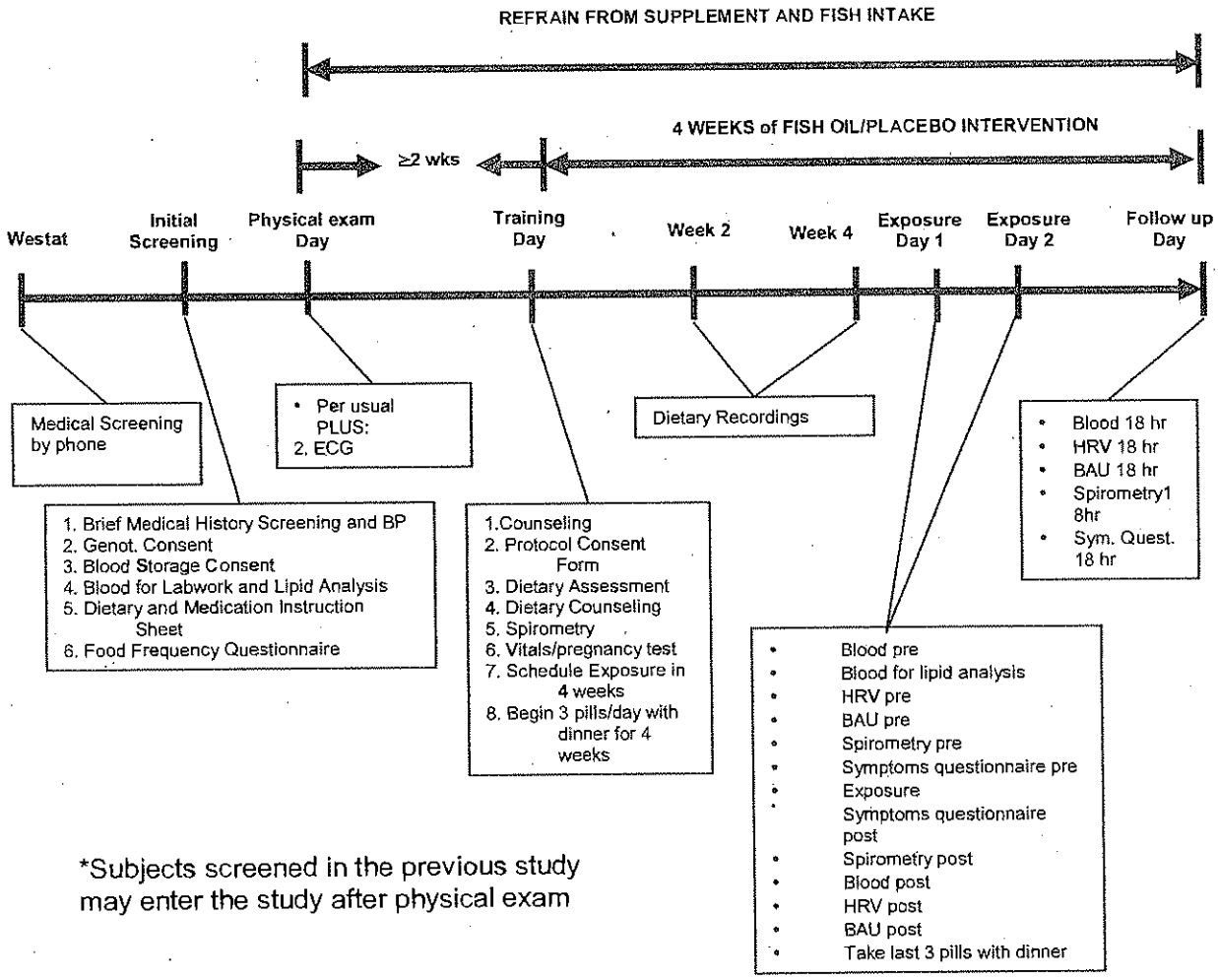
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

First Review of IRB Submission Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 4/06/2009 Expiration Date : 2/01/2010 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Coordinator :

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☑ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Wyomie Milliken

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter: 4/9/09
  - ☐ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date: 4/9/09)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent
- Consent forms attached: 2
- Other attachments: 0

FINAL ACTIONS: 4/9/09

- ☑ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

RECEIVED
APR 06 2009
IRB UNC-CH
RECEIVED
4/6/09
CTRC

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
☐	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
☐	4. The sponsor's document describing the amendment, if any.	1
☐	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1). Modification of genotyping consent form (consent form #1). In order to enroll the subjects who have been genotyped previously, we have added "If you have been genotyped before, there will be no genotyping this time. You will be given the information below." on page 2.

2). Modification of study consent from (consent form #3).

a). The minute ventilation measurement during exposures will be moved up to before the face mask is put on.
b). Blood pressure and heart rate measurements by a BP monitor will be added on the exposure days.

Please see the highlighted changes.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: April 6, 2009**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD
(if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

Signature of Principal Investigator or designee — Date: 4/6/09

Signature of Faculty Advisor (if applicable) — Date: 4-6-2009

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 5/06/2009
Expiration Date of Approval: 2/01/2010

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment dated 4/30/09 addresses:

1. moving the minute ventilation measurement to training day from exposure day
2. addition of instruction & risk associated with blood pressure monitor
3. addition of payment for blood pressure monitor so total payment $1543
4. changes to recruitment materials
5. changes to dietary restriction

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet & Robert Truckner, (EPA), Non-IRB Review Contact; Wanda Simmons,

(CTRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 5-6-09 AND 2-1-10 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

3

Biomedical Form

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: April 30, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if...

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 30 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and two exposure sessions which last approximately 8 hours each, and one session 18 hours after air pollution exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. Then we will check your resting breathing rate. You will tour the exposure chambers (one for air exposure and the other for air pollution exposure) and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

Exposure days

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and

Initial/Date _____

shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 48 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor. You will wear a pressure cuff and a monitor which is about the size of the Holter monitor. Please keep your arm relaxed and still when the pressure cuff is inflated. We will remove the monitor and pressure cuff at the end of the exposure days.
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to clean air on the first day then to concentrated Chapel Hill air particles on the second day; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently.

If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return on the follow-up day, for your safety you should not shower or bathe until after the monitor is removed. However, you will be given an instruction to follow if you need to have a shower or bath.

Eighteen hours after PM exposure follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after PM exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the

Initial/Date _____

appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Blood pressure monitor: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any

Initial/Date _____

employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1543

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
	Pre-study qualification total = $60
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
	Training day total =$61

Initial/Date _____

Exposures (2 x 8 hours)

Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Blood pressure monitor (2@$50 each)	$100
Time (16h @$12/h)	$192
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus	$25

Total for completion of 2 exposures=$1181

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25

Total for completion of post-exposure =$141
Protocol Completion Bonus $100

Approximate TOTAL for completion of study=$1543

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Initial/Date _____

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ________________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

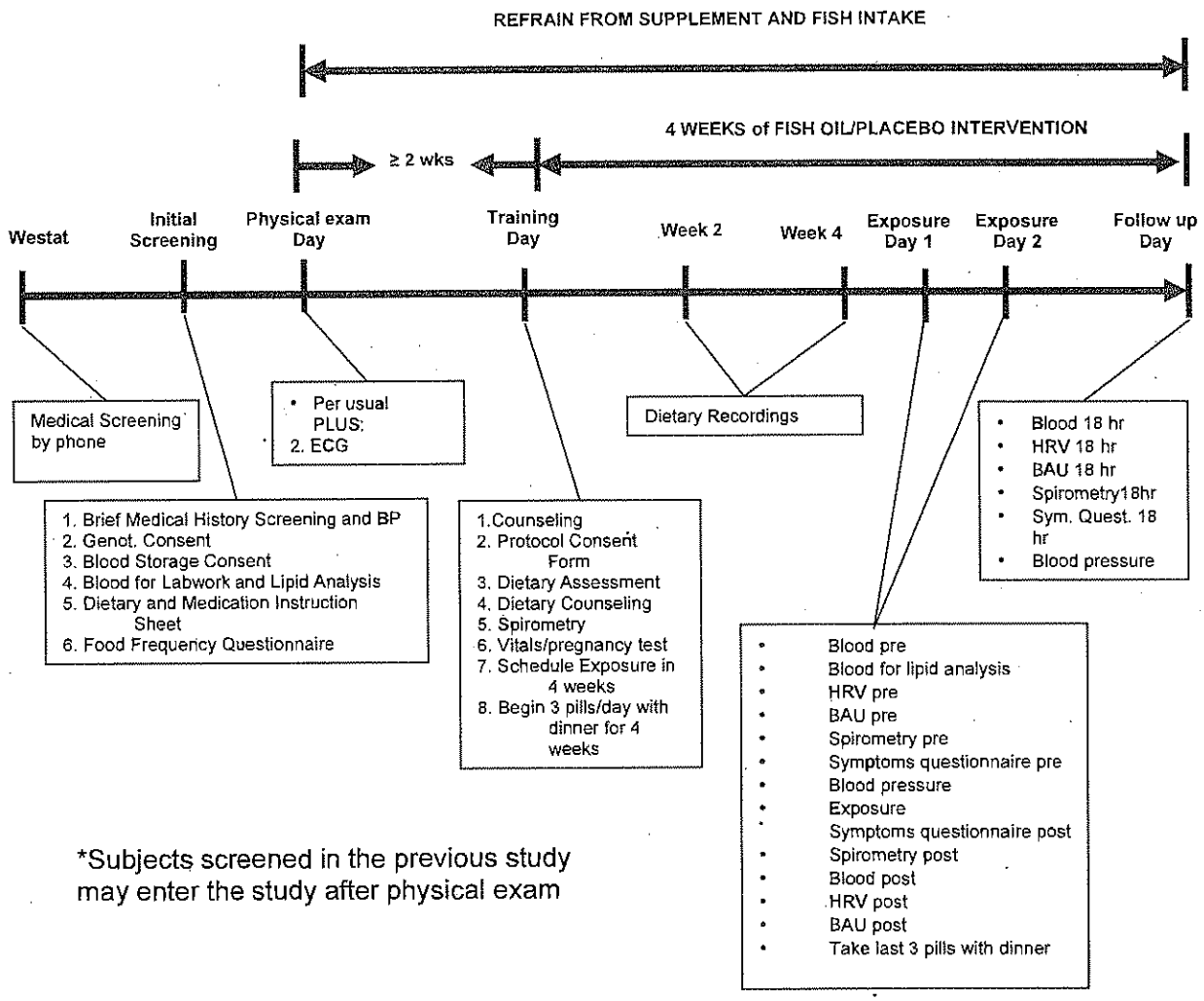
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

APPROVED - [illegible] UNC-CH
MAY 06 2009

OMEGA CON STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1543.

Procedures:

Volunteers initially undergo a blood test and genotype screening, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genotyping visit and $30 for the additional medical screening. If you are eligible for the study your participation will last approximately 6 to 7 weeks. Participation will require 6 weeks of dietary restrictions and four weeks of fish oil or olive oil treatment.

The study will begin with a training visit that will last about 3 hours and two exposure sessions which last about 8 hours each, with one 3-hour follow up session 18 hours after Exposure 2. Following training you will begin the treatment (taken as capsules) for 4weeks prior to your exposure sessions. You will be asked to record your food intake twice for 3 days.

Procedures involved in this study include breathing tests, heart rate monitoring, including wearing an overnight monitors after the exposure, blood draws, blood pressure monitoring, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be able refrain from certain foods and medications for at least six weeks and to take the supplements for four weeks prior to the exposure. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-0518, previously 95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposures. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Genotyping Visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (1 hour)

Physical Exam: days and times TBD (2 hours)

Training (3 hours)

Dietary Supplementation (4 weeks)

Dietary Recordings, week 2 and week 4 (3 days/week)

Two Exposure Days (end of 4 weeks supplementation): 8:00 AM (8 hours each)

Follow-up Day: 18 hours after Exposure Day 2 (3 hours)

14161

NATIONAL INSTITUTES OF HEALTH
DIET HISTORY QUESTIONNAIRE

APPROVED - IRB, UNC-CH
MAY 06 2009

GENERAL INSTRUCTIONS

- Answer each question as best you can. Estimate if you are not sure. A guess is better than leaving a blank.
- Use only a blue or black ball point pen. Do not use pencil or felt-tip pen. Do not fold, staple, or tear the pages.
- Be sure to completely blacken in each of the answers.
- If you make any changes, cross out the incorrect answer and blacken the correct answer. Also draw a circle around the correct answer.
- Do not make any stray marks on this form.
- If you mark NEVER, NO, or DON'T KNOW for a question, please follow any arrows or instructions that direct you to the next question.

BEFORE TURNING THE PAGE, PLEASE COMPLETE THE FOLLOWING QUESTIONS

Subject ID:

BAR CODE LABEL OR SUBJECT ID HERE

Today's Date:

MONTH	DAY	YEAR
○ JAN		○ 2007
○ FEB		○ 2008
○ MAR	○ 0 ○ 0	○ 2009
○ APR	○ 1 ○ 1	○ 2010
○ MAY	○ 2 ○ 2	○ 2011
○ JUN	○ 3 ○ 3	
○ JUL	○ 4	
○ AUG	○ 5	
○ SEP	○ 6	
○ OCT	○ 7	
○ NOV	○ 8	
○ DEC	○ 9	

In what month were you born?

○ JAN
○ FEB
○ MAR
○ APR
○ MAY
○ JUN
○ JUL
○ AUG
○ SEP
○ OCT
○ NOV
○ DEC

In what year were you born?

1 9 ___ ___

○ 0 ○ 0
○ 1 ○ 1
○ 2 ○ 2
○ 3 ○ 3
○ 4 ○ 4
○ 5 ○ 5
○ 6 ○ 6
○ 7 ○ 7
○ 8 ○ 8
○ 9 ○ 9

Are you male or female?

○ Male
○ Female

14161

1. Over the past 12 months, how often did you drink **tomato juice** or **vegetable juice**?

O NEVER (GO TO QUESTION 2)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

1a. Each time you drank **tomato juice** or **vegetable juice**, how much did you usually drink?

O Less than 3/4 cup (6 ounces)
O 3/4 to 1 1/4 cups (6 to 10 ounces)
O More than 1 1/4 cups (10 ounces)

2. Over the past 12 months, how often did you drink **orange juice** or **grapefruit juice**?

O NEVER (GO TO QUESTION 3)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

2a. Each time you drank **orange juice** or **grapefruit juice**, how much did you usually drink?

O Less than 3/4 cup (6 ounces)
O 3/4 to 1 1/4 cups (6 to 10 ounces)
O More than 1 1/4 cups (10 ounces)

3. Over the past 12 months, how often did you drink **other 100% fruit juice** or **100% fruit juice mixtures** (such as apple, grape, pineapple, or others)?

O NEVER (GO TO QUESTION 4)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

3a. Each time you drank **other fruit juice** or **fruit juice mixtures**, how much did you usually drink?

O Less than 3/4 cup (6 ounces)
O 3/4 to 1 1/2 cups (6 to 12 ounces)
O More than 1 1/2 cups (12 ounces)

Question 4 appears in the next column.

Over the past 12 months...

4. How often did you drink other **fruit drinks** (e.g. cranberry cocktail, Hi-C, lemonade, or Kool-Aid, diet or regular)?

O NEVER (GO TO QUESTION 5)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

4a. Each time you drank **fruit drinks**, how much did you usually drink?

O Less than 1 cup (8 ounces)
O 1 to 2 cups (8 to 16 ounces)
O More than 2 cups (16 ounces)

4b. How often were your fruit drinks **diet** or **sugar-free drinks**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

5. How often did you drink **milk as a beverage** (NOT in coffee, NOT in cereal)? (Please include chocolate milk and hot chocolate.)

O NEVER (GO TO QUESTION 6)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

5a. Each time you drank **milk as a beverage**, how much did you usually drink?

O Less than 1 cup (8 ounces)
O 1 to 1 1/2 cups (8 to 12 ounces)
O More than 1 1/2 cups (12 ounces)

5b. What kind of **milk** did you usually drink?

O Whole milk
O 2% fat milk
O 1% fat milk
O Skim, nonfat, or 1/2% fat milk
O Soy milk
O Rice milk
O Other

Question 6 appears on the next page.

14161

Over the past 12 months...

6. How often did you drink **meal replacement, energy, or high-protein beverages** such as Instant Breakfast, Ensure, Slimfast, Sustacal or others?

- O NEVER (GO TO QUESTION 7)
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

6a. Each time you drank **meal replacement beverages**, how much did you usually drink?

- O Less than 1 cup (8 ounces)
- O 1 to 1 1/2 cups (8 to 12 ounces)
- O More than 1 1/2 cups (12 ounces)

7. Over the past 12 months, did you drink **soft drinks, soda, or pop**?

- O NO (GO TO QUESTION 8)
- O YES

7a. How often did you drink **soft drinks, soda, or pop IN THE SUMMER**?

- O NEVER
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

7b. How often did you drink **soft drinks, soda, or pop DURING THE REST OF THE YEAR**?

- O NEVER
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

7c. Each time you drank **soft drinks, soda, or pop**, how much did you usually drink?

- O Less than 12 ounces or less than 1 can or bottle
- O 12 to 16 ounces or 1 can or bottle
- O More than 16 ounces or more than 1 can or bottle

Question 8 appears in the next column.

7d. How often were these soft drinks, soda, or pop **diet or sugar-free**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

7e. How often were these soft drinks, soda, or pop **caffeine-free**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

8. Over the past 12 months, did you drink **beer**?

- O NO (GO TO QUESTION 9)
- O YES

8a. How often did you drink **beer IN THE SUMMER**?

- O NEVER
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

8b. How often did you drink **beer DURING THE REST OF THE YEAR**?

- O NEVER
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

8c. Each time you drank **beer**, how much did you usually drink?

- O Less than a 12-ounce can or bottle
- O 1 to 3 12-ounce cans or bottles
- O More than 3 12-ounce cans of bottles

Question 9 appears on the next page.

14161

Over the past 12 months...

9. How often did you drink **wine** or **wine coolers**?

- O NEVER (GO TO QUESTION 10)
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

9a. Each time you drank **wine** or **wine coolers**, how much did you usually drink?

- O Less than 5 ounces or less than 1 glass
- O 5 to 12 ounces or 1 to 2 glasses
- O More than 12 ounces or more than 2 glasses

10. How often did you drink **liquor** or **mixed drinks**?

- O NEVER (GO TO QUESTION 11)
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

10a. Each time you drank **liquor** or **mixed drinks**, how much did you usually drink?

- O Less than 1 shot of liquor
- O 1 to 3 shots of liquor
- O More than 3 shots of liquor

11. Over the past 12 months, did you eat **oatmeal, grits**, or **other cooked cereal**?

- O NO (GO TO QUESTION 12)
- O YES

11a. How often did you eat **oatmeal, grits**, or **other cooked cereal IN THE WINTER**?

- O NEVER
- O 1-6 times per winter
- O 7-11 times per winter
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 12 appears in the next column.

11b. How often did you eat oatmeal, grits, or **other cooked cereal** DURING THE REST OF THE YEAR?

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

11c. Each time you ate **oatmeal, grits, or other cooked cereal**, how much did you usually eat?

- O Less than 3/4 cup
- O 3/4 to 1 1/4 cups
- O More than 1 1/4 cups

12. How often did you eat **cold cereal**?

- O NEVER (GO TO QUESTION 13)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

12a. Each time you ate **cold cereal**, how much did you usually eat?

- O Less than 1 cup
- O 1 to 2 1/2 cups
- O More than 2 1/2 cups

12b. How often was the cold cereal you ate **Total, Product 19**, or **Right Start**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

12c. How often was the cold cereal you ate **All Bran, Fiber One, 100% Bran**, or **Bran Buds**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 13 appears on the next page.

14161

Over the past 12 months...

12d. How often was the cold cereal you ate **some other bran** or **fiber cereal** (such as Cheerios, Shredded Wheat, Raisin Bran, Bran Flakes, Grape-Nuts, Granola, Wheaties, or Healthy Choice)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

12e. How often was the cold cereal you ate any **other type of cold cereal** (such as Corn Flakes, Rice Krispies, Frosted Flakes, Special K, Froot Loops, Cap'n Crunch, or others)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

12f. Was **milk** added to your cold cereal?

- O NO (GO TO QUESTION 13)
- O YES

12g. What kind of **milk** was usually added?

- O Whole Milk
- O 2% fat milk
- O 1% fat milk
- O Skim, nonfat, or 1/2% fat milk
- O Soy milk
- O Rice milk
- O Other

12h. Each time **milk was added to your cold cereal,** how much was usually added?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

13. How often did you eat **applesauce**?

- O NEVER (GO TO QUESTION 14)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 14 appears in the next column.

13a. Each time you ate **applesauce**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

14. How often did you eat **apples**?

- O NEVER (GO TO QUESTION 15)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

14a. Each time you ate **apples**, how many did you usually eat?

- O Less than 1 apple
- O 1 apple
- O More than 1 apple

15. How often did you eat **pears** (fresh, canned, or frozen)?

- O NEVER (GO TO QUESTION 16)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

15a. Each time you ate **pears**, how many did you usually eat?

- O Less than 1 pear
- O 1 pear
- O More than 1 pear

16. How often did you eat **bananas**?

- O NEVER (GO TO QUESTION 17)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 17 appears on the next page.

14161

Over the past 12 months...

16a. Each time you ate **bananas**, how many did you usually eat?

- O Less than 1 banana
- O 1 banana
- O More than 1 banana

17. How often did you eat **dried fruit**, such as prunes or raisins (not including dried apricots)?

- O NEVER (GO TO QUESTION 18)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

17a. Each time you ate **dried fruit** how much did you usually eat (not including dried apricots)?

- O Less than 2 tablespoons
- O 2 to 5 tablespoons
- O More than 5 tablespoons

18. Over the past 12 months, did you eat **peaches, nectarines,** or **plums**?

- O NO (GO TO QUESTION 19)
- O YES

18a. How often did you eat **fresh peaches, nectarines,** or **plums WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

18b. How often did you eat **peaches, nectarines,** or **plums** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR?**

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 19 appears in the next column.

18c. Each time you ate **peaches, nectarines,** or **plums**, how much did you usually eat?

- O Less than 1 fruit or less than 1/2 cup
- O 1 to 2 fruits or 1/2 to 3/4 cup
- O More than 2 fruits or more than 3/4 cup

19. How often did you eat **grapes**?

- O NEVER (GO TO QUESTION 20)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

19a. Each time you ate **grapes,** how much did you usually eat?

- O Less than 1/2 cup or less than 10 grapes
- O 1/2 to 1 cup or 10 to 30 grapes
- O More then 1 cup or more than 30 grapes

20. Over the past 12 months, did you eat **cantaloupe**?

- O NO (GO TO QUESTION 21)
- O YES

20a. How often did you eat **fresh cantaloupe WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

20b. How often did you eat **fresh or frozen cantaloupe DURING THE REST OF THE YEAR?**

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 21 appears on the next page.

14161

Over the past 12 months...

20c. Each time you ate **cantaloupe**, how much did you usually eat?

- O Less than 1/4 melon or less than 1/2 cup
- O 1/4 melon or 1/2 to 1 cup
- O More than 1/4 melon or more than 1 cup

21. Over the past 12 months, did you eat **melon, other than cantaloupe** (such as watermelon or honeydew)?

- O NO (GO TO QUESTION 22)
- O YES

21a. How often did you eat **fresh melon, other than cantaloupe** (such as watermelon or honeydew) **WHEN IN SEASON?**

O NEVER

- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

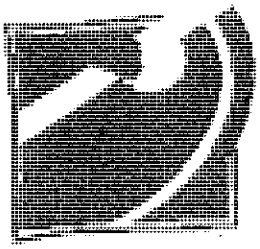
21b. How often did you eat **fresh or frozen melon, other than cantaloupe** (such as watermelon or honeydew) **DURING THE REST OF THE YEAR?**

O NEVER

- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

21c. Each time you ate **melon other than cantaloupe**, how much did you usually eat?

- O Less than 1/2 cup or 1 small wedge
- O 1/2 to 2 cups or 1 medium wedge
- O More than 2 cups or 1 large wedge



Question 23 appears in the next column.

22. Over the past 12 months, did you eat **strawberries?**

- O NO (GO TO QUESTION 23)
- O YES

22a. How often did you eat **fresh strawberries WHEN IN SEASON?**

O NEVER

- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

22b. How often did you eat **fresh or frozen strawberries DURING THE REST OF THE YEAR?**

O NEVER

- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

22c. Each time you ate **strawberries**, how much did you usually eat?

- O Less than 1/4 cup or or less than 3 berries
- O 1/4 to 3/4 cup or 3 to 8 berries
- O More than 3/4 cup or more than 8 berries

23. Over the past 12 months, did you eat **oranges, tangerines, or tangelos?**

- O NO (GO TO QUESTION 24)
- O YES

23a. How often did you eat **fresh oranges, tangerines, or tangelos WHEN IN SEASON?**

O NEVER

- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 24 appears on the next page.

14161

Over the past 12 months...

23b. How often did you eat **oranges, tangerines,** or **tangelos** (fresh or canned) **DURING THE REST OF THE YEAR?**

O NEVER

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

23c. Each time you ate **oranges, tangerines,** or **tangelos,** how much did you usually eat?

O Less than 1 fruit
O 1 fruit
O More than 1 fruit

24. Over the past 12 months, did you eat **grapefruit**?

O NO (GO TO QUESTION 25)

O YES

24a. How often did you eat **fresh grapefruit WHEN IN SEASON**?

O NEVER

O 1-6 times per season
O 7-11 times per season
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

24b. How often did you eat **grapefruit** (fresh or canned) **DURING THE REST OF THE YEAR?**

O NEVER

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

24c. Each time you ate **grapefruit**, how much did you usually eat?

O Less than 1/2 grapefruit
O 1/2 grapefruit
O More than 1/2 grapefruit

Question 25 appears in the next column.

25. How often did you eat **other kinds of fruit**?

O NEVER (GO TO QUESTION 26)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

25a. Each time you ate **other kinds of fruit**, how much did you usually eat?

O Less than 1/4 cup
O 1/4 to 3/4 cup
O More than 3/4 cup

26. How often did you eat **COOKED greens** (such as spinach, turnip, collard, mustard, chard, or kale)?

O NEVER (GO TO QUESTION 27)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

26a. Each time you ate **COOKED greens**, how much did you usually eat?

O Less than 1/2 cup
O 1/2 to 1 cup
O More than 1 cup

27. How often did you eat **RAW greens** (such as spinach, turnip, collard, mustard, chard, or kale)? (*We will ask about lettuce later*).

O NEVER (GO TO QUESTION 28)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

27a. Each time you ate **RAW greens**, how much did you usually eat?

O Less than 1/2 cup
O 1/2 to 1 cup
O More than 1 cup

Question 28 appears on the next page.

14161

Over the past 12 months...

28. How often did you eat coleslaw?

- O NEVER (GO TO QUESTION 29)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

28a. Each time you ate coleslaw, how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 3/4 cup
- O More than 3/4 cup

29. How often did you eat **sauerkraut** or **cabbage** (other than coleslaw)?

- O NEVER (GO TO QUESTION 30)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

29a. Each time you ate **sauerkraut** or **cabbage**, how much did you usually eat?

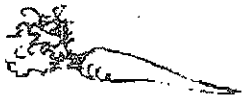
- O Less than 1/4 cup
- O 1/4 to 1 cup
- O More than 1 cup

30. How often did you eat **carrots** (fresh, canned, or frozen)?

- O NEVER (GO TO QUESTION 31)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

30a. Each time you ate **carrots**, how much did you usually eat?

- O Less then 1/4 cup or less than 2 baby carrots
- O 1/4 to 1/2 cup or 2 to 5 baby carrots
- O More than 1/2 cup or more than 5 baby carrots



Question 31 appears in the next column.

31. How often did you eat **string beans** or **green beans** (fresh, canned, or frozen)?

- O NEVER (GO TO QUESTION 32)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

31a. Each time you ate **string beans** or **green beans**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

32. How often did you eat **peas** (fresh, canned, or frozen)?

- O NEVER (GO TO QUESTION 33)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

32a. Each time you ate **peas**, how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 3/4 cup
- O More than 3/4 cup

33. Over the past 12 months, did you eat **corn**?

- O NO (GO TO QUESTION 34)
- O YES

33a. How often did you eat **fresh corn WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 34 appears on the next page.

14161

Over the past 12 months...

33b. How often did you eat **corn** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR?**

O NEVER

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

33c. Each time you ate **corn**, how much did you usually eat?

O Less than 1 ear or less than 1/2 cup
O 1 ear or 1/2 to 1 cup
O More than 1 ear or more than 1 cup

34. Over the past 12 months, how often did you eat **broccoli** (fresh or frozen)?

O NEVER (GO TO QUESTION 35)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

34a. Each time you ate **broccoli**, how much did you usually eat?

O Less than 1/4 cup
O 1/4 to 1 cup
O More than 1 cup

35. How often did you eat **cauliflower** or **Brussels sprouts** (fresh or frozen)?

O NEVER (GO TO QUESTION 36)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

35a. Each time you ate **cauliflower** or **Brussels sprouts**, how much did you usually eat?

O Less than 1/4 cup
O 1/4 to 1/2 cup
O More than 1/2 cup

Question 36 appears in the next column.

36. How often did you eat **mixed vegetables**?

O NEVER (Go to Question 37)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

36a. Each time you ate **mixed vegetables**, how much did you usually eat?

O Less than 1/2 cup
O 1/2 to 1 cup
O More than 1 cup

37. How often did you eat **onions**?

O NEVER (GO TO QUESTION 38)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

37a. Each time you ate **onions**, how much did you usually eat?

O Less than 1 slice or less than 1 tablespoon
O 1 slice or 1 to 4 tablespoons
O More than 1 slice or more than 4 tablespoons

38. Now think about all the **cooked vegetables** you ate in the past 12 months and how they were prepared. How often were your vegetables **COOKED WITH** some sort of **fat**, including oil spray? *(Please do not include potatoes.)*

O NEVER (GO TO QUESTION 39)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

Question 39 appears on the next page.

14161

Over the past 12 months...

38a. Which fats were usually added to your vegetables **DURING COOKING?** *(Please do not include potatoes. **Mark all that apply.**)*

- O Margarine (including low-fat)
- O Butter (including low-fat)
- O Lard, fatback, or bacon fat
- O Olive Oil
- O Corn Oil
- O Canola or rapeseed oil
- O Oil spray, such as Pam or others
- O Other kinds of oils
- O None of the above

39. Now, thinking again about all the **cooked vegetables** you ate in the past 12 months, how often was some sort of fat, sauce, or dressing added **AFTER COOKING OR AT THE TABLE?** (*Please do not include potatoes.*)

- O NEVER (GO TO QUESTION 40)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 times per day
- O 3 or more times per day

39a. Which fats, sauces, or dressings were usually added **AFTER COOKING OR AT THE TABLE?** (*Please do not include potatoes. **Mark all that apply.***)

- O Margarine (including low-fat)
- O Butter (including low-fat)
- O Lard, fatback, or bacon fat
- O Salad dressing
- O Cheese sauce
- O White sauce
- O Other

39b. If margarine, butter, lard, fatback, or bacon fat was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- O Did not usually add these
- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

39c. If salad dressing, cheese sauce, or white sauce was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- O Did not usually add these
- O Less than 1 tablespoon
- O 1 to 3 tablespoons
- O More than 3 tablespoons

Question 40 appears in the next column.

40. Over the past 12 months, how often did you eat **sweet peppers** (green, red, or yellow)?

- O NEVER (GO TO QUESTION 41)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

40a. Each time you ate **sweet peppers**, how much did you usually eat?

- O Less than 1/8 pepper
- O 1/8 to 1/4 pepper
- O More than 1/4 pepper

41. Over the past 12 months, did you eat **fresh tomatoes** (including those in salads)?

- O NO (GO TO QUESTION 42)
- O YES

41a. How often did you eat **fresh tomatoes** (including those in salads) **WHEN IN SEASON?**

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

41b. How often did you eat **fresh tomatoes** (including those in salads) **DURING THE REST OF THE YEAR?**

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

41c. Each time you ate **fresh tomatoes**, how much did you usually eat?

- O Less than 1/4 tomato
- O 1/4 to 1/2 tomato
- O More than 1/2 tomato

Question 42 appears on the next page.

14161

Over the **past 12 months**...

42. How often did you eat **lettuce salads** (with or without other vegetables)?

O NEVER (GO TO QUESTION 43)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

42a. Each time you ate **lettuce salads**, how much did you usually eat?

O Less than 1/4 cup
O 1/4 to 1 1/4 cups
O More than 1 1/4 cups

43. How often did you eat **salad dressing** (including low-fat) on salads?

O NEVER (GO TO QUESTION 44)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

43a. Each time you ate **salad dressing** on salads, how much did you usually eat?

O Less than 2 tablespoons
O 2 to 4 tablespoons
O More than 4 tablespoons

44. How often did you eat **sweet potatoes** or **yams**?

O NEVER (GO TO QUESTION 45)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

44a. Each time you ate **sweet potatoes** or **yams**, how much did you usually eat?

O 1 small potato or less than 1/4 cup
O 1 medium potato or 1/4 to 3/4 cup
O 1 large potato or more than 3/4 cup

Question 45 appears in the next column.

45. How often did you eat **French fries, home fries, hash browned potatoes,** or **tater tots**?

O NEVER (GO TO QUESTION 46)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

45a. Each time you ate **French fries, home fries, hash browned potatoes,** or **tater tots**, how much did you usually eat?

O Less than 10 fries or less than 1/2 cup
O 10 to 25 fries or 1/2 to 1 cup
O More than 25 fries or more than 1 cup

46. How often did you eat **potato salad**?

O NEVER (GO TO QUESTION 47)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

46a. Each time you ate **potato salad**, how much did you usually eat?

O Less than 1/2 cup
O 1/2 to 1 cup
O More than 1 cup

47. How often did you eat **baked, boiled,** or **mashed potatoes**?

O NEVER (GO TO QUESTION 48)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

47a. Each time you ate **baked, boiled,** or **mashed potatoes**, how much did you usually eat?

O 1 small potato or less than 1/2 cup
O 1 medium potato or 1/2 to 1 cup
O 1 large potato or more than 1 cup

Question 48 appears on the next page.

14161

Over the past 12 months...

47b. How often was **sour cream** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE?**

- O Almost never or never (GO TO QUESTION 47d)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

47c. Each time **sour cream** was added to your potatoes, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 3 tablespoons
- O More than 3 tablespoons

47d. How often was **margarine** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE?**

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

47e. How often was **butter** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE?**

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

47f. Each time **margarine** or **butter** was added to your potatoes, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

47g. How often was **cheese** or **cheese sauce** added to your potatoes, **EITHER IN COOKING OR AT THE TABLE?**

- O Almost never or never (GO TO QUESTION 48)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 48 appears in the next column.

47h. Each time **cheese** or **cheese sauce** was added to your potatoes, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 3 tablespoons
- O More than 3 tablespoons

48. How often did you eat **salsa**?

- O NEVER (GO TO QUESTION 49)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

48a. Each time you ate **salsa**, how much did you usually eat?

- O Less than 1 tablespoon
- O 1 to 5 tablespoons
- O More than 5 tablespoons

49. How often did you eat **catsup**?

- O NEVER (GO TO QUESTION 50)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

49a. Each time you ate **catsup**, how much did you usually eat?

- O Less than 1 teaspoon
- O 1 to 6 teaspoons
- O More than 6 teaspoons

50. How often did you eat **stuffing, dressing**, or **dumplings**?

- O NEVER (GO TO QUESTION 51)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

50a. Each time you ate **stuffing, dressing**, or **dumplings**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

Question 51 appears on the next page.

14161

Over the **past 12 months**...

51. How often did you eat **chili**?

- O NEVER (GO TO QUESTION 52)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

51a. Each time you ate **chili**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 3/4 cup
- O More than 1 3/4 cup

52. How often did you eat **Mexican foods** (such as tacos, tostados, burritos, tamales, fajitas, enchiladas, quesadillas, and chimichangas)?

- O NEVER (GO TO QUESTION 53)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

52a. Each time you ate **Mexican foods**, how much did you usually eat?

- O Less than 1 taco, burrito, etc.
- O 1 to 2 tacos, burritos, etc.
- O More than 2 tacos, burritos, etc.

53. How often did you eat **cooked dried beans** (such as baked beans, pinto, kidney, blackeyed peas, lima, lentils, soybeans, or refried beans)? (*Please don't include bean soups or chili.*)

- O NEVER (GO TO QUESTION 54)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

53a. Each time you ate **beans**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

Question 54 appears in the next column.

53b. How often were the beans you ate refried **beans, beans prepared with any type of fat,** or **with meat added**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

54. How often did you eat **other kinds of vegetables**?

- O NEVER (GO TO QUESTION 55)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

54a. Each time you ate **other kinds of vegetables**, how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 1/2 cup
- O More than 1/2 cup

55. How often did you eat **rice** or **other cooked grains** (such as bulgar, cracked wheat, or millet)?

- O NEVER (GO TO QUESTION 56)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

55a. Each time you ate **rice** or **other cooked grains**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 1/2 cups
- O More than 1 1/2 cups

55b. How often was **butter, margarine,** or **oil** added to your rice **IN COOKING OR AT THE TABLE**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 56 appears on the next page.

14161

Over the **past 12 months**...

56. How often did you eat **pancakes, waffles, or French toast**?

- O NEVER (GO TO QUESTION 57)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

56a. Each time you ate **pancakes, waffles, or French toast**, how much did you usually eat?

- O Less than 1 medium piece
- O 1 to 3 medium pieces
- O More than 3 medium pieces

56b. How often was **margarine** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE?**

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

56c. How often was **butter** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE?**

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

56d. Each time **margarine** or **butter** was added to your pancakes, waffles, or French toast, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

56e. How often was **syrup** added to your pancakes, waffles, or French toast?

- O Almost never or never (GO TO QUESTION 57)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 57 appears in the next column.

56f. Each time **syrup** was added to your pancakes, waffles, or French toast, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 4 tablespoons
- O More than 4 tablespoons

57. How often did you eat **lasagna, stuffed shells, stuffed manicotti, ravioli, or tortellini**? (*Please do not include spaghetti or other pasta.*)

- O NEVER (GO TO QUESTION 58)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

57a. Each time you ate **lasagna, stuffed shells, stuffed manicotti, ravioli, or tortellini**, how much did you usually eat?

- O Less than 1 cup
- O 1 to 2 cups
- O More than 2 cups

58. How often did you eat **macaroni and cheese**?

- O NEVER (GO TO QUESTION 59)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

58a. Each time you ate **macaroni and cheese**, how much did you usually eat?

- O Less than 1 cup
- O 1 to 1 1/2 cups
- O More than 1 1/2 cups

59. How often did you eat **pasta salad** or **macaroni salad**?

- O NEVER (GO TO QUESTION 60)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 60 appears on the next page.

14161

Over the **past 12 months**...

59a. Each time you ate **pasta salad** or **macaroni salad**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

60. Other than the pastas listed in Question 57, 58, and 59, how often did you eat **pasta**, **spaghetti**, or **other noodles**?

O NEVER (GO TO QUESTION 61)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

60a. Each time you ate **pasta**, **spaghetti** or **other noodles**, how much did you usually eat?

- O Less than 1 cup
- O 1 to 3 cups
- O More than 3 cups

60b. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITH meat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

60c. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITHOUT meat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

60d. How often did you eat your pasta, spaghetti, or other noodles with **margarine**, **butter**, **oil**, or **cream sauce**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 61 appears in the next column.

61. How often did you eat **bagels** or **English muffins**?

O NEVER (GO TO INTRODUCTION TO QUESTION 62)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

61a. Each time you ate **bagels** or **English muffins**, how much did you usually eat?

- O Less than 1 bagel or English muffin
- O 1 bagel or English muffin
- O More than 1 bagel or English muffin

61b. How often was **margarine** (including low-fat) added to your bagels or English muffins?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

61c. How often was **butter** (including low-fat) added to your bagels or English muffins?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

61d. Each time **margarine** or **butter** was added to your bagels or English muffins, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 2 teaspoons
- O More than 2 teaspoons

61e. How often was **cream cheese** (including low-fat) spread on your bagels or English muffins?

- O Almost never or never (GO TO INTRODUCTION TO QUESTION 62)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Introduction to Question 62 appears on the next page.

14161

Over the past 12 months...

61f. Each time **cream cheese** was added to your bagels or English muffins, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 2 tablespoons
- O More than 2 tablespoons

The next questions ask about your intake of breads other than bagels or English muffins. First, we will ask about bread you ate as part of sandwiches only. Then we will ask about all other bread you ate.

62. How often did you eat **breads** or **rolls AS PART OF SANDWICHES** (including burger and hot dog rolls)?

- O NEVER (GO TO QUESTION 63)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

62a. Each time you ate **breads** or **rolls AS PART OF SANDWICHES**, how many did you usually eat?

- O 1 slice or 1/2 roll
- O 2 slices or 1 roll
- O More than 2 slices or more than 1 roll

62b. How often were the breads or rolls that you used for your sandwiches **white bread** (including burger and hot dog rolls)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

62c. How often was **mayonnaise** or **mayonnaise-type dressing** (including low-fat) added to your sandwich bread or rolls?

- O Almost never or never (GO TO QUESTION 62e)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 62e appears in the next column

Question 63 appears in the next column.

62d. Each time **mayonnaise** or **mayonnaise-type dressing** was added to your sandwich breads or rolls, how much was usually added?

- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

62e. How often was **margarine** (including low-fat) added to your sandwich bread or rolls?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

62f. How often was **butter** (including low-fat) added to your sandwich bread or rolls?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

62g. Each time **margarine** or **butter** was added to your sandwich breads or rolls, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 2 teaspoons
- O More than 2 teaspoons

63. How often did you eat **breads** or **dinner rolls, NOT AS PART OF SANDWICHES?**

- O NEVER (GO TO INTRODUCTION TO QUESTION 64)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

63a. Each time you ate **breads** or **dinner rolls, NOT AS PART OF SANDWICHES**, how much did you usually eat?

- O 1 slice or 1 dinner roll
- O 2 slices or 2 dinner rolls
- O More than 2 slices or more than 2 dinner rolls

Question 64 appears on the next page.

14161

Over the past 12 months...

63b. How often were the breads or rolls you ate **white bread**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

63c. How often was **margarine** (including low-fat) added to your breads or rolls?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

63d. How often was **butter** (including low-fat) added to your breads or rolls?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

63e. Each time **margarine** or **butter** was added to your breads or rolls, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 2 teaspoons
- O More than 2 teaspoons

63f. How often was **cream cheese** (including low-fat) added to your breads or rolls?

- O Almost never or never (GO TO QUESTION 64)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

63g. Each time **cream cheese** was added to your breads or rolls, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 2 tablespoon
- O More than 2 tablespoons

Question 64 appears in the next column.

64. How often did you eat **jam, jelly, or honey** on bagels, muffins, bread, rolls, or crackers?

- O NEVER (GO TO QUESTION 65)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

64a. Each time you ate **jam, jelly, or honey**, how much did you usually eat?

- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

65. How often did you eat **peanut butter** or **other nut butter**?

- O NEVER (GO TO QUESTION 66)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

65a. Each time you ate **peanut butter** or **other nut butter**, how much did you usually eat?

- O Less than 1 tablespoon
- O 1 to 2 tablespoons
- O More than 2 tablespoons

66. How often did you eat **roast beef** or **steak IN SANDWICHES**?

- O NEVER (GO TO QUESTION 67)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

66a. Each time you ate **roast beef** or **steak IN SANDWICHES**, how much did you usually eat?

- O Less than 1 slice or less than 2 ounces
- O 1 to 2 slices or 2 to 4 ounces
- O More than 2 slices or more than 4 ounces

Question 67 appears on the next page.

14161

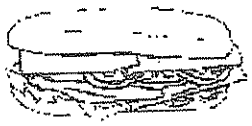
Over the past 12 months...

67. How often did you eat **turkey** or **chicken COLD CUTS** (such as loaf, luncheon meat, turkey ham, turkey salami, or turkey pastrami)? *(We will ask about other turkey or chicken later.)*

- O NEVER (GO TO QUESTION 68)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

67a. Each time you ate **turkey** or **chicken COLD CUTS**, how much did you usually eat?

- O Less than 1 slice
- O 1 to 3 slices
- O More than 3 slices



68. How often did you eat **luncheon** or **deli-style ham**? *(We will ask about other ham later.)*

- O NEVER (GO TO QUESTION 69)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

68a. Each time you ate **luncheon** or **deli-style ham**, how much did you usually eat?

- O Less than 1 slice
- O 1 to 3 slices
- O More than 3 slices

68b. How often was the luncheon or deli-style ham you ate **light**, **low-fat**, or **fat-free**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 69 appears in the next column.

69. How often did you eat other **cold cuts** or **luncheon meats** (such as bologna, salami, corned beef, pastrami, or others, including low-fat)? *(Please do not include ham, turkey, or chicken cold cuts.)*

- O NEVER (GO TO QUESTION 70)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

69a. Each time you ate **other cold cuts** or **luncheon meats**, how much did you usually eat?

- O Less than 1 slice
- O 1 to 3 slices
- O More than 3 slices

69b. How often were the other cold cuts or luncheon meats you ate **light**, **low-fat**, or **fat-free cold cuts** or **luncheon meats**? *(Please do not include ham, turkey, or chicken cold cuts.)*

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

70. How often did you eat **canned tuna** (including in salads, sandwiches, or casseroles)?

- O NEVER (GO TO QUESTION 71)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

70a. Each time you ate **canned tuna**, how much did you usually eat?

- O Less than 1/4 cup or less than 2 ounces
- O 1/4 to 1/2 cup or 2 to 3 ounces
- O More than 1/2 cup or more than 3 ounces

70b. How often was the canned tuna you ate **water-packed tuna**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 71 appears on the next page.

14161

Over the past 12 months...

70c. How often was the canned tuna you ate **prepared with mayonnaise** or **other dressing** (including low-fat)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

71. How often did you eat **GROUND chicken** or **turkey**? *(We will ask about other chicken and turkey later.)*

- O NEVER (GO TO QUESTION 72)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

71a. Each time you ate **GROUND chicken** or **turkey**, how much did you usually eat?

- O Less than 2 ounces or less than 1/2 cup
- O 2 to 4 ounces or 1/2 to 1 cup
- O More than 4 ounces or more than 1 cup

72. How often did you eat **beef hamburgers** or **cheeseburgers**?

- O NEVER (GO TO QUESTION 73)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

72a. Each time you ate **beef hamburgers** or **cheeseburgers**, how much did you usually eat?

- O Less than 1 patty or less than 2 ounces
- O 1 patty or 2 to 4 ounces
- O More than 1 patty or more than 4 ounces

72b. How often were the beef hamburgers or cheesburgers you ate made with **lean ground beef**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 73 appears in the next column.

73. How often did you eat **ground beef in mixtures** (such as meatballs, casseroles, chili, or meatloaf)?

- O NEVER (GO TO QUESTION 74)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

73a. Each time you ate **ground beef in mixtures**, how much did you usually eat?

- O Less than 3 ounces or less than 1/2 cup
- O 3 to 8 ounces or 1/2 to 1 cup
- O More than 8 ounces or more than 1 cup

74. How often did you eat **hot dogs** or **frankfurters**? *(Please do not include sausages or vegetarian hot dogs.)*

- O NEVER (GO TO QUESTION 75)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

74a. Each time you ate **hot dogs** or **frankfurters**, how many did you usually eat?

- O Less than 1 hot dog
- O 1 to 2 hot dogs
- O More than 2 hot dogs

74b. How often were the hot dogs or frankfurters you ate **light** or **low-fat hot dogs**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 75 appears on the next page.

14161

Over the past 12 months...

75. How often did you eat beef mixtures such as **beef stew, beef pot pie, beef and noodles**, or **beef and vegetables**?

- O NEVER (GO TO QUESTION 76)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

75a. Each time you ate **beef stew, beef pot pie, beef and noodles**, or **beef and vegetables**, how much did you usually eat?

- O Less than 1 cup
- O 1 to 2 cups
- O More than 2 cups

76. How often did you eat **roast beef** or **pot roast**? *(Please do not include roast beef or pot roast in sandwiches.)*

- O NEVER (GO TO QUESTION 77)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

76a. Each time you ate **roast beef** or **pot roast** (including in mixtures), how much did you usually eat?

- O Less than 2 ounces
- O 2 to 5 ounces
- O More than 5 ounces

77. How often did you eat **steak** (beef)? *(Do not include steak in sandwiches.)*

- O NEVER (GO TO QUESTION 78)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

77a. Each time you ate **steak** (beef), how much did you usually eat?

- O Less than 3 ounces
- O 3 to 7 ounces
- O More than 7 ounces

Question 78 appears in the next column.

77b. How often was the steak you ate **lean steak**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

78. How often did you eat **pork** or **beef spareribs**?

- O NEVER (GO TO QUESTION 79)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

78a. Each time you ate **pork** or **beef spareribs**, how much did you usually eat?

- O Less than 4 ribs
- O 4 to 12 ribs
- O More than 12 ribs

79. How often did you eat **roast turkey, turkey cutlets**, or **turkey nuggets** (including in sandwiches)?

- O NEVER (GO TO QUESTION 80)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

79a. Each time you ate **roast turkey, turkey cutlets**, or **turkey nuggets**, how much did you usually eat? *(Please note: 4 to 8 turkey nuggets = 3 ounces)*

- O Less than 2 ounces
- O 2 to 4 ounces
- O More than 4 ounces

80. How often did you eat **chicken** as part of **salads, sandwiches, casseroles, stews**, or **other mixtures**?

- O NEVER (GO TO QUESTION 81)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 81 appears on the next page.

14161

Over the past 12 months...

80a. Each time you ate **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 1/2 cups
- O More than 1 1/2 cups

81. How often did you eat **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets)? *(Please do not include chicken in mixtures.)*

- O NEVER (GO TO QUESTION 82)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

81a. Each time you ate **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets), how much did you usually eat?

- O Less than 2 drumsticks or wings, less than 1 breast or thigh, or less than 4 nuggets
- O 2 drumsticks or wings, 1 breast or thigh, or 4 to 8 nuggets
- O More than 2 drumsticks or wings, more than 1 breast or thigh, or more than 8 nuggets

81b. How often was the chicken you ate **fried chicken** (including deep fried) or **chicken nuggets**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

81c. How often was the chicken you ate **WHITE meat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

81d. How often did you eat chicken **WITH skin**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question appears 82 in the next column.

82. How often did you eat **baked ham** or **ham steak**?

- O NEVER (GO TO QUESTION 83)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

82a. Each time you ate **baked ham** or **ham steak**, how much did you usually eat?

- O Less than 1 ounce
- O 1 to 3 ounces
- O More than 3 ounces

83. How often did you eat **pork** (including chops, roasts, and in mixed dishes)? *(Please do not include ham, ham steak, or sausage.)*

- O NEVER (GO TO QUESTION 84)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

83a. Each time you ate **pork**, how much did you usually eat?

- O Less than 2 ounces or less than 1 chop
- O 2 to 5 ounces or 1 chop
- O More than 5 ounces or more than 1 chop

84. How often did you eat **gravy** on meat, chicken, potatoes, rice, etc.?

- O NEVER (GO TO QUESTION 85)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

84a. Each time you ate **gravy** on meat, chicken, potatoes, rice, etc., how much did you usually eat?

- O Less than 1/8 cup
- O 1/8 to 1/2 cup
- O More than 1/2 cup

Question 85 appears on the next page.

14161

Over the past 12 months...

85. How often did you eat **liver** (all kinds) or **liverwurst**?

- ○ NEVER (GO TO QUESTION 86)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

85a. Each time you ate **liver** or **liverwurst**, how much did you usually eat?

- ○ Less than 1 ounce
- ○ 1 to 4 ounces
- ○ More than 4 ounces

86. How often did you eat **bacon** (including low-fat)?

- ○ NEVER (GO TO QUESTION 87)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

86a. Each time you ate **bacon**, how much did you usually eat?

- ○ Fewer than 2 slices
- ○ 2 to 3 slices
- ○ More than 3 slices

86b. How often was the bacon you ate **light**, **low-fat**, or **lean bacon**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

87. How often did you eat **sausage** (including low-fat)?

- ○ NEVER (GO TO QUESTION 88)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

Question 88 appears in the next column.

87a. Each time you ate **sausage**, how much did you usually eat?

- ○ Less than one patty or 2 links
- ○ 1 to 3 patties or 2 to 5 links
- ○ More than 3 patties or 5 links

87b. How often was the sausage you ate **light**, **low-fat**, or **lean sausage**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

88. How often did you eat **fish sticks** or **fried fish** (including fried seafood or shellfish)?

- ○ NEVER (GO TO QUESTION 89)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

88a. Each time you ate **fish sticks** or **fried fish**, how much did you usually eat?

- ○ Less than 2 ounces or less than 1 fillet
- ○ 2 to 7 ounces or 1 fillet
- ○ More than 7 ounces or more than 1 fillet

89. How often did you eat **fish** or **seafood that was NOT FRIED** (including shellfish)?

- ○ NEVER (GO TO INTRODUCTION TO QUESTION 90)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

89a. Each time you ate **fish** or **seafood that was not fried**, how much did you usually eat?

- ○ Less than 2 ounces or less than 1 fillet
- ○ 2 to 5 ounces or 1 fillet
- ○ More than 5 ounces or more than 1 fillet

Introduction to Question 90 appears on the next page.

14161

Over the past 12 months...

Now think about all the meat, poultry, and fish you ate in the past 12 months and how they were prepared.

90. How often was **oil, butter, margarine, or other fat** used to **FRY, SAUTE, BASTE, OR MARINATE** any meat, poultry, or fish you ate? *(Please do not include deep frying.)*

- O NEVER (GO TO QUESTION 91)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

90a. Which of the following **fats** were regularly used to prepare your meat, poultry, or fish? ***(Mark all that apply.)***

- O Margarine (including low-fat)
- O Butter (including low-fat)
- O Lard, fatback, or bacon fat
- O Olive Oil
- O Corn Oil
- O Canola or rapeseed oil
- O Oil spray, such as Pam or others
- O Other kinds of oils
- O None of the above

91. How often did you eat **tofu, soy burgers, or soy meat-substitutes**?

- O NEVER (GO TO QUESTION 92)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

91a. Each time you ate **tofu, soy burgers, or soy meat-substitutes**, how much did you usually eat?

- O Less than 1/4 cup or less than 2 ounces
- O 1/4 to 1/2 cup or 2 to 4 ounces
- O More than 1/2 cup or more than 4 ounces

Question 92 appears in the next column.

92. Over the past 12 months, did you eat soups?

- O NO (Go To Question 93)
- O YES

92a. How often did you eat **soup DURING THE WINTER?**

- O NEVER
- O 1-6 times per winter
- O 7-11 times per winter
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

92b. How often did you eat **soup DURING THE REST OF THE YEAR?**

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

92c. Each time you ate **soup**, how much did you usually eat?

- O Less than 1 cup
- O 1 to 2 cups
- O More than 2 cups

92d. How often were the soups you ate **bean soups**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

92e. How often were the soups you ate **cream soups** (including chowders)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 93 appears on the next page.

14161

Over the **past 12 months**...

92f. How often were the soups you ate **tomato** or **vegetable soups**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

92g. How often were the soups you ate **broth soups** (including chicken) **with** or **without noodles** or **rice**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

93. How often did you eat **pizza**?

- O NEVER (GO TO QUESTION 94)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

93a. Each time you ate **pizza**, how much did you usually eat?

- O Less than 1 slice or less than 1 mini pizza
- O 1 to 3 slices or 1 mini pizza
- O More than 3 slices or more than 1 mini pizza

93b. How often did you eat pizza with **pepperoni**, **sausage**, or **other meat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

94. How often did you eat **crackers**?

- O NEVER (GO TO QUESTION 95)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 95 appears in the next column.

94a. Each time you ate **crackers**, how many did you usually eat?

- O Fewer than 4 crackers
- O 4 to 10 crackers
- O More than 10 crackers

95. How often did you eat **corn bread** or **corn muffins**?

- O NEVER (GO TO QUESTION 96)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

95a. Each time you ate **corn bread** or **corn muffins**, how much did you usually eat?

- O Less than 1 piece or muffin
- O 1 to 2 pieces or muffins
- O More than 2 pieces or muffins

96. How often did you eat **biscuits**?

- O NEVER (GO TO QUESTION 97)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

96a. Each time you ate **biscuits**, how many did you usually eat?

- O Fewer than 1 biscuit
- O 1 to 2 biscuits
- O More than 2 biscuits

97. How often did you eat **potato chips**, **tortilla chips**, or **corn chips** (including low-fat, fat-free, or low-salt)?

- O NEVER (GO TO QUESTION 98)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 98 appears on the next page.

14161

Over the past 12 months...

97a. Each time you ate **potato chips, tortilla chips**, or **corn chips**, how much did you usually eat?

- O Fewer than 10 chips or less than 1 cup
- O 10 to 25 chips or 1 to 2 cups
- O More than 25 chips or more than 2 cups

97b. How often were the chips you ate **Wow chips** or other **chips made with fat substitute** (Olean or Olestra)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

97c. How often were the chips you ate other **low-fat** or **fat-free chips**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

98. How often did you eat **popcorn** (including low-fat)?

O NEVER (GO TO QUESTION 99)

- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

98a. Each time you ate **popcorn**, how much did you usually eat?

- O Less than 2 cups, popped
- O 2 to 5 cups, popped
- O More than 5 cups, popped

99. How often did you eat **pretzels**?

O NEVER (GO TO QUESTION 100)

- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 100 appears in the next column.

99a. Each time you ate **pretzels**, how many did you usually eat?

- O Fewer than 5 average twists
- O 5 to 20 average twists
- O More than 20 average twists

100. How often did you eat **peanuts, walnuts, seeds**, or **other nuts**?

O NEVER (GO TO QUESTION 101)

- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

100a. Each time you ate **peanuts, walnuts, seeds**, or **other nuts**, how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 1/2 cup
- O More than 1/2 cup

101. How often did you eat **energy bars, high-protein**, or **breakfast bars** such as **Power bars, Balance, Clif**, or **others**?

O NEVER (GO TO QUESTION 102)

- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

101a. Each time you ate **energy, high-protein**, or **breakfast bars**, how much did you usually eat?

- O Less than 1 bar
- O 1 bar
- O More than 1 bar

102. How often did you eat **yogurt** (NOT including frozen yogurt)?

O NEVER (GO TO QUESTION 103)

- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 103 appears on the next page.

14161

Over the past 12 months...

102a. Each time you ate **yogurt**, how much did you usually eat?

- ○ Less than 1/2 cup or less than 1 container
- ○ 1/2 to 1 cup or 1 container
- ○ More than 1 cup or more than 1 container

103. How often did you eat **cottage cheese** (including low-fat)?

- ○ NEVER (GO TO QUESTION 104)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

103a. Each time you ate **cottage cheese**, how much did you usually eat?

- ○ Less than 1/4 cup
- ○ 1/4 to 1 cup
- ○ More than 1 cup

104. How often did you eat **cheese** (including low-fat; including on cheeseburgers or in sandwiches or subs)?

- ○ NEVER (GO TO QUESTION 105)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

104a. Each time you ate **cheese**, how much did you usually eat?

- ○ Less than 1/2 ounce or less than 1 slice
- ○ 1/2 to 1 1/2 ounces or 1 slice
- ○ More than 1 1/2 ounces or more than 1 slice

104b. How often was the cheese you ate **light** or **low-fat cheese**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

Question 105 appears in the next column.

104c. How often was the cheese you ate **fat-free cheese**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

105. How often did you eat **frozen yogurt, sorbet,** or **ices** (including low-fat or fat-free)?

- ○ NEVER (GO TO QUESTION 106)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

105a. Each time you ate frozen **yogurt, sorbet,** or **ices**, how much did you usually eat?

- ○ Less than 1/2 cup or less than 1 scoop
- ○ 1/2 to 1 cup or 1 to 2 scoops
- ○ More than 1 cup or more than 2 scoops

106. How often did you eat **ice cream, ice cream bars,** or **sherbet** (including low-fat or fat-free)?

- ○ NEVER (GO TO QUESTION 107)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

106a. Each time you ate **ice cream, ice cream bars,** or **sherbet**, how much did you usually eat?

- ○ Less than 1/2 cup or less than 1 scoop
- ○ 1/2 to 1 1/2 cups or 1 to 2 scoops
- ○ More than 1 1/2 cups or more than 2 scoops

106b. How often was the ice cream you ate **light, low-fat,** or **fat-free ice cream** or **sherbet**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

Question 107 appears on the next page.

14161

Over the **past 12 months**...

107. How often did you eat **cake** (including low-fat or fat-free)?

- O NEVER (GO TO QUESTION 108)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

107a. Each time you ate **cake**, how much did you usually eat?

- O Less than 1 medium piece
- O 1 medium piece
- O More than 1 medium piece

107b. How often was the cake you ate **light, low-fat**, or **fat-free cake**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

108. How often did you eat **cookies** or **brownies** (including low-fat or fat-free)?

- O NEVER (GO TO QUESTION 109)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

108a. Each time you ate **cookies** or **brownies**, how much did you usually eat?

- O Less than 2 cookies or 1 small brownie
- O 2 to 4 cookies or 1 medium brownie
- O More than 4 cookies or 1 large brownie

108b. How often were the cookies or brownies you ate **light, low-fat**, or **fat-free cookies** or **brownies**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 109 appears in the next column.

109. How often did you eat **doughnuts, sweet rolls, Danish**, or **pop-tarts**?

- O NEVER (GO TO QUESTION 110)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

109a. Each time you ate **doughnuts, sweet rolls, Danish**, or **pop-tarts**, how much did you usually eat?

- O Less than 1 piece
- O 1 to 2 pieces
- O More than 2 pieces

110. How often did you eat **sweet muffins** or **dessert breads** (including low-fat or fat-free)?

- O NEVER (GO TO QUESTION 111)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

110a. Each time you ate **sweet muffins** or **dessert breads**, how much did you usually eat?

- O Less than 1 medium piece
- O 1 medium piece
- O More than 1 medium piece

110b. How often were the sweet muffins or dessert breads you ate **light, low-fat**, or **fat-free sweet muffins** or **dessert breads**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

111. How often did you eat **fruit crisp, cobbler**, or **strudel**?

- O NEVER (GO TO QUESTION 112)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 112 appears on the next page.

14161

Over the past 12 months...

111a. Each time you ate **fruit crisp, cobbler,** or **strudel**, how much did you usually eat?

- ○ Less than 1/2 cup
- ○ 1/2 cup to 1 cup
- ○ More than 1 cup

112. How often did you eat **pie**?

- ○ NEVER (GO TO QUESTION 113)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

112a. Each time you ate **pie**, how much did you usually eat?

- ○ Less than 1/8 of a pie
- ○ About 1/8 of a pie
- ○ More than 1/8 of a pie

The next four questions ask about the kinds of pie you ate. Please read all four questions before answering.

112b. How often were the pies you ate **fruit pie** (such as apple, blueberry, others)?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

112c. How often were the pies you ate **cream, pudding, custard,** or **meringue pie**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

112d. How often were the pies you ate **pumpkin** or **sweet potato pie**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

Question 113 appears in the next column.

112e. How often were the pies you ate **pecan pie**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

113. How often did you eat **chocolate candy**?

- ○ NEVER (GO TO QUESTION 114)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

113a. Each time you ate **chocolate candy**, how much did you usually eat?

- ○ Less than 1 average bar or less than 1 ounce
- ○ 1 average bar or 1 to 2 ounces
- ○ More than 1 average bar or more than 2 ounces

114. How often did you eat **other candy**?

- ○ NEVER (GO TO QUESTION 115)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

114a. Each time you ate **other candy**, how much did you usually eat?

- ○ Fewer than 2 pieces
- ○ 2 to 9 pieces
- ○ More than 9 pieces

115. How often did you eat **eggs, egg whites,** or **egg substitutes** (NOT counting eggs in baked goods and desserts)? *(Please include eggs in salads, quiche, and souffles.)*

- ○ NEVER (GO TO QUESTION 116)
- ○ 1-6 times per year
- ○ 7-11 times per year
- ○ 1 time per month
- ○ 2-3 times per month
- ○ 1 time per week
- ○ 2 times per week
- ○ 3-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2 or more times per day

Question 116 appears on the next page.

14161

Over the past 12 months...

115a. Each time you ate eggs, how many did you usually eat?

- O 1 egg
- O 2 eggs
- O 3 or more eggs

115b. How often were the eggs you ate **egg substitutes**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

115c. How often were the eggs you ate **egg whites only**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

115d. How often were the eggs you ate **regular whole eggs**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

115e. How often were the eggs you ate **cooked in oil, butter, or margarine**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

115f. How often were the eggs you ate part of **egg salad**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

116. How many cups of coffee, caffeinated or decaffeinated, did you drink?

- O NONE (GO TO QUESTION 117)
- O Less than 1 cup per month
- O 1-3 cups per month
- O 1 cup per week
- O 2-4 cups per week
- O 5-6 cups per week
- O 1 cup per day
- O 2-3 cups per day
- O 4-5 cups per day
- O 6 or more cups per day

116a. How often was the coffee you drank **decaffeinated**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

117. How many glasses of **ICED tea**, caffeinated or decaffeinated, did you drink?

- O NONE (GO TO QUESTION 118)
- O Less than 1 cup per month
- O 1-3 cups per month
- O 1 cup per week
- O 2-4 cups per week
- O 5-6 cups per week
- O 1 cup per day
- O 2-3 cups per day
- O 4-5 cups per day
- O 6 or more cups per day

117a. How often was the iced tea you drank **decaffeinated** or **herbal tea**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

118. How many cups of **HOT tea**, caffeinated or decaffeinated, did you drink?

- O NONE (GO TO QUESTION 119)
- O Less than 1 cup per month
- O 1-3 cups per month
- O 1 cup per week
- O 2-4 cups per week
- O 5-6 cups per week
- O 1 cup per day
- O 2-3 cups per day
- O 4-5 cups per day
- O 6 or more cups per day

118a. How often was the hot tea you drank **decaffeinated** or **herbal tea**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 119 appears on the next page.

14161

Over the past 12 months...

119. How often did you add **sugar** or **honey** to your coffee or tea?

- ○ NEVER (GO TO QUESTION 120)
- ○ Less than 1 time per month
- ○ 1-3 times per month
- ○ 1 time per week
- ○ 2-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2-3 times per day
- ○ 4-5 times per day
- ○ 6 or more times per day

119a. Each time **sugar** or **honey** was added to your coffee or tea, how much was usually added?

- ○ Less than 1 teaspoon
- ○ 1 to 3 teaspoons
- ○ More than 3 teaspoons

120. How often did you add **artificial sweetener** to your coffee or tea?

- ○ NEVER (GO TO QUESTION 121)
- ○ Less than 1 time per month
- ○ 1-3 times per month
- ○ 1 time per week
- ○ 2-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2-3 times per day
- ○ 4-5 times per day
- ○ 6 or more times per day

120a. What kind of **artificial sweetener** did you usually use?

- ○ Equal or aspartame
- ○ Sweet N Low or saccharin

121. How often was **non-dairy creamer** added to your coffee or tea?

- ○ NEVER (GO TO QUESTION 122)
- ○ Less than 1 time per month
- ○ 1-3 times per month
- ○ 1 time per week
- ○ 2-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2-3 times per day
- ○ 4-5 times per day
- ○ 6 or more times per day

121a. Each time **non-dairy creamer** was added to your coffee or tea, how much was usually used?

- ○ Less than 1 teaspoon
- ○ 1 to 3 teaspoons
- ○ More than 3 teaspoons

Question 122 appears in the next column.

121b. What kind of **non-dairy creamer** did you usually use?

- ○ Regular powered
- ○ Low-fat or fat-free powered
- ○ Regular liquid
- ○ Low-fat or fat-free liquid

122. How often was **cream** or **half and half** added to your coffee or tea?

- ○ NEVER (GO TO QUESTION 123)
- ○ Less than 1 time per month
- ○ 1-3 times per month
- ○ 1 time per week
- ○ 2-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2-3 times per day
- ○ 4-5 times per day
- ○ 6 or times more per day

122a. Each time **cream** or **half and half** was added to your coffee or tea, how much was usually added?

- ○ Less than 1 tablespoon
- ○ 1 to 2 tablespoons
- ○ More than 2 tablespoons

123. How often was **milk** added to your coffee or tea?

- ○ NEVER (GO TO QUESTION 124)
- ○ Less than 1 time per month
- ○ 1-3 times per month
- ○ 1 time per week
- ○ 2-4 times per week
- ○ 5-6 times per week
- ○ 1 time per day
- ○ 2-3 times per day
- ○ 4-5 times per day
- ○ 6 or times more per day

123a. Each time **milk** was added to your coffee or tea, how much was usually added?

- ○ Less than 1 tablespoon
- ○ 1 to 3 tablespoons
- ○ More than 3 tablespoons

123b. What kind of **milk** was usually added to your coffee or tea?

- ○ Whole milk
- ○ 2% milk
- ○ 1% milk
- ○ Skim, nonfat, or 1/2% milk
- ○ Evaporated or condensed (canned) milk
- ○ Soy milk
- ○ Rice milk
- ○ Other

Question 124 appears on the next page.

14161

Over the past 12 months...

124. How often was **sugar** or **honey** added to foods you ate? (Please do not include sugar in coffee, tea, other beverages, or baked goods.)

- O NEVER (GO TO INTRODUCTION TO QUESTION 125)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

124a. Each time **sugar** or **honey** was added to foods you ate, how much was usually added?

- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

The following questions are about the kinds of margarine, mayonnaise, sour cream, cream cheese, and salad dressing that you eat. If possible, please check the labels of these foods to help you answer.

125. Over the past 12 months, did you eat **margarine?**

- O NO (GO TO QUESTION 126)
- O YES

125a. How often was the margarine you ate **regular-fat margarine** (stick or tub)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

125b. How often was the margarine you ate **light or low-fat margarine** (stick or tub)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 126 appears in the next column.

125c. How often was the margarine you ate **fat-free margarine?**

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

126. Over the past 12 months, did you eat **butter?**

- O NO (GO TO QUESTION 127)
- O YES

126a. How often was the butter you ate **light** or **low-fat butter?**

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

127. Over the past 12 months, did you eat **mayonnaise or mayonnaise-type dressing?**

- O NO (GO TO QUESTION 128)
- O YES

127a. How often was the mayonnaise you ate **regular-fat mayonnaise?**

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

127b. How often was the mayonnaise you ate **light or low-fat mayonnaise?**

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 128 appears on the next page.

14161

Over the past 12 months...

127c. How often was the mayonnaise you ate **fat-free mayonnaise**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

128. Over the past 12 months, did you eat **sour cream**?

- ○ NO (GO TO QUESTION 129)
- ○ YES

128a. How often was the sour cream you ate **regular-fat sour cream**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

128b. How often was the sour cream you ate **light, low-fat, or fat free sour cream**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

129. Over the past 12 months, did you eat **cream cheese**?

- ○ NO (GO TO QUESTION 130)
- ○ YES

129a. How often was the cream cheese you ate **regular-fat cream cheese**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

Question 130 appears in the next column.

129b. How often was the cream cheese you ate **light, low-fat, or fat-free cream cheese**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

130. Over the past 12 months, did you eat **salad dressing**?

- ○ NO (GO TO INTRODUCTION TO QUESTION 131)
- ○ YES

130a. How often was the salad dressing you ate **regular-fat salad dressing** (including oil and vinegar dressing)?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

130b. How often was the salad dressing you ate **light or low-fat salad dressing**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

130c. How often was the salad dressing you ate **fat-free salad dressing**?

- ○ Almost never or never
- ○ About 1/4 of the time
- ○ About 1/2 of the time
- ○ About 3/4 of the time
- ○ Almost always or always

The following two questions ask you to summarize your usual intake of vegetables and fruits. Please do not include salads, potatoes, or juices.

131. Over the past 12 months, how many servings of **vegetables** (not including salad or potatoes) did you eat per week or per day?

- ○ Less than 1 per week
- ○ 1-2 per week
- ○ 3-4 per week
- ○ 5-6 per week
- ○ 1 per day
- ○ 2 per day
- ○ 3 per day
- ○ 4 per day
- ○ 5 or more per day

14161

Over the past 12 months...

132. Over the past 12 months, how many servings of **fruit** (not including juices) did you eat per week or per day?

- O Less than 1 per week
- O 1-2 per week
- O 3-4 per week
- O 5-6 per week
- O 1 per day
- O 2 per day
- O 3 per day
- O 4 per day
- O 5 or more per day

133. Over the past month, which of the following foods did you eat **AT LEAST THREE TIMES**? ***(Mark all that apply.)***

- O Avocado, guacamole
- O Cheesecake
- O Chocolate, fudge, or butterscotch toppings or syrups
- O Chow mein noodles
- O Croissants
- O Dried apricots
- O Egg rolls
- O Granola bars
- O Hot peppers
- O Jello, gelatin
- O Milkshakes or ice-cream sodas
- O Olives
- O Oysters
- O Pickles or pickled vegetables or fruit
- O Plantains
- O Pork neckbones, hock, head, or feet
- O Pudding or custard
- O Veal, venison, lamb
- O Whipped cream, regular
- O Whipped cream, substitute
- O NONE

134. For **ALL** of the past 12 months, have you followed any type of **vegetarian diet?**

- O NO (GO TO INTRODUCTION TO QUESTION 135)
- O YES

134a. Which of the following foods did you **TOTALLY EXCLUDE** from your diet? ***(Mark all that apply.)***

- O Meat (beef, pork, lamb etc.)
- O Poultry (chicken, turkey, duck)
- O Fish and seafood
- O Eggs
- O Dairy products (milk, cheese, etc.)

Introduction to Question 135 appears in the next column.

The next questions are about your use of fiber supplements or vitamin pills.

135. Over the past 12 months, did you take any of the following types of **fiber** or **fiber supplements** on a regular basis (more than once per week for at least 6 of the last 12 months)? ***(Mark all that apply.)***

- O NO, didn't take any fiber supplements on a regular basis (GO TO QUESTION 136)
- O YES, psyllium products (such as Metamucil, Fiberall, Serutan, Perdiem, Correctol)
- O YES, methylcellulose/cellulose products (such as Citrucel, Unifiber)
- O YES, Fibercon
- O YES, Bran (such as wheat bran, oat bran, or bran wafers)

136. Over the past 12 months, did you take any **multivitamins**, such as One-a-Day-, Theragran-, or Centrum-type multivitamins (as pills, liquids, or packets)?

- O NO (GO TO INTRODUCTION TO QUESTION 138)
- O YES

137. How often did you take **One-a-Day-**, **Theragran-**, or **Centrum-type** multivitamins?

- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

137a. Does your **multivitamin** usually contain **minerals** (such as iron, zinc, etc.)?

- O NO
- O YES
- O Don't know

137b. For how many years have you taken **multivitamins?**

- O Less than 1 year
- O 1-4 years
- O 5-9 years
- O 10 or more years

Question 138 appears on the next page.

14161

Over the past 12 months...

137c. Over the past 12 months, did you take any vitamins, minerals, or other herbal supplements other than your multivitamin?

O No

Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- **Did not skip any pages,**
- **Completely blackened-in each answer, and**
- **Crossed out the incorrect answer and circled the correct answer if you made any changes.**

O YES (GO TO INTRODUCTION TO QUESTION 138)

These last questions are about the vitamins, minerals, or herbal supplements you took that are NOT part of One-a-Day-, Theragran-, or Centrum-type of multivitamin.

Please include vitamins taken as part of an antioxidant supplement.

138. How often did you take **Beta-carotene** (NOT as part of a multivitamin in Question 137)?

O NEVER (GO TO QUESTION 139)
O Less than 1 day per month
O 1-3 days per month
O 1-3 days per week
O 4-6 days per week
O Every day

138a. When you took **Beta-carotene**, about how much did you take in one day?

O Less than 10,000 IU
O 10,000 - 14,999 IU
O 15,000 - 19,999 IU
O 20,000 - 24,999 IU
O 25,000 IU or more
O Don't know

138b. For how many years have you taken **Beta-carotene**?

O Less than 1 year
O 1-4 years
O 5-9 years
O 10 or more years

Question 139 appears in the next column.

139. How often did you take **Vitamin A** (NOT as part of a multivitamin in Question 137)?

O NEVER (GO TO QUESTION 140)
O Less than 1 day per month
O 1-3 days per month
O 1-3 days per week
O 4-6 days per week
O Every day

139a. When you took **Vitamin A**, about how much did you take in one day?

O Less than 8,000 IU
O 8,000 - 9,999 IU
O 10,000 - 14,999 IU
O 15,000 - 24,999 IU
O 25,000 IU or more
O Don't know

139b. For how many years have you taken **Vitamin A**?

O Less than 1 year
O 1-4 years
O 5-9 years
O 10 or more years

140. How often did you take **Vitamin C** (NOT as part of a multivitamin in Question 137)?

O NEVER (GO TO QUESTION 141)
O Less than 1 day per month
O 1-3 days per month
O 1-3 days per week
O 4-6 days per week
O Every day

140a. When you took **Vitamin C**, about how much did you take in one day?

O Less than 500 mg
O 500 - 999 mg
O 1,000 - 1,499 mg
O 1,500 - 1,999 mg
O 2,000 mg or more
O Don't know

140b. For how many years have you taken **Vitamin C**?

O Less than 1 year
O 1-4 years
O 5-9 years
O 10 or more years

Question 141 appears on the next page.

Over the past **12 months**...

141. How often did you take **Vitamin E** (NOT as part of a multivitamin in Question 137)?

- O NEVER (GO TO QUESTION 142)
- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

141a. When you took **Vitamin E**, about how much did you take in one day?

- O Less than 400 IU
- O 400-799 IU
- O 800-999 IU
- O 1,000 IU or more
- O Don't know

141b. For how many years have you taken **Vitamin E**?

- O Less than 1 year
- O 1-4 years
- O 5-9 years
- O 10 or more years

142. How often did you take **Calcium or Calcium-containing antacids** (NOT as part of a multivitamin in Question 137)?

- O NEVER (GO TO QUESTION 143)
- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

142a. When you took **Calcium** or **Calcium-containing antacids**, about how much elemental calcium did you take in one day? *(If possible, please check the label for elemental calcium.)*

- O Less than 500 mg
- O 500 - 599 mg
- O 600 - 999 mg
- O 1,000 mg or more
- O Don't know

Question 143 appears in the next column.

142b. For how many years have you taken Calcium or Calcium-containing antacids?

- O Less than 1 year
- O 1-4 years
- O 5-9 years
- O 10 or more years

The next two questions ask you about other supplements you took more than once per week.

143. Please mark any of the following **single supplements** you took more than once per week (**NOT** as part of a multivitamin in Question 137):

- O B-6
- O B-complex
- O Brewer's yeast
- O Cod liver oil
- O Coenzyme Q
- O Fish Oil (Omega-3 fatty acids)
- O Folic acid/folate
- O Glucosamine
- O Hydroxytryptophan (HTP)
- O Iron
- O Niacin
- O Selenium
- O Zinc

144. Please mark any of the following **herbal** or **botanical supplements** you took more than once per week.

- O Aloe vera
- O Astragalus
- O Bilberry
- O Cascara sagrada
- O Cat's claw
- O Cayenne
- O Cranberry
- O Dong Kuai (Tangkwei)
- O Echinacea
- O Evening primrose oil
- O Feverfew
- O Garlic
- O Ginger
- O Ginkgo biloba
- O Ginseng (American or Asian)
- O Goldenseal
- O Grapeseed extract
- O Kava, kava
- O Milk thistle
- O Saw palmetto
- O Siberian ginseng
- O St. John's wort
- O Valerian
- O Other

Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- **Did not skip any pages,**
- **Completely blackened-in each answer, and**
- **Crossed out the incorrect answer and circled the correct answer if you made any changes.**

Web Site Advertising: www.epastudies.org

OMEGA CON: Elder Diet and Air Pollution Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as PM, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

Participation in this study will require six weeks of dietary restrictions and four weeks of fish oil or olive oil treatments, as well as one exposure to clean air and one to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. Study procedures include breathing tests, heart rate and blood pressure monitoring, blood tests, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. Total time commitment after screening is about 22 hours, over 6-7 weeks. Compensation up to $ 1543 is provided.

APPROVED - IRB, UNC-CH
MAY 06 2009

APPROVED - IRB, UNC-CH
MAY 06 2009

Dietary restrictions

Participants are asked to eliminate use of nutritional supplements containing omega-3 fatty acids and antioxidants (e.g. beta-carotene, selenium, vitamin C, vitamin E, zinc) for **two weeks** prior to beginning the intervention and during the intervention.

During the intervention, participants are asked to refrain from consuming the following foods:

1. All types of fish and shellfish
2. Walnuts
3. Flaxseed and flaxseed oil
4. Rapeseed oil
5. Canola oil
6. Soybeans and soy products
7. Eggland's Best eggs
8. Red wine
9. All foods and beverages supplemented with omega-3 fatty acids

APPROVED - IRB UNC-CH

MAY 06 2009

The US Environmental Protection Agency is seeking

ADULT VOLUNTEERS

Ages 50 to 75 for Research

This is a research study about genetics, diet supplementation and exposure to air pollution.

We are looking for healthy older adults to study diet supplementation and the effects of air pollution exposure on heart and lung function.

Total time commitment after screening is about 22 hours over 6 to 7 weeks. You will receive payment for screening, the study, and out of town travel. Parking is provided.

1-888-279-9353 or
919-966-0604
www.epastudies.org

In care of the environment • WESTAT • EPA Recruiting

The Human Studies Facility is located on the UNC-CH campus

First Review of IRB Submission Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 5/01/2009 **Expiration Date :** 2/01/2010 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Coordinator :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ? ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Wyomie Milliken

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter: 5/6/09
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 5/6/09)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☒ Hard copy sent
- Consent forms attached:
- Other attachments:

FINAL ACTIONS: 5/6/09

- ☒ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

RECEIVED 5-1-09 CTRC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1. Modification of study consent from (consent form #3).

a). The minute ventilation measurement will be moved to the training day from the exposure day.

b). The instruction and the risk associated with the blood pressure monitor have been added.

c). The payment for the blood pressure monitor has been added and the total payment will be increased to $1543.

2. Modification of the recruitment materials.

3. Modification of the dietary restriction.

Please see the highlighted changes.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: April 30, 2009**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

_____ _____

Signature of Principal Investigator or designee Date

_____ _____

Signature of Faculty Advisor (if applicable) Date

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
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Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 5/29/2009
Expiration Date of Approval: 2/01/2010

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Reviewed Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment dated 5/29/09 addresses:

1. addition of food record instructions & forms for training day
2. on days that air pollution particle concentrtions are too low on scheduled PM exposure day, they may be rescheduled

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, EPA; Robert Truckner, (EPA), Non-IRB Review Contact; Wanda Simmons, (CTRC), Non-IRB Review Contact

Food Record Instructions (OMEGACON)

APPROVED - IRB UNC-CH
MAY 29 2009

During the course of the study, we are asking you to complete food records for three days each week. Please record everything you consume (food, beverage, and/or supplements) on the following sheets for three days each week, with two of the three days being weekdays, and one of the three days being a weekend day.

For each eating occasion, please write down the following:
1) date
2) time of day
3) location (if not at home, please provide name of restaurant or eating establishment).
Then list all items consumed in as much detail as possible. Next to each item, write down the estimated portion size. Use the portion size booklet provided to guide you in estimating the serving size of each food and beverage item. For mixed dishes, please list all ingredients and describe the preparation method (e.g., baked or fried entrée?; cooked or raw vegetables?). Remember to list all beverages, including water. If you need additional space, you may write on the back of the page. Please write as clearly as possible. You may be contacted by phone by the study nutritionist for clarification if needed.

An **example** of a food record for a full day is provided below.
EXAMPLE:

January 5, 2009 6:30am at home
2 cups coffee, black
1/3 cup Kashi Go Lean Crunch cereal
½ cup Cheerios, regular
½ cup skim milk (on cereal)
4 ounces cranberry-grape juice (100% juice)

January 5, 2009 11:00am at work
6 ounce container fat-free Stonyfield Farm vanilla yogurt

January 5, 2009 12:30pm at work
1 pimento cheese sandwich, with 2 slices 100% whole wheat break, ¼ cup pimento cheese (store-bought pimento cheese)
1 medium-sized Gala red apple
8 ounces water

January 5, 2009 6:00pm at Mexican restaurant
2 cups tortilla chips
2 tbsp mild red salsa
1 chicken quesadilla (2 tortillas, 5 ounces chicken, 2 ounces cheese, 2 tbsp sour cream)
8 ounces water

January 5, 2009 8:00pm at home
½ cup ice cream, Ben and Jerry's mint chocolate cookie

Food Record **Week 2, Day 1** **Name:** **Subject #:**

Food Record **Week 2, Day 2** **Name:** **Subject #:**

Food Record **Week 2, Day 3** **Name:** **Subject #:**

Food Record **Week 4, Day 1** **Name:** **Subject #:**

Food Record **Week 4, Day 2** **Name:** **Subject #:**

Food Record **Week 4, Day 3** **Name:** **Subject #:**

First Review of IRB Submission
Modification

Training Not Met (Haiyan Tong, Michael Schmitt, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 5/29/2009 **Expiration Date :** 2/01/2010 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Admin :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full-IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Laura O Curry

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter: 5/29/09
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Termination

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HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

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IRB UNC-CH

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5/29/09
CTRC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1. Addition of food record instructions and forms which will be administered on the training day.

2. In consideration of some special circumstances (i. e., raining or snowing day) that the air pollution particle concentrations will be too low on the scheduled PM exposure day, we may reschedule the PM exposure to another day. We will make this minor modification on the IRB protocol accordingly and this modification will not affect the consent forms, therefore we do not submit revised IRB protocol and consent forms with this modification.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: May 29, 2009**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD (if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Signature of Principal Investigator or designee	Date: 5/29/09
Signature of Faculty Advisor (if applicable)	Date: 5-29-09

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
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CB #7097
Chapel Hill, NC 27599-7097
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Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 6/22/2009
Expiration Date of Approval: 2/01/2010

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Approved Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment dated 6/18/09 addresses:

1. addition of overnight blood pressure monitoring in addition to the daytime monitoring
2. total payment for the study is $1668
3. addition of James Scott

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, EPA; Robert Truckner, (EPA), Non-IRB Review Contact; Wanda Simmons, (CTRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 6/22/09 AND 2/01/10 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form 3

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: June 16, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 30 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and two exposure sessions which last approximately 8 hours each, and one session 18 hours after air pollution exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. Then we will check your resting breathing rate. You will tour the exposure chambers (one for air exposure and the other for air pollution exposure) and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

Exposure days

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and

Initial/Date _____

shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 48 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor. You will wear a pressure cuff and a monitor which is about the size of the Holter monitor. Please keep your arm relaxed and still when the pressure cuff is inflated. We will remove the monitor and pressure cuff at about 48 hr later.
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to clean air on the first day then to concentrated Chapel Hill air particles on the second day; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently.

If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return on the follow-up day, for your safety you should not shower or bathe until after the monitor is removed. However, you will be given an instruction to follow if you need to have a shower or bath.

Eighteen hours after PM exposure follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after PM exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor and removed when you leave.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the

Initial/Date _____

appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Blood pressure monitor: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any

Initial/Date _____

employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1668.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
	Pre-study qualification total = $60
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
	Training day total =$61

Initial/Date _____

Exposures (2 x 8 hours)

Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Blood pressure monitor (2@$100 each)	$200
Time (16h @$12/h)	$192
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50

Total for completion of 2 exposures = $1306

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25

Total for completion of post-exposure =$141
Protocol Completion Bonus $100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Initial/Date _____

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ____________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

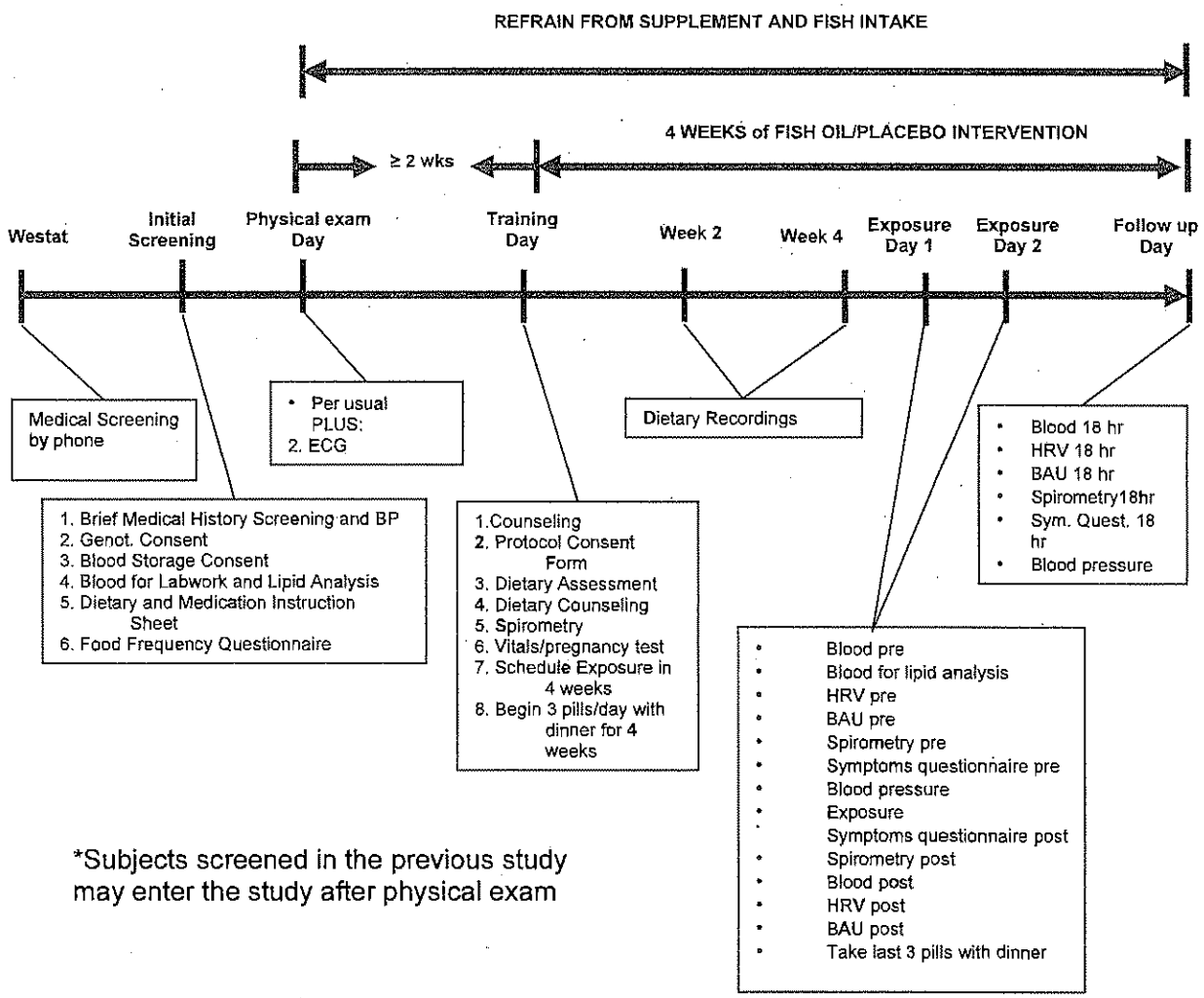
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

APPROVED - IRB, HMC-CH
JUN 22 2009

Web Site Advertising: www.epastudies.org

OMEGA CON: Elder Diet and Air Pollution Study

This is a study for healthy 50 to 75 year old adults. The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases. As a volunteer you will undergo a blood test to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as PM, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

Participation in this study will require six weeks of dietary restrictions and four weeks of fish oil or olive oil treatments, as well as one exposure to clean air and one to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. Study procedures include breathing tests, heart rate and blood pressure monitoring, blood tests, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. Total time commitment after screening is about 22 hours, over 6-7 weeks. Compensation up to $1668 is provided.

APPROVED - IRB, UNC-CH
JUN 22 2009

OMEGA CON STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

This study is for healthy adults ages 50 to 75. Payment for the study is incremental with a maximum total of $1668.

Procedures:

Volunteers initially undergo a blood test and genotype screening, followed by routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the genotyping visit and $30 for the additional medical screening. If you are eligible for the study your participation will last approximately 6 to 7 weeks. Participation will require 6 weeks of dietary restrictions and four weeks of fish oil or olive oil treatment.

The study will begin with a training visit that will last about 3 hours and two exposure sessions which last about 8 hours each, with one 3-hour follow up session 18 hours after Exposure 2. Following training you will begin the treatment (taken as capsules) for 4weeks prior to your exposure sessions. You will be asked to record your food intake twice for 3 days.

Procedures involved in this study include breathing tests, heart rate and blood pressure monitoring, including wearing overnight monitors after the exposure, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be able refrain from certain foods and medications for at least six weeks and to take the supplements for four weeks prior to the exposure. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-0518, previously 95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposures. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Genotyping Visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (1 hour)

Physical Exam: days and times TBD (2 hours)

Training (3 hours)

Dietary Supplementation (4 weeks)

Dietary Recordings, week 2 and week 4 (3 days/week)

Two Exposure Days (end of 4 weeks supplementation): 8:00 AM (8 hours each)

Follow-up Day: 18 hours after Exposure Day 2 (3 hours)

First Review of IRB Submission Modification

Training Not Met (Haiyan Tong, James Scott, Michael Schmitt, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 6/18/2009 Expiration Date : 2/01/2010 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Admin :

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Wyomie Milliken

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter: 6/19/09
  - ☒ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date: 6/19/09)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☒ Hard copy sent

Consent forms attached: 1

Other attachments: 2

FINAL ACTIONS:

- ☒ Approved 6/19/09
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Closure

RECEIVED
JUN 18 2009
IRB UNC-CH

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
version 29-Apr-2008b

RECEIVED 6/18/09
CTRC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
☐	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
☐	4. The sponsor's document describing the amendment, if any.	1
☐	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1. Addition of overnight blood pressure monitoring in addition to the daytime monitoring.
2. The total payment for the study will be $1668.
3. Adding James Scott in the co-investigators list.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes inerease risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: June 16, 2009**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Signature of Principal Investigator or designee

6/17/09
Date

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 9/17/2009
Expiration Date of Approval: 2/01/2010

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Approved Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment received 9/16/09 addresses the termination procedure of air pollution exposure to minimize the risk

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, Environmentally Protection Agency; Robert Truckner, (EPA), Non-IRB Review Contact; Wanda Simmons, (CTRC), Non-IRB Review Contact

First Review of IRB Submission
Modification

Training Not Met (Haiyan Tong, James Scott, Michael Schmitt, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 9/16/2009 **Expiration Date :** 2/01/2010 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Admin :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

☐ Not-HSR ☐ Exempt (Category:) ☑ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Laura Curry Munn

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter: 9/17/09
  - ☑ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Closure

RECEIVED
SEP 16 2009
IRB UNC-CH

RECEIVED 9/16/09
CTRC

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

version 29-Apr-2008b

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
□	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
x	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1. Modification of the termination procedure of air pollution exposure to minimize the risk to the study subjects. All changes are highlighted on pages 14 and 15 in the protocol.

2. Is this modification being submitted in response to an unanticipated problem or adverse event? ___yes __x_no

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 GCRC #: 2579 **Date: September 14, 2009**

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Signature of Principal Investigator or designee — Date 9/15/09

Signature of Faculty Advisor (if applicable) — Date 9/15/09

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

RECEIVED
SEP 16 2009
IRB UNC-CH

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

Part A.1. Contact Information, Agreements, and Signatures

Date: September 14, 2009

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _x_ postdoc __ resident __ other

Name of faculty advisor: James Samet, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD; Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):

__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
x	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
x	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
☐	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
☐	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*

	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for* ***Consent for Stored Samples***.	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.
Participants Thirty healthy 50-75 year-old male and female subjects will be involved in the study.
Procedures (methods): The subjects will be exposed to clean air for 2 hours on the first day then concentrated ambient fine and ultrafine PM for 2 hours on the second day after being randomly supplemented with four weeks of omega-3 fatty acid or olive oil.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac

autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (15-17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (20). Therefore, the role of GSTs in mediating the cardiovascular effects following acute PM exposure will also be studied in the protocol in GSTM1 null and GSTM1 positive healthy older adults.

The respiratory effects of PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.

6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
15. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal is approximately 30 subjects for the study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially

by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This will be a randomized, double-blinded study. Thirty study subjects will be randomly given fish oil (15 subjects) or olive oil (15 subjects) supplements for 4 weeks. The subjects will be given a container filled with 4 weeks supply of enteric-coated either 3g/day of fish oil or 3g/day of olive oil capsules. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 410 mg of eicosapentaenoic acid (EPA) and 274 mg of docosahexaenoic acid (DHA). Each 1000 mg of this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. Each subject will be exposed to clean air for 2 hour on the first day then air containing fine and ultrafine PM (PM<2.5) for 2 hours on the second day in an exposure chamber after the supplementation. If the PM concentrations are too low on the scheduled exposure day, they may be rescheduled. (See the study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotyping consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 25, methods of recruiting) will not need genotyping. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 80 ml of blood collected. A portion of the blood will be used for genotyping of GSTM1 and another portion of the blood will be used for the cholesterol levels, phospholipid and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back to the medical station.

- ***GSTM1 genotyping***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null.
- ***Food frequency questionnaire in the study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency

Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. The height of subject's mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. The resting minute ventilation will be measured. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the study:*** Subjects will be randomly given an approximately 4 weeks supply of either fish oil or olive oil supplements. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record every other week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 4 weeks of supplementation period. We will give the subjects another 4 weeks supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the exposure, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU), pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 48 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.
- **Blood pressure and heart rate measurement** will be measured intermittently by a BP monitor. A blood pressure cuff and a monitor which is about the size of the Holter monitor will be placed and will remain in place until the subjects leave for the day. The subjects will be instructed to keep arm relaxed and still when the pressure cuff is inflated.
- ***Brachial artery ultrasound:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation.

The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Pulmonary function*** will be measured by spirometry.
- ***Blood sampling***: approximately 80 ml of blood sample will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

Exposure:

The subjects will be exposed to clean air on the first day then concentrated ambient fine and ultrafine PM on the second day after the dietary supplementation. The PM exposure day may be rescheduled if the PM concentrations are too low on the scheduled day.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. During the exposure, subject will be monitored continuously by visual camera monitor, continuous pulse oximetry, blood pressure every 15 minutes, and telemetry. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours.

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.
The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will be up to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously. ~~by a DataRAM monitor (DR4000; Thermo Electron Corp) calibrated to filter obtained measurements of particle ug/m^3.~~ Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The particle mass of the air entering the chamber will be monitored continuously. If it is confirmed that particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, exposure will be terminated. The process for confirmation may take up to additional 10 minutes. ~~The computer is programmed to initiate a shutdown procedure if chamber~~

~~particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM.~~ The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 80 ml of blood samples will be collected.

Eighteen Hours Post-PM exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function (BAU) measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 80 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for two times of 24 hours duration using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Blood Pressure and Heart Rate will be measured intermittently on the exposure days and follow up day by a BP monitor. Systemic, diastolic, and mean blood pressure and heart rate data will be recorded.

Flow-Mediated Dilatation (brachial artery ultrasound) Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before and immediately after (within 1 hr after exposure ends) each exposure, and approximately 18 hrs after PM exposure, blood will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level,

biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In this study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single

inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Blood pressure and heart rate measurement**: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.
- **Brachial artery ultrasound:** There are no known risks associated with imaging of the brachial artery. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 3g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will be up to 600 ug/m^3 and the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject.

During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test the hypothesis that PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.

We will measure a number of endpoints (e.g., neutrophils, inflammation markers, and lung function). Several markers of the cardiovascular response to PM will also be measured (e.g., blood pressure, HRV, brachial arterial diameter, change in blood vasoactivators, and coagulation factors). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect 0.13 units of change in BAU diameter as observed in the pilot study and estimated that 15 subjects per group are needed to achieve type I error rate 0.05 at power of 80%. We used estimates of variance in BAU diameter (0.27) and correlation among the repeated measurements (0.97) from the OMEGA pilot study. We used the equation for comparing two groups (placebo and treatment) with a single explanatory variables (PM concentration).

Other Exploratory Observations:

We hypothesize that GSTM1 null subjects will have increased responses to PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of PM on changes in: heart rate variability in both time and frequency domains, blood CBC, fibrinogen, D-dimer, and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply:*

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data.** Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission**. Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials**. Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no

Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no

Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the study will take approximately 12 months. Participant recruitment is expected to be continuous throughout the study until the intended number of participants is reached.

The individual will have 6 visits to the research facility over approximately 6-7 weeks. The pre-enrollment and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after the blood work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment will require approximately 3 hours. Each exposure day will last approximately 8 hours. Eighteen hours after the PM exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study. If the subject is qualified and finished the entire study, the total compensation for completion of this study will be approximate $1668.

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total = $60	
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
Training day total = $61	
Exposures (2 x 8 hours)	
Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Time (16h @$12/h)	$192
Blood pressure monitor	$200
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50
Total for completion of 2 exposures =$1306	
Eighteen hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25
Total for completion of post-exposure = $141	
Protocol Completion Bonus	$100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

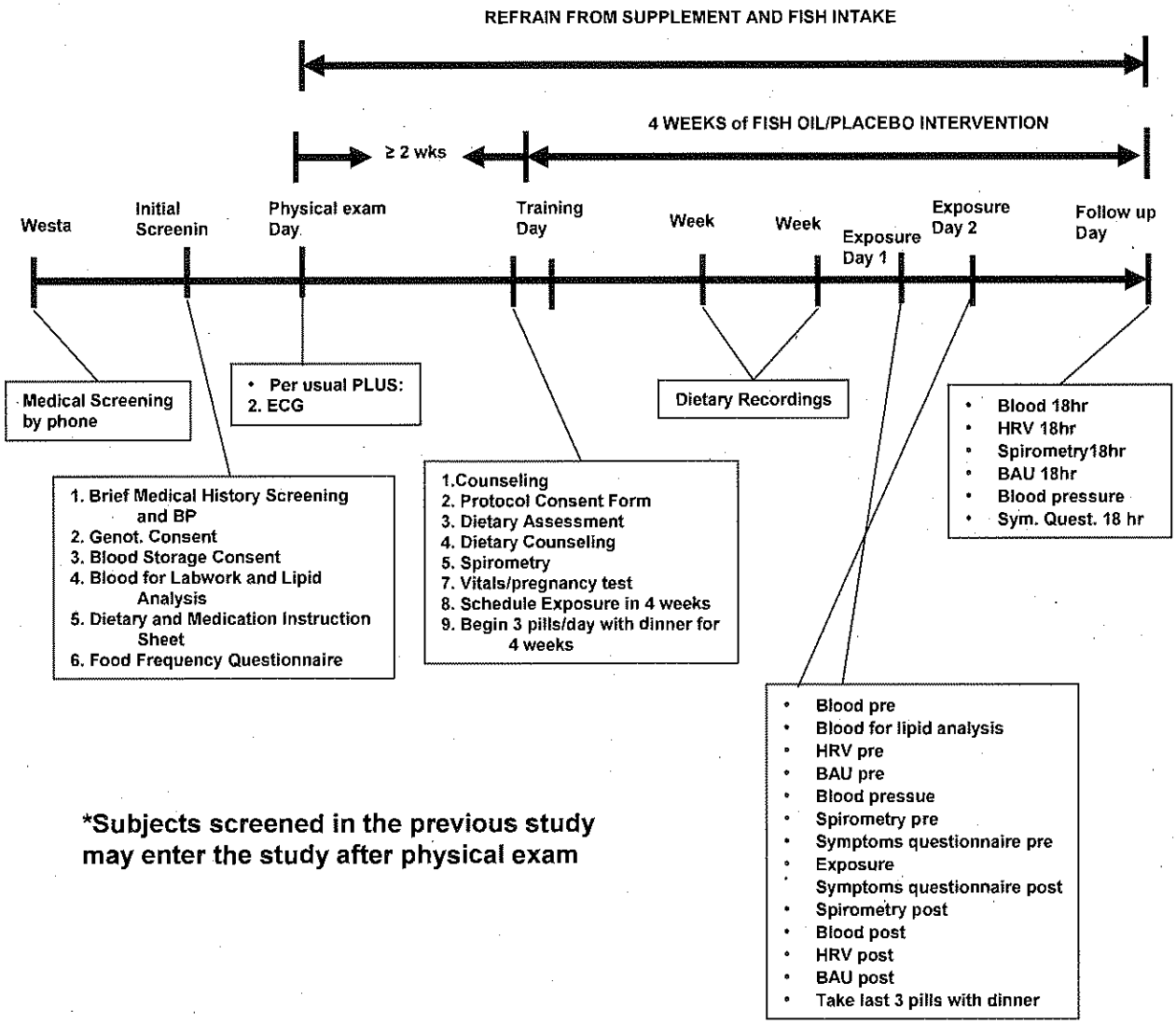
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



STUDY FLOW DIAGRAM

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Jerb Patterson /AE
Authorized signature on behalf of IRB

Approval Date: 1/27/2010
Expiration Date of Approval: 1/24/2011

RE: Notice of IRB Approval by Full Board Review
Submission Type: Renewal
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Investigator's Responsibilities:

Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Any unanticipated problem involving risks to subjects or others (including adverse events reportable under UNC-Chapel Hill policy) should be reported to the IRB using the web portal at https://irbis.unc.edu/irb.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

THE NORTH CAROLINA
TRANSLATIONAL AND CLINICAL SCIENCES INSTITUTE
DISCOVERY • CLINICAL PRACTICE • COMMUNITIES

RECEIVED
FEB 15 2010
IRB UNC-CH

TO: Dr. Haiyan Tong

FROM: Dr. Michael W. Fried, Director

DATE: January 28, 2010

SUBJECT: Approval of Renewal for **GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust IRB Study #: 07-0190**

Both the Clinical and Translational Research Center Oversight Committee and the Institutional Review Board (IRB) have reviewed this proposal and have approved it until **January 24, 2011.**

REMEMBER:
This approval is contingent on your citing the CTSA grant number (UL1RR025747) in any publications that might ensue from the work conducted on this NIH funded center.
For recommended language to use in your publications, please see http://tracs.unc.edu/index.php?option=com_content&view=article&id=130&Itemid=193

Inpatient admissions should be scheduled with Ms. Uli Blankenship (843-0560) and outpatient visits with Ms. Sherry McLaurin (6-1437). The approved consent form must be signed by each patient before any studies are done.

Should any significant side effects occur in any volunteer participating in this study, you are expected to inform the Director of the Clinical and Translational Research Center and the Chairman of the Human Rights Committee immediately.

Michael W. Fried, MD, Director

Clinical and Translational Research Center
Campus Box #7600
Phone: 919-966-1435
Fax: 919-966-1576
Web site: tracs.unc.edu

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 1/27/10 AND 1/24/11
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotyping With Identifying Information 1

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: January 5, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about this research study?
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to air pollution. The purpose of the genotyping is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 30 participants in this study.

How long will your participation last?
Participation in this genetic session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

If you have been genotyped before, there will be no genotyping this time. You will be given the information below.

At the end, we will give you a copy of the Medical History Form and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and bring back the FFQ on the physical exam day. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Are there any reasons you should not participate?

Initial/Date _____

- You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol). You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?
There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?
The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?
You will be paid $30 for completing this procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate.

Who owns the blood samples?

Initial/Date _____

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board,

Initial/Date _____

University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 1/27/10 AND 1/24/11
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Storing Biological Specimens <u>With</u> Identifying Information **2**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: January 5, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; BA; Dave Peden, MD, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

<u>What are some general things you should know about this research study?</u>
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Air pollution exposure has been found to cause a range of lung and systemic changes in normal subjects. We will be collecting blood samples which will help us to further study this condition. In order to do so, we will need to collect and store blood. Blood samples will be collected in order to look for but not limited to indicators of inflammation due to the Air pollution exposure. We may further isolate RNA from blood for genetic analysis. RNA may be isolated for genetic analysis to see if there is a difference between pre- and post Air pollution exposures.

All samples will be stored where only project members will have access to the samples. There is a need to store samples in such a repository because this will be an ongoing study where samples from subjects will be collected over an extended period of time. Storing of samples allows for all samples to be processed at the same time and also allows our scientist the opportunity to further study these samples with as yet unknown questions and techniques.

How will the specimen be collected?
The description of the samples to be collected and the manner in which this will be done have been described in the main study consent.

What will happen to the specimen?
Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the Air pollution exposure. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens (or data) for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained form subjects, these specimens (or data) will be provided without identifiers to these other researchers by employing a data use agreement.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding the cardiovascular effects of Air pollution exposure in older population.

What are the possible risks or discomforts involved with being in this study?
This study might involve the following risks and/or discomforts to you:

1. Blood sampling will be performed by medical personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be

Initial/Date _____

entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you for storage of the specimens?
There will be no cost to you for the storage and use of the specimens for research purposes.

Will you receive anything for being in this study?
You will not receive anything for taking part in this study with regard to storage of excess specimens. Reimbursement for participation in the main study is addressed in the detailed consent for that study.

Who owns the specimens?
Any blood, body fluids, or tissue specimens obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Initial/Date _____

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the specimens from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board, University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Initial/Date _____

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 1/27/10 AND 1/24/11
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form

3

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: January 5, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 30 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and two exposure sessions which last approximately 8 hours each, and one session 18 hours after air pollution exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. Then we will check your resting breathing rate. You will tour the exposure chambers (one for air exposure and the other for air pollution exposure) and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

Exposure days

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and

Initial/Date _____

shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 48 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor. You will wear a pressure cuff and a monitor which is about the size of the Holter monitor. Please keep your arm relaxed and still when the pressure cuff is inflated. We will remove the monitor and pressure cuff at about 48 hr later.
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to clean air on the first day then to concentrated Chapel Hill air particles on the second day; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently.

If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return on the follow-up day, for your safety you should not shower or bathe until after the monitor is removed. However, you will be given an instruction to follow if you need to have a shower or bath.

Eighteen hours after PM exposure follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after PM exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor and removed when you leave.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the

Initial/Date _____

appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Blood pressure monitor: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any

Initial/Date _____

employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1668.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
	Pre-study qualification total = $60
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
	Training day total =$61

Initial/Date _____

Exposures (2 x 8 hours)

Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Blood pressure monitor (2@$100 each)	$200
Time (16h @$12/h)	$192
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50

Total for completion of 2 exposures = $1306

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25

Total for completion of post-exposure =$141
Protocol Completion Bonus $100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Initial/Date _____

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

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Initial/Date ____________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Initial/Date _____

Full Committee Review
Renewal

Receipt Date : 1/06/2010 Expiration Date : 2/01/2010 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Admin :

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Agenda Date 1/25/2010 Reviewer 1: **Roche, Myra** Reviewer 2: **Patterson, J. Herbert** (chair)

Additional Findings and/or Special Populations: ☐ Children ☐ Prisoners ☐ Pregnant Women

☐ Sensitive Info ☑ IND ☐ IDE ☐ DNA/Gene transfer ☑ Genetics ☑ GCRC ☐ ORC ☐ LAR

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Meeting Notes and Required Findings : 30 → 20 → ? → 10

- 2 withdrawals → OK

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter: AS
  - ☑ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date: HP | AR)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

JR

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☑ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Deferred
- ☐ Disapproved
- ☐ Return to sender
- ☐ Closure

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
REQUEST FOR RENEWAL OF IRB APPROVAL OR STUDY CLOSURE
Version August 26, 2009

RECEIVED
JAN 06 2010
IRB UNC-CH

If the research is continuing:

- Check the relevant items.
- Include two collated sets of copies (sorted in the order listed) of checked items.

→ **Submissions will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This form (renewal or closure).	2
x	2. Any items specifically requested in questions # 4 through 9 (in that order).	2
x	3. The most recent application submitted for IRB approval. This application should be updated to include any modifications since the study was initially approved or last renewed. **If there are any new modifications included with this renewal, highlight the proposed modifications by underlining.**	2
x	4. Clean copies of all consent document(s) to be used in the upcoming approval period, for stamping.	2
x	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

IRB study #: 07-0190 **Date:** 1-5-2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor** (if applicable): James Samet, PhD

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

1. In a few sentences, describe the past year's work, and describe what you plan for the upcoming year, including data analysis, if relevant.

Twenty subjects have been enrolled and finished the study since the last renewal of IRB protocol. We are proposing to enroll 10 more subjects in the coming year to finish the data collection phase.

2. Number of subjects involved through direct contact or use of their data (for multi-site studies, include only subjects covered by this IRB) (Note: **b+d** should not be larger than **a**)
   a. Total projected number as approved by IRB: 30
   b. Total number of subjects involved to date (for clinical trials include "screen failures"): 20
   c. Number of subjects added since last renewal:
   d. Number to be included in upcoming year: 10

Answer the following questions based on information since initial approval or last renewal. Only include subjects covered by this IRB.

3. Have there been any modifications approved since the last review? If your IRB application has not already been updated to reflect these changes, do so now and attach any revised documents, including application and/or consent documents.	_x_ yes __no
4. Have any subjects withdrawn voluntarily or been withdrawn from the study? **If yes, explain**; give number and reasons for withdrawals. Two study subjects have been withdrawn from the study after they enrolled. One subject (subject ID number OMC-012) experienced bradycardia during a 2-hr filtered clean air exposure on September 21, 2009 and the other subject (subject ID number OMC-017) had high blood pressure during a 2-hr filtered clean air exposure on November 16, 2009. We do not expect any adverse health effect from filtered clean air exposure so these subjects' symptoms were not related to the research. The subjects were discharged from EPA Medical Station with normal vital signs and reportedly feeling fine. It was recommended that the subjects follow up with their primary care physicians for better management.	_x_ yes __no
5. Have there been any complaints about the research from subjects or others? **If yes, explain**	__ yes _x_no
6. Have there been any findings (e.g., publications, new information) that alter the risk/benefit ratio or otherwise impact the study? **If yes, explain, including whether these new findings are relevant to participants' willingness to continue.**	__ yes _x_no
7. Have there been any relevant multi-center reports? **If yes, provide a copy of the report.**	__ yes _x_no
8. Does this study have a Data and Safety Monitoring Committee (DSMC or DSMB)? **If yes, provide a report** from the DSMC.	__ yes _x_no
9. Have there been unanticipated problems or serious adverse events since the last renewal? **If yes, include all copies** of local Adverse Event reports with this submission.	__ yes _x_no
10. Has this study been audited by external sponsor or monitor since approved or last renewed? **If yes, include a copy** of the audit report.	__ yes _x_no
11. Are you requesting any changes to the study or consent documents? **If yes, include the form** requesting *Modification of Approved Human Subjects Research* and underline the proposed change in the updated application and/or consent documents.	__ yes _x_no
12. Will you be enrolling, consenting or re-consenting subjects in the upcoming approval period? **If yes, include clean copies** of consents/assents/fact sheets to be used to receive a new stamp. **Include any new recruitment materials** to be used with subjects.	_x_ yes __ no

Action requested by Principal Investigator (choose only one):

Renew approval:

__ Study has always involved *only* analysis of existing data or specimens. Continue as approved.

Study involves(ed) direct interaction/intervention or contact with subjects:

_x Continue as approved: Enrollment of new subjects continues.

__ Enrollment of new subjects closed; interaction/intervention with previously enrolled subjects continues.

__ Direct interaction with subjects completed but subsequent monitoring or follow up continues.

__ Subjects' involvement completed but renewal is requested for data analysis.

Closure of Study:

__ Research completed: Identifiable data or human biological specimens are stored according to plan already approved by the IRB.

__ Research completed: All data or human biological specimens are deidentified.

__ Lack of funding or other *(specify)*:

Signature of Principal Investigator	Date 1/6/2010
Signature of Faculty Advisor (if applicable)	Date 1/6/2010

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

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APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

Part A.1. Contact Information, Agreements, and Signatures

Date: January 5, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _x_ postdoc __ resident __ other

Name of faculty advisor: James Samet, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD; Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):

__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
x	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
x	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
□	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
□	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator — Date: 1/6/2010

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor — Date: 1/6/2010

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee — Date

Print Name of Department Chair or designee — Department

Part A.2. Summary Checklist *Are the following involved?*

	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? *If yes, see instructions for **Consent for Stored Samples**.*	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? *If yes, approval by the **UNC-CH Radiation Safety Committee** is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? *If yes, approval by the **UNC-CH Institutional Biosafety Committee** is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? *If yes, submit this application directly to the **Oncology Protocol Review Committee**.*	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? *If yes, obtain the **GCRC Addendum** from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of iuterest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.
Participants Thirty healthy 50-75 year-old male and female subjects will be involved in the study.
Procedures (methods): The subjects will be exposed to clean air for 2 hours on the first day then concentrated ambient fine and ultrafine PM for 2 hours on the second day after being randomly supplemented with four weeks of omega-3 fatty acid or olive oil.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac

autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (15-17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (20). Therefore, the role of GSTs in mediating the cardiovascular effects following acute PM exposure will also be studied in the protocol in GSTM1 null and GSTM1 positive healthy older adults.

The respiratory effects of PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.

6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
15. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal is approximately 30 subjects for the study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially

by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This will be a randomized, double-blinded study. Thirty study subjects will be randomly given fish oil (15 subjects) or olive oil (15 subjects) supplements for 4 weeks. The subjects will be given a container filled with 4 weeks supply of enteric-coated either 3g/day of fish oil or 3g/day of olive oil capsules. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 410 mg of eicosapentaenoic acid (EPA) and 274 mg of docosahexaenoic acid (DHA). Each 1000 mg of this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. Each subject will be exposed to clean air for 2 hour on the first day then air containing fine and ultrafine PM (PM<2.5) for 2 hours on the second day in an exposure chamber after the supplementation. If the PM concentrations are too low on the scheduled exposure day, they may be rescheduled. (See the study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotyping consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 25, methods of recruiting) will not need genotyping. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 80 ml of blood collected. A portion of the blood will be used for genotyping of GSTM1 and another portion of the blood will be used for the cholesterol levels, phospholipid and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back to the medical station.

- ***GSTM1 genotyping***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null.
- ***Food frequency questionnaire in the study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency

Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. The height of subject's mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. The resting minute ventilation will be measured. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the study:*** Subjects will be randomly given an approximately 4 weeks supply of either fish oil or olive oil supplements. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record every other week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 4 weeks of supplementation period. We will give the subjects another 4 weeks supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the exposure, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU), pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 48 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.
- **Blood pressure and heart rate measurement** will be measured intermittently by a BP monitor. A blood pressure cuff and a monitor which is about the size of the Holter monitor will be placed and will remain in place until the subjects leave for the day. The subjects will be instructed to keep arm relaxed and still when the pressure cuff is inflated.
- ***Brachial artery ultrasound:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation.

The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Pulmonary function*** will be measured by spirometry.
- ***Blood sampling***: approximately 80 ml of blood sample will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

Exposure:

The subjects will be exposed to clean air on the first day then concentrated ambient fine and ultrafine PM on the second day after the dietary supplementation. The PM exposure day may be rescheduled if the PM concentrations are too low on the scheduled day.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. During the exposure, subject will be monitored continuously by visual camera monitor, continuous pulse oximetry, blood pressure every 15 minutes, and telemetry. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours.

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.
The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will be up to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The particle mass of the air entering the chamber will be monitored continuously. If it is confirmed that particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, exposure will be terminated. The process for confirmation may take up to additional 10 minutes. The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to

removal of the subject from the chamber. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 80 ml of blood samples will be collected.

Eighteen Hours Post-PM exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function (BAU) measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 80 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for two times of 24 hours duration using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Blood Pressure and Heart Rate will be measured intermittently on the exposure days and follow up day by a BP monitor. Systemic, diastolic, and mean blood pressure and heart rate data will be recorded.

Flow-Mediated Dilatation (brachial artery ultrasound) Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before and immediately after (within 1 hr after exposure ends) each exposure, and approximately 18 hrs after PM exposure, blood will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In this study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Blood pressure and heart rate measurement**: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.
- **Brachial artery ultrasound:** There are no known risks associated with imaging of the brachial artery. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 3g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will be up to 600 ug/m^3 and the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are

occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test the hypothesis that PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.

We will measure a number of endpoints (e.g., neutrophils, inflammation markers, and lung function). Several markers of the cardiovascular response to PM will also be measured (e.g., blood pressure, HRV, brachial arterial diameter, change in blood vasoactivators, and coagulation factors). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect 0.13 units of change in BAU diameter as observed in the pilot study and estimated that 15 subjects per group are needed to achieve type I error rate 0.05 at power of 80%. We used estimates of variance in BAU diameter (0.27) and correlation among the repeated measurements (0.97) from the OMEGA pilot study. We used the equation for comparing two groups (placebo and treatment) with a single explanatory variables (PM concentration).

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4%

decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of PM on changes in: heart rate variability in both time and frequency domains, blood CBC, fibrinogen, D-dimer, and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data**. Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no
Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the study will take approximately 12 months. Participant recruitment is expected to be continuous throughout the study until the intended number of participants is reached.

The individual will have 6 visits to the research facility over approximately 6-7 weeks. The pre-enrollment and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after the blood work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment will require approximately 3 hours. Each exposure day will last approximately 8 hours. Eighteen hours after the PM exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study. If the subject is qualified and finished the entire study, the total compensation for completion of this study will be approximate $1668.

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total = $60	
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
Training day total = $61	
Exposures (2 x 8 hours)	
Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Time (16h @$12/h)	$192
Blood pressure monitor	$200
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50
Total for completion of 2 exposures =$1306	
Eighteen hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25
Total for completion of post-exposure = $141	
Protocol Completion Bonus	$100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

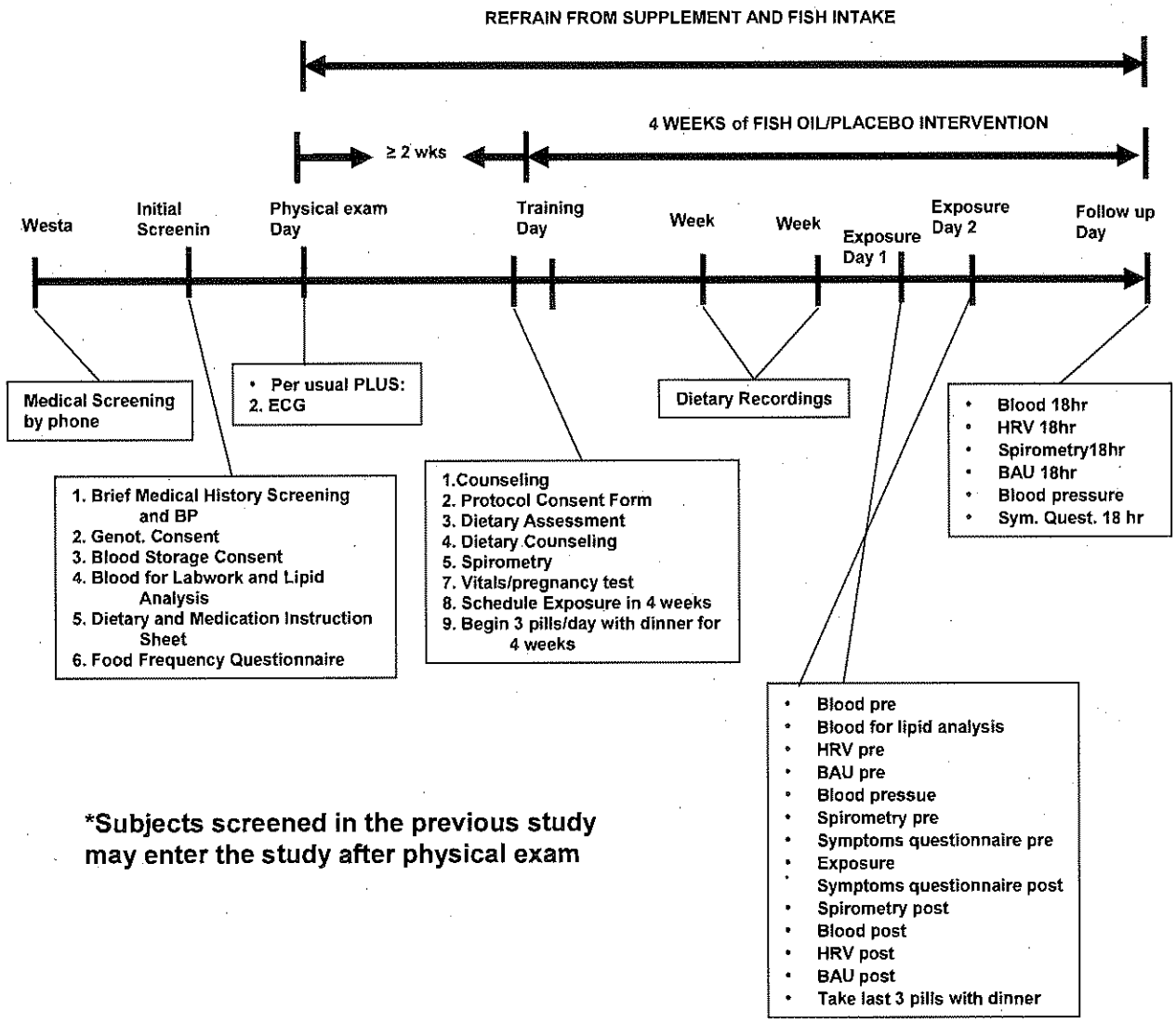
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



STUDY FLOW DIAGRAM

Full Committee Review
Renewal

Receipt Date : 1/06/2010 Expiration Date : 2/01/2010 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Admin :

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Agenda Date 1/25/2010 Reviewer 1: **Roche, Myra** Reviewer 2: **Patterson, J. Herbert** (chair)

Additional Findings and/or Special Populations: ☐ Children ☐ Prisoners ☐ Pregnant Women

☐ Sensitive Info ☑ IND ☐ IDE ☐ DNA/Gene transfer ☑ Genetics ☑ GCRC ☐ ORC ☐ LAR

Findings

Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Meeting Notes and Required Findings :

PROCESSING STEPS (OFFICE USE ONLY):

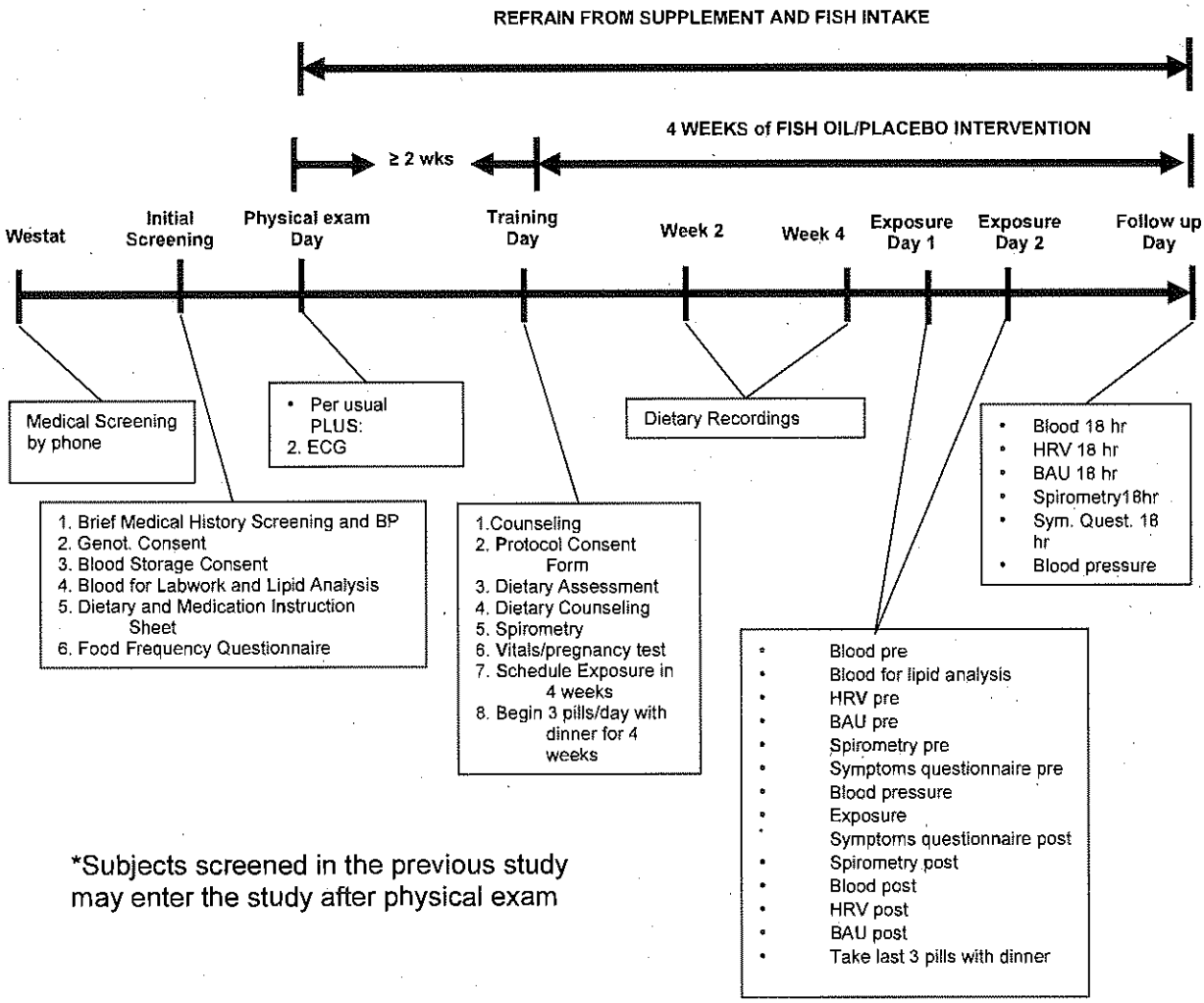
- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date: _____)
  - ☐ Approved by chair, see edits (Initials/Date: _____)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date: _____)
  - ☐ Approved by chair, see edits (Initials/Date: _____)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached: _____

Other attachments: _____

FINAL ACTIONS:

- ☐ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Deferred
- ☐ Disapproved
- ☐ Return to sender
- ☐ Closure



STUDY FLOW DIAGRAM

Initial/Date _____

CTRC RENEWAL/CLOSURE

STUDY: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

IRB #: 07-0190

P.I.: Haiyan Tong, MD, PhD

DATE REVIEWED: January 6, 2010

RECEIVED
JAN 06 2010
IRB UNC-CH

RECOMMENDATION OF REVIEWER:

☒ **Approval:** Enrollment of new subjects is continues.

☐ Contingent approval—Defer to IRB (explain):

☐ Returned for re-submission (explain)):

☐ Disapproval (explain):

Duration of Approval OR STUDY CLOSURE:

☐ 12 months (12 months or less)

☐ **Study closed**

Diane Tonke _____ January 6, 2010

Regulatory Core Signature (Date)

_____ _____

Michael Fried, MD (Date)

CLINICAL AND TRANSLATIONAL RESEARCH CENTER
UNC HOSPITAL ROOM 3005
CAMPUS BOX #7600 • CHAPEL HILL, NC • 27599-7600
PHONE: 919.966.1435 • FAX: 919.966.1576

THE NORTH CAROLINA
TRANSLATIONAL AND CLINICAL SCIENCES INSTITUTE
DISCOVERY • CLINICAL PRACTICE • COMMUNITIES

RECEIVED
JAN 06 2010
IRB UNC-CH

TO: Office of Human Research Ethics
Institutional Review Board

FROM: Dr. Michael Fried
Clinical and Translational Research Center (CTRC)

DATE: January 6, 2010

STUDY: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

IRB #: 07-0190

P.I.: Haiyan Tong, MD, PhD

The CTRC has now received the enclosed project from the Principal Investigator requesting study closure). Copies of the project are enclosed for your committee.

please call Haiyan @ 966-4413 when ready to pick up. Thanks, Haiyan Tong

IRB Coordinator: Please note Title not same as in IRBIS — I did not ✓ all mods. Diane

Clinical and Translational Research Center
Campus Box #7600
Phone: 919-966-1435
Fax: 919-966-1576
Web site: tracs.unc.edu

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 4/27/2010
Expiration Date of Approval: 1/24/2011

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Approved Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment, dated 4/20/2010, seeks to increase the number of subjects from 30 to 32. In addition, recruitment materials are revised.

The consent document now reflects the above changes as necessary.

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 4/27/10 AND 1/24/11 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina at Chapel Hill

Consent for Genotyping <u>With</u> Identifying Information 1

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: April 21, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

<u>What are some general things you should know about this research study?</u>
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to air pollution. The purpose of the genotyping is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene. If you do not qualify for the study, your participation will end. If you do qualify you will complete the entire study.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 32 participants in this study.

How long will your participation last?
Participation in this genetic session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

If you have been genotyped before, there will be no genotyping this time. You will be given the information below.

At the end, we will give you a copy of the Medical History Form and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and bring back the FFQ on the physical exam day. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Initial/Date _____

Are there any reasons you should not participate?

- You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol). You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate.

Who owns the blood samples?

Initial/Date _____

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board,

Initial/Date _____

University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 4/27/10 AND 1/28/11
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form **3**

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: April 21, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 32 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and two exposure sessions which last approximately 8 hours each, and one session 18 hours after air pollution exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. Then we will check your resting breathing rate. You will tour the exposure chambers (one for air exposure and the other for air pollution exposure) and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

Exposure days

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and

Initial/Date _____

shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 48 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor. You will wear a pressure cuff and a monitor which is about the size of the Holter monitor. Please keep your arm relaxed and still when the pressure cuff is inflated. We will remove the monitor and pressure cuff at about 48 hr later.
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to clean air on the first day then to concentrated Chapel Hill air particles on the second day; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently.

If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return on the follow-up day, for your safety you should not shower or bathe until after the monitor is removed. However, you will be given an instruction to follow if you need to have a shower or bath.

Eighteen hours after PM exposure follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after PM exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor and removed when you leave.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the

Initial/Date _____

appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Blood pressure monitor: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any

Initial/Date _____

employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1668.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
	Pre-study qualification total = $60
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
	Training day total =$61

Initial/Date _____

Exposures (2 x 8 hours)

Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Blood pressure monitor (2@$100 each)	$200
Time (16h @$12/h)	$192
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50

Total for completion of 2 exposures = $1306

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25

Total for completion of post-exposure =$141
Protocol Completion Bonus $100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Initial/Date _____

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

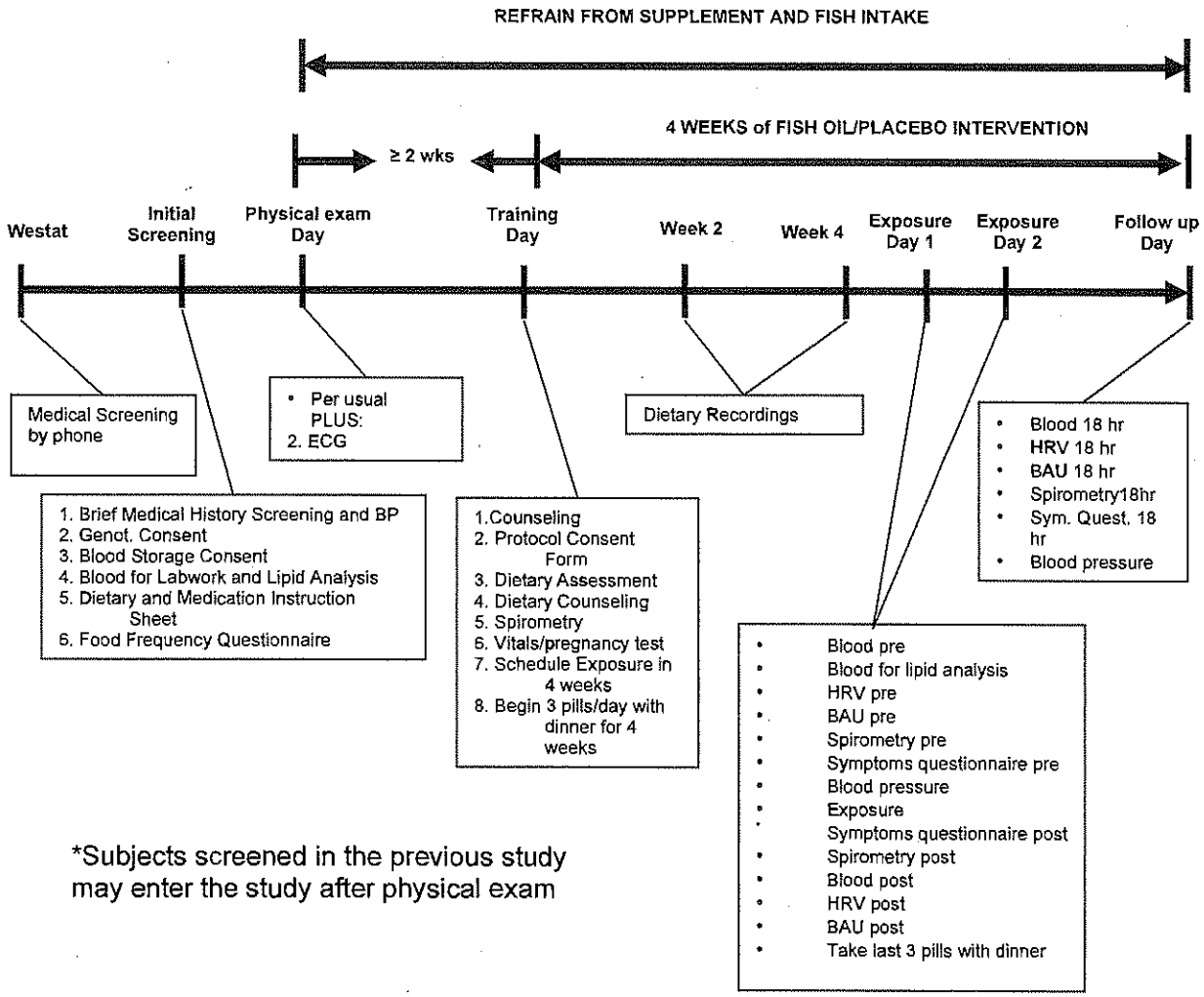
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

APPROVED - IRB, UNC-CH
APR 27 2010

OMEGA CON STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

The current phase of this study is for healthy adults ages 50 to 75 who have the GSTM1 Null genotype. Volunteers initially undergo a blood test and genotype screening, for which you will be paid $30. If after the initial genotyping visit you do not qualify for the study, your participation will end. If you do qualify for and complete the entire study, payment is incremental with a maximum total of $1668.

Procedures:

If you qualify by genotype screening, you will have routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 for the additional medical screening. If you are eligible for the study your participation will last approximately 6 to 7 weeks. Participation will require 6 weeks of dietary restrictions and four weeks of fish oil or olive oil treatment.

The study will begin with a training visit that will last about 3 hours and two exposure sessions which last about 8 hours each, with one 3-hour follow up session 18 hours after Exposure 2. Following training you will begin the treatment (taken as capsules) for 4weeks prior to your exposure sessions. You will be asked to record your food intake twice for 3 days.

Procedures involved in this study include breathing tests, heart rate and blood pressure monitoring, including wearing overnight monitors after the exposure, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be able refrain from certain foods and medications for at least six weeks and to take the supplements for four weeks prior to the exposure. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-0518, previously 95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposures. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

Genotyping Visit, days/times TBD (less than 1 hour)

Medical History Screening, to be completed at home and mailed (1 hour)

Physical Exam: days and times TBD (2 hours)

Training (3 hours)

Dietary Supplementation (4 weeks)

Dietary Recordings, week 2 and week 4 (3 days/week)

Two Exposure Days (end of 4 weeks supplementation): 8:00 AM (8 hours each)

Follow-up Day: 18 hours after Exposure Day 2 (3 hours)

APPROVED - IRB, UNC-CH
APR 27 2010

OMEGACON

What is the purpose of this research study?

This study will find out if a component of ambient air pollution to which we are all exposed, particulate matter (PM) produced by care and coal-fired power plants, increases the risks of changes in the heart and whether fish oil supplements will lessen the risks caused by PM. Researchers are studying the gene GSTM1, glutathione-S-transferase, that is one of several genes responsible for protecting your body against oxidants such as PM. Some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants. Results from this study will increase the understanding of how gaseous and particulate air pollutants (which cases the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with heart diseases.

Can I take part in the study?

You may be able to take part in the study if you are between the ages of 50 and 75.

What will I be asked to do?

- Have blood drawn to determine the type of GSTM1 gene you carry. Currently only those who are GSTM1 Null will be eligible.
- Eat a special diet for six weeks.
- Take fish oil or olive oil treatments for four weeks.
- Breathe clean filtered air and air with carefully controlled amounts of pollution particles.
- Take part in breathing tests, including one called spirometry (link provided to explanation).
- Have your heart rate monitored (link provided to explanation).
- Have a brachial artery ultrasound (BAU, link provided to explanation).
- Take a dose of nitroglycerine, a short-acting vasodilator.

How long will it take?

Following initial screening, the study will take between 15 and 22 hours over 6 or 7 weeks.

What will I get for volunteering?

If you complete all visits and procedures, we will pay you $1668.

How can I sign up or get more information?

Call us or send and email. Our office hours are Monday-Friday from 8 am to 5 pm EST. After hours, please leave a message on voice mail, and we will return your call promptly.

- (919 966-0604 (local)
- 1-888-279-9353 (toll free)
- recruitment@epa.gov

APPROVED - IRB, UNC-CH
APR 27 2010

OMEGA CON STUDY (IRB #07-0190)
E-mail Announcement

SUBJECT: INFORMATIONAL: Research study for Healthy Older Adults

The main purpose of this research study is to determine if a component of air pollution, particulate matter (PM) elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Volunteers ages 50 to 75 will be tested to determine the type of a particular gene they carry, one of several genes responsible for protecting the body against oxidants. For this phase of the study only individuals with the GSTM1 Null genotype will be eligible. Participation will include four-weeks of fish oil or olive oil treatments and two exposures, to a controlled amount of PM. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Office of Human Research Protection (OHRE) Biomedical Institutional Review Board. IRB # 07-0190 (GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

First Review of IRB Submission Modification

Training Not Met (Haiyan Tong, James Scott, Michael Schmitt, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 4/26/2010 Expiration Date : 1/24/2011 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Admin :

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

☐ Not-HSR ☐ Exempt (Category:) ☑ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: WTP 4/26/10 Reviewer 2:

Entered by: Wyomie Milliken

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter: WTP 4/27/10
  - ☐ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date: 4/27/10)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent
  - ☑ Hard copy sent

Consent forms attached: 3

Other attachments: 3

FINAL ACTIONS:

- ☑ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Closure

RECEIVED
APR 2 6 2010
IRB UNC-CH

RECEIVED 4/26/10 CTRC

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
MODIFICATION OF APPROVED
HUMAN SUBJECTS RESEARCH
Version May 22, 2009

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. If adding personnel, include name, location (UNC or specific outside location), role, and email address for each person who should receive electronic copies of IRB correspondence to PI. For those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/) include documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1). Adding two more subjects to the study. We planed to enroll 30 subjects in the study and 22 have finished the study. However two subjects were exposed to lower concentrations of air pollution particles which were far below the target level. Based on our previous observation that lower air pollution concentrations do not produce significant cardiovascular effects thus we are increasing the number of subjects to 32. We will screen more potential volunteers for qualification.

2). Study consent forms and recruitment materials were modified accordingly.

2. Is this modification being submitted in response to an unanticipated problem/adverse event or new findings? ___yes _x__no
If yes, explain, including whether these events or findings are relevant to participants' willingness to continue.

3. Do any of the proposed changes increase risk? ___yes _x__no If yes, explain.

IRB study #: 07-0190 CTRC #: 2579 **Date:** April 20, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Faculty advisor: James Samet, PhD

(if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Signature of Principal Investigator or designee — Date: 4/21/10

Signature of Faculty Advisor (if applicable) — Date: 4/22/10

OMEGA CON STUDY
IRB # 07-0190 GCRC #2579

Purpose:

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular and respiratory systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, (GSTM1, glutathione-S-transferase) is one of several genes responsible for protecting your body against oxidants such as air pollutants, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive, may be more susceptible to the effects of air pollutants.

The current phase of this study is for healthy adults ages 50 to 75 who have the GSTM1 Null genotype. Volunteers initially undergo a blood test and genotype screening, for which you will be paid $30. If after the initial genotyping visit you do not qualify for the study, your participation will end. If you do qualify for and complete the entire study, payment ~~for the study~~ is incremental with a maximum total of $1668.

Procedures:

~~Volunteers initially undergo a blood test and~~ If you qualify by genotype screening, ~~followed by~~ you will have routine medical screening at the EPA Human Studies Facility, which includes completing medical history forms and a physical exam. You will be paid $30 ~~for the genotyping visit and $30~~ for the additional medical screening. If you are eligible for the study your participation will last approximately 6 to 7 weeks. Participation will require 6 weeks of dietary restrictions and four weeks of fish oil or olive oil treatment.

The study will begin with a training visit that will last about 3 hours and two exposure sessions which last about 8 hours each, with one 3-hour follow up session 18 hours after Exposure 2. Following training you will begin the treatment (taken as capsules) for 4weeks prior to your exposure sessions. You will be asked to record your food intake twice for 3 days.

Procedures involved in this study include breathing tests, heart rate and blood pressure monitoring, including wearing overnight monitors after the exposure, blood draws, and ultrasound imaging of an artery in your arm. During the ultrasound procedure you will also be given a dose of nitroglycerine, which is a short-acting vasodilator. You will be exposed to a controlled amount of air pollution particles, no more than you might encounter if you visited a large city such as Los Angeles, New York, or Mexico City on a smoggy day. You will be asked to wear a heart rate monitor over night following exposure sessions.

The timing of this study is important: you must be able refrain from certain foods and medications for at least six weeks and to take the supplements for four weeks prior to the exposure. Does this sound like something that you would be interested in doing?

If yes, proceed to eligibility screening (IRB #95-0518, previously 95-EPA-66). Study specific inclusion/exclusion criteria appear in the IRB application (#GCRC 2579).

Study Restrictions:

In addition to the timing of the study, you must be willing to observe several important restrictions. They are:

- No use of over the counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposures. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No intake of omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the study.
- No intake of antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Must be willing to use olive oil for cooking, dressings, and sauces during the study.
- Must avoid drinking red wine during the study.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the exposure day, you should eat a light breakfast.
- Must not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- No caffeine for 12 hours prior to all study visits.
- Due to the overnight heart monitor must be willing to refrain from bathing between exposure and follow-up.

Would you be able to comply with these restrictions? If so, we can schedule you for an office visit to complete the initial part of the screening.

Study Schedule

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Dietary Recordings, week 2 and week 4 (3 days/week)

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Follow-up Day: 18 hours after Exposure Day 2 (3 hours)

Web Site Advertising (www.epastudies.org): IRB #07-0190

OMEGACON

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Can I take part in the study?

You may be able to take part in the study if you are between the ages of 50 and 75.

What will I be asked to do?

- Have blood drawn to determine the type of GSTM1 gene you carry. Currently only those who are GSTM1 Null will be eligible.
- Eat a special diet for six weeks.
- Take fish oil or olive oil treatments for four weeks.
- Breathe clean filtered air and air with carefully controlled amounts of pollution particles.
- Take part in breathing tests, including one called spirometry (link provided to explanation).
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- Have a brachial artery ultrasound (BAU, link provided to explanation).
- Take a dose of nitroglycerine, a short-acting vasodilator.

How long will it take?

Following initial screening, the study will take between 15 and 22 hours over 6 or 7 weeks.

What will I get for volunteering?

If you complete all visits and procedures, we will pay you $1668.

How can I sign up or get more information?

Call us or send and email. Our office hours are Monday-Friday from 8 am to 5 pm EST. After hours, please leave a message on voice mail, and we will return your call promptly.

- (919 966-0604 (local)
- 1-888-279-9353 (toll free)
- recruitment@epa.gov

OMEGA CON STUDY (IRB #07-0190)
E-mail Announcement

SUBJECT: INFORMATIONAL: Research study for Healthy Older Adults

The main purpose of this research study is to determine if a component of air pollution, particulate matter (PM) elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Volunteers ages 50 to 75 will be tested to determine the type of a particular gene they carry, one of several genes responsible for protecting the body against oxidants. For this phase of the study only individuals with the GSTM1 Null genotype will be eligible. Participation will include four-weeks of fish oil or olive oil treatments and two exposures, to a controlled amount of PM. For more information, please visit our web site at **www.epastudies.org** or call Westat EPA Recruiting at 966-0604.

Approved [date] by the Office of Human Research Protection (OHRE) Biomedical Institutional Review Board. IRB # 07-0190 (GCRC #2579).

This email is sponsored by: U.S. Environmental Protection Agency Human Studies Division located on the UNC-Chapel Hill campus.

University of North Carolina at Chapel Hill

Consent for Genotyping <u>With</u> Identifying Information **1**

IRB Study # 07-0190 **GCRC #: 2579**
Consent Form Version Date: April 21, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Michael Schmitt, MS; Susan Steck, PhD, RD; Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: (919) 966-4993
Study Contact email: tong.haiyan@epa.gov

<u>What are some general things you should know about this research study?</u>
Research studies are designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Research with blood, tissue and/or body fluids (specimens) can help researchers understand how the human body works. Research using specimens can also answer other questions. Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat diseases. In the future, some research may help to develop new products, such as drugs.

You may refuse to allow us to have or store your specimen. If you are a patient with an illness, you do not have to be in the research study in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

Initial/Date _____

What is the purpose of this study?
Recent reports have shown that people with a particular gene, known as the GSTM1 null gene, are more susceptible to air pollutants. The purpose of this study is to look at the cardioprotective effects of fish oil when people exposed to air pollution. The purpose of the genotyping is to determine whether you carry a GSTM1 sufficient gene or GSTM1 null gene. If you do not qualify for the study, your participation will end. If you do qualify you will complete the entire study.

How many subjects will participate in this study?
If you decide to participate, you will be one of approximately 32 participants in this study.

How long will your participation last?
Participation in this genetic session of the study will last for approximately 30 minutes.

What will happen if you participate in this study?
We will briefly review your medical history and any medical conditions that you have or medications that you are currently taking. You will sign 2 copies of the study consent form. We will measure your blood pressure and draw about 80 ml of blood for genotyping and blood analysis.

If you have been genotyped before, there will be no genotyping this time. You will be given the information below.

At the end, we will give you a copy of the Medical History Form and then fill it out and mail it back to the Westat recruitment office. We will also give you a copy of Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. If selected to participate you will be asked to fill out the FFQ and bring back the FFQ on the physical exam day. You will be given a dietary and medication instruction sheet to follow if you are selected and pass the physical exam.

How will the blood sample be collected?
You will have about 80 ml (about 5 1/3 tablespoon) of blood taken by our trained nursing staff.

What will happen to the blood?
A portion of the blood sample (about 5 ml) collected will be used to look at the GSTM1 gene and another portion of the blood will be used for cholesterol and lipid analysis. If there are excess samples left over after use for the purposes of this specific study, they will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill North Carolina. Only project members of the study will have access to the samples.

During the course of this research, other researchers may request access to specimens for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate IRB approved consent has been obtained from subjects, these specimens will be provided without identifiers to these other researchers by employing a data use agreement.

Initial/Date _____

Are there any reasons you should not participate?

- You should not participate in this portion of the study if you are not a candidate for the subsequent portions. The inclusion and exclusion criteria will be described. Briefly, you should not have chronic diseases including allergies, lung diseases, diabetes, need for a heart pacemaker, a previous chest pain and heart attack or coronary bypass surgery and uncontrolled high blood pressure. You are on doctor's orders to take fish oil that we do not want to interfere with your therapy. You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, acebutolol). You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history. The investigators and medical stuff will explain other potential exclusionary conditions in detail to you.

What are the possible benefits to you?

There are no direct benefits to you for completing this portion of the study. However, you will know your blood cholesterol levels and you will know your genotype of GSTM1 gene.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following minimal risks and/or discomforts to you:

1. The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

2. Risk of breach of confidentiality is minimal. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers

Will there be any cost to you to participate?

The U.S. EPA will pay the costs of this research. You will not be billed for any procedures.

Will you receive anything for being in this study?

You will be paid $30 for completing this procedure. We will give you parking coupons to cover the cost of parking. If you live more than 30 miles outside of the Chapel Hill/Carrboro area, you will be reimbursed for mileage at the current government rate.

Who owns the blood samples?

Initial/Date _____

Any blood samples obtained for the purpose of this study become the exclusive property of The U.S. Environmental Protection Agency. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records which use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Will researchers seek approval from you to do future studies involving the blood samples?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants.

In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive study results of future research involving your blood samples?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you individually. In rare cases, you may be offered the opportunity to receive information about the results of research in which the specimens were used (for example, findings that would affect your medical care).

Can you withdraw the blood samples from the research study?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. You may also contact the Institutional Review Board,

Initial/Date _____

University of North Carolina at Chapel Hill, 919-966-3113, Medical School Building 52, CB 7097, Chapel Hill, NC 27599, or by email at IRB_subjects@unc.edu. It is best to make your request in writing.

Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

Initial/Date _____

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Biomedical Form **3**

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: April 21, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).
- You are pregnant, attempting to become pregnant or breastfeeding.

Initial/Date _____

- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 32 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and two exposure sessions which last approximately 8 hours each, and one session 18 hours after air pollution exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. Then we will check your resting breathing rate. You will tour the exposure chambers (one for air exposure and the other for air pollution exposure) and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

Exposure days

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and

Initial/Date _____

shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 48 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor. You will wear a pressure cuff and a monitor which is about the size of the Holter monitor. Please keep your arm relaxed and still when the pressure cuff is inflated. We will remove the monitor and pressure cuff at about 48 hr later.
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to clean air on the first day then to concentrated Chapel Hill air particles on the second day; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently.

If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return on the follow-up day, for your safety you should not shower or bathe until after the monitor is removed. However, you will be given an instruction to follow if you need to have a shower or bath.

Eighteen hours after PM exposure follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after PM exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor and removed when you leave.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the

Initial/Date _____

appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Blood pressure monitor: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any

Initial/Date _____

employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1668.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
	Pre-study qualification total = $60
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
	Training day total =$61

Initial/Date _____

Exposures (2 x 8 hours)

Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Blood pressure monitor (2@$100 each)	$200
Time (16h @$12/h)	$192
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50

Total for completion of 2 exposures = $1306

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25

Total for completion of post-exposure =$141

Protocol Completion Bonus $100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Initial/Date _____

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ______________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

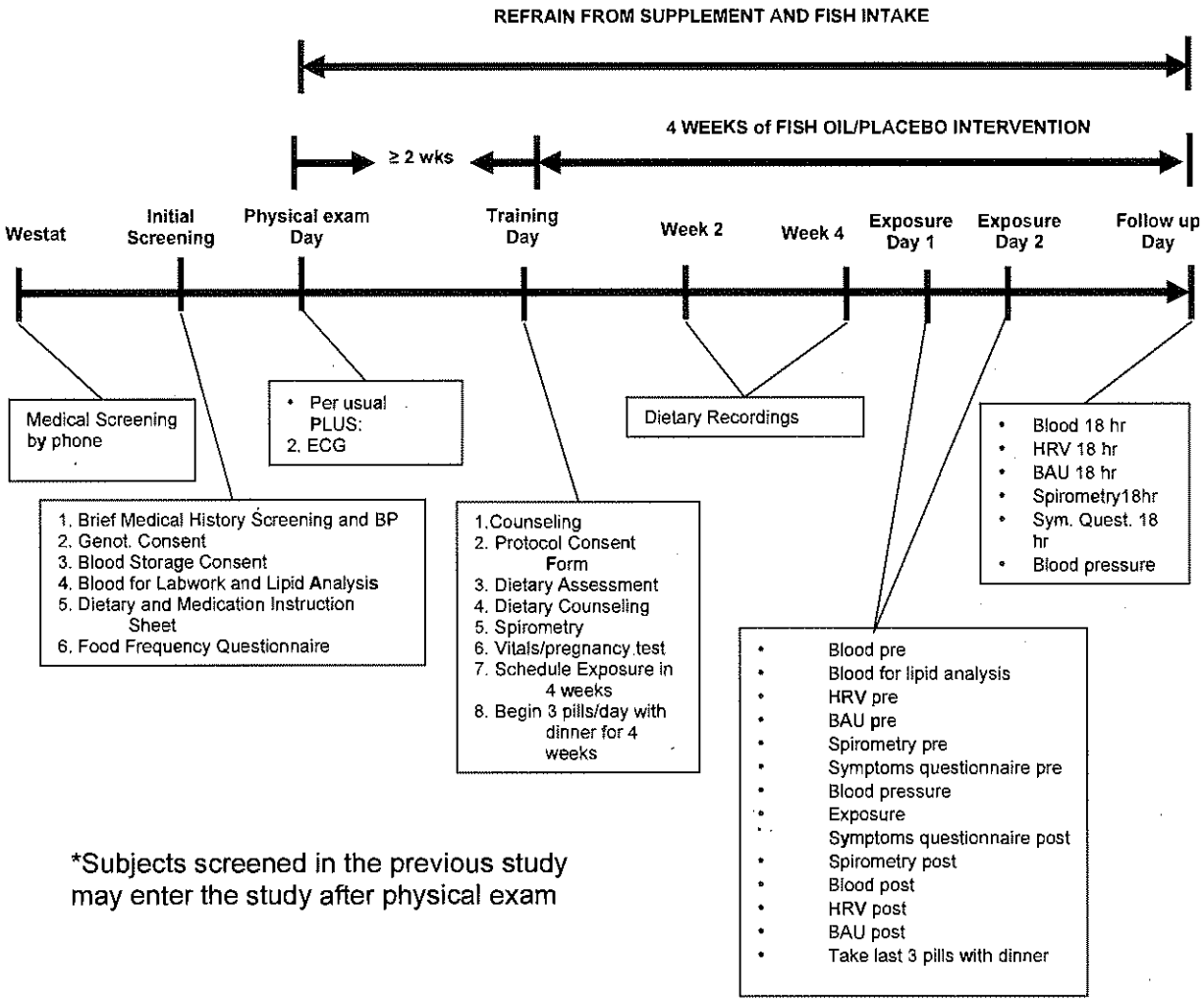
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Copy

Authorized signature on behalf of IRB

Date: 5/11/2010

RE: IRB review of Unanticipated Problem report
Submission Type: Unanticipated Problem
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Submission Description:

A female study subject (OMC-028) with a history of migraine headache had migraine headache after air pollution exposure on May 4, 2010. The symptom disappeared the next day. Protocol to be modified to exclude persons with a history of migraine headache.

Based on the IRB's review of your report of an unanticipated problem, it has been determined that no additional information is required and no changes in the study are warranted.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

Response to Minor Stipulations
Adverse Event

Receipt Date : 5/05/2010 Expiration Date : 1/24/2011 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Admin : Ina Friedman

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

☐ Full Board Review ☑ Not Full Board Review

Study Description :

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description :

A female study subject (OMC-028) with a history of migraine headache had migraine headache after air pollution exposure on May 4, 2010. The symptom disappeared the next day. Protocol to be modified to exclude persons with a history of migraine headache.

Please process.
Thx

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date: _____)
  - ☐ Approved by chair, see edits (Initials/Date: _____)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter:
  - ☐ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date: [illegible] 5/11/10)
  - ☐ Approved by chair, see edits (Initials/Date: _____)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached: _____

Other attachments: _____

FINAL ACTIONS: [illegible] 5/7/10

- ☑ Approved
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to Full IRB
- ☐ Return to sender
- ☐ Closure

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
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Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Date: 5/06/2010

RE: Contingencies to be addressed following IRB review of unanticipated problem report
Submission Type: Unanticipated Problem
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Submission Description:

A female study subject (OMC-028) with a history of migraine headache had migraine headache after air pollution exposure on May 4, 2010. The symptom disappeared the next day. Protocol to be modified to exclude persons with a history of migraine headache.

The IRB has reviewed your unanticipated problem report and determined that changes to the study are warranted as described below. Please address these changes in a cover memo that includes a point-by-point response to the item(s) listed and attach any revised study documents, including protocol, consent forms, recruitment materials, etc.

When you respond, please be sure to cite the IRB study number noted above, and indicate that you are responding to an unanticipated problem report. Failure to link your response to this study, in general, and this memo, in particular, may result in delays. Any additional changes beyond what has been requested (including new materials) must also be listed and discussed in the memo. Please submit 2 copies of all revised materials, one in which all changes are underlined and the other a "clean" copy with no underlining.

Study Specific Details:

Thank you for the report of a subject with migraine headache post exposure. Please submit a protocol modification (paper form) to include migraine heade as an exclusion criterion.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

THE UNIVERSITY
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(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 5/12/2010
Expiration Date of Approval: 1/24/2011

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Approved Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment received 5/6/10 addresses addition of exclusion criteria of history of migraine headache in response to SAE.

Investigator's Responsibilities:
When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: James Samet, Environmentally Protection Agency; Robert Truckner, (EPA), Non-IRB Review Contact; Wanda Simmons, (CTRC), Non-IRB Review Contact

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

THIS CONSENT DOCUMENT SHOULD BE USED ONLY BETWEEN 5/12/10 AND 1/24/11 APPROVED BY INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

Biomedical Form

3

IRB Study #____07-0190____ GCRC #: 2579
Consent Form Version Date: May 6, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acid Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Principal Investigator: Haiyan Tong, MD, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-0665, (919) 966-4993
Email Address: tong.haiyan@epa.gov, samet.james@epa.gov
Co-Investigators: Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.
Faculty Advisor: James M Samet, PhD, MPH
Funding Source: US Environmental Protection Agency Intramural Federal Research
Study Contact telephone number: 966-4993
Study Contact email: tong.haiyan@epa.gov

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason at any time.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Your participation is voluntary. Deciding not to be in the study or leaving the study before it is completed will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a

Initial/Date _____

copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The main purpose of this research study is to determine if a component of ambient air pollution to which we are all exposed, particulate matter (PM), elevates the risks of cardiac changes and whether fish oil supplements will lessen the risks caused by PM. Results from this study may increase the understanding of how gaseous and particulate air pollutants (which causes the haze seen in some polluted cities) may adversely affect the functioning of the human cardiovascular (heart and blood vessels) and respiratory (lungs) systems. This understanding may be especially important for patients with cardiopulmonary diseases.

Some of your blood will also be used to determine the type of a particular gene you carry. This gene, glutathione-S-transferase (GSTM1) is one of several genes responsible for protecting your body against oxidants such as air pollutant, and some recent studies have shown that people carrying a mutation in this specific gene, which renders this gene inactive maybe more susceptible to the effects of air pollutants.

You are being asked to be in this study because:

- You are 50-75 years old, generally healthy.
- You have a normal resting electrocardiogram (ECG).
- Your oxygen saturation is greater than 94% at the time of physical exam.

Are there any reasons you should not be in this study?

You should not participate in this study if…

- You have a history of chest pain, irregular heart beats, heart failure, and heart attack or coronary bypass surgery.
- You have a heart pacemaker.
- You have untreated high blood pressure (> 150 systolic, > 90 diastolic).
- You have a history of chronic lung diseases such as chronic obstructive pulmonary disease or severe asthma.
- You have a history of migraine headache.
- A history of rheumatologic diseases or immunodeficiency state,
- You have allergies to fish or omega-3 fatty acids.
- You are on doctor's orders to take fish oil that we do not want to interfere with your therapy.
- You are currently taking β-blockers (such as atenolol, metoprolol, propanolol, and acebutolol).
- You have a history of bleeding or coagulation disorders and are taking blood thinner medication.
- You are currently smoking or have a smoking history within 1 year of the study (defmed as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- You are a diabetic.
- You have cancer (exception for history of nonmelanoma skin cancer).

Initial/Date _____

- You are pregnant, attempting to become pregnant or breastfeeding.
- You have an allergy to latex.
- History of skin allergy to tape or electrodes.

You should **NOT** participate if you are unable to comply with the following requirements:

- No over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications for 2 weeks prior to exposure. Low-dose aspirin and Tylenol (acetaminophen) are permitted.
- No omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, Eggland's Best eggs, and cod liver oil for two weeks before and during the intervention period.
- No antioxidants (eg, beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the intervention period.
- Use olive oil for cooking, dressings, and sauces during the intervention period.
- Avoid drinking red wine during fish oil/placebo intervention period.
- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Eat a light breakfast on the exposure day.
- Not eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not consume caffeine for 12 hours prior to all study visits.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 32 people in this research study.

How long will your participation in this study last?

You will have up to 6 visits to the research facility over approximately 6-7 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this study will include two parts: 1) four-week of fish oil/olive oil treatments, and 2) exposure part. Exposure part include one training session (today) for about 3 hours, and two exposure sessions which last approximately 8 hours each, and one session 18 hours after air pollution exposure which last approximately 3 hours.

Storage of some of your blood samples in this study may be indefinite.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone an initial screening visit and a general physical examination to ensure that you are a candidate for this study. If you are a female participating in this study, you should have been asked about your menstrual history. You will have a pregnancy test today

Initial/Date _____

and you will have another pregnancy test on exposure day if it is more than 7 days since today's pregnancy test.

Today's visit is expected to last about 3 hours. We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we will expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign 2 copies of the study consent form and we will give one copy to you.

We will then train you on a breathing instrument to prepare you for your exposure session. This is known as spirometry, and you will breathe through a filter into the instrument. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. Then we will check your resting breathing rate. You will tour the exposure chambers (one for air exposure and the other for air pollution exposure) and the height of your mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted.

After these tests have been successfully performed, and if you are deemed to be a suitable candidate and you decide to participate in this study, you will undergo a dietary assessment on what food you have had in the last two weeks. You will be scheduled for a four-week fish oil or olive oil supplementation and the exposure session. You will be given a four-week supply of either fish oil or olive oil tablets. Please take 3 pills at dinner each day. You and your research team will not know what supplements you have received. A list of medications, environmental exposures, and foods to avoid in the next four weeks will be given to you. You will be asked to keep 3-day food records (one 3-day record per week in the study) 2 times during supplementation. We will provide a handout for you with instructions on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes. You will be contacted by phone for clarification of ambiguous information. You will be rescheduled if you have experienced an illness and you have to stop taking the pills during this four weeks of supplementation period. We will give you another four weeks supply of either fish oil or olive oil tablets and you will need to restart the pills again.

Exposure days

We will call you a few days before the exposure session to remind you of your scheduled visit. We will also remind you to refrain from alcohol, excessive amounts of caffeine, and from any activities where you could be exposed to high levels of pollutants (e.g., cigarette smoke, paint fumes) for a couple of days before your visit. You should not eat pan fried or grilled meat after midnight of the exposure day.

You will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level, and a symptom questionnaire).
- Have your heart rate viability (HRV) measured by a Holter monitor. You will have several ECG leads attached to your chest. It may be necessary to clean and

Initial/Date _____

shave the areas of your chest where these leads will be placed. Excessive deodorant, skin lotions, and body sprays may interfere with the function of some of these leads so we will ask you to not apply these to your chest area on the day you report to the HSF. The leads will be connected to 2 monitors (small recording devices about the size and weight of a portable tape player) to obtain readings of your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on you until you return the next day. You will be asked to recline quietly and breathe at a constant rate for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm. It is important that you do not fall asleep during this 30-minute period. The following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate and then the monitor will be removed. These monitors will allow us to determine if PM causes small changes in the ability of your nervous system to regulate how your heart beats. You will return to the HSF about 48 hr later to get the monitor removed. Please avoid any strenuous activities while you are wearing this monitor.

- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor. You will wear a pressure cuff and a monitor which is about the size of the Holter monitor. Please keep your arm relaxed and still when the pressure cuff is inflated. We will remove the monitor and pressure cuff at about 48 hr later.
- Have about 80 ml blood drawn (about 5 1/3 tablespoons). We will test this blood to see if PM affects the ability of your blood to clot correctly, or changes proteins on the surface of blood cells. **With your permission, we may also store some of your blood we obtained during the study for yet-to-be-determined tests in the future.**
- We will take you to the General Clinical Research Center (GCRC) of North Carolina Memorial Hospital (NCMH) where we will conduct an ultrasound of an artery in your arm. The ultrasound operator will scan your arm with probe and then place a tourniquet on your arm, much like a cuff used to measure blood pressure. Measurement of the size of the artery will be made four times. First, you will be asked to rest quietly for 15 minutes, and then the first 90 second scan will be performed. Then the blood pressure cuff on your arm will be inflated for 5 minutes in order to stop the flow of blood. You may feel sensations similar to those when your foot "goes to sleep", such as "pins and needles" and tingling. After the pressure is released, a second scan will be taken of the artery. You will rest quietly for another 10 minutes, and a third ultrasound scan will be taken at the end of this rest period. You will then be given a dose of nitroglycerin under your tongue. This drug takes effect very quickly and is sometimes associated with a short-lasting headache or dizziness. Three minutes later, the final ultrasound scan will be made. You will then be asked to rest quietly for 5 minutes so that the effects of the drug will wear off before you leave the laboratory.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.

Initial/Date _____

- You will then enter the exposure chamber and be exposed to PM.

During the exposure, you will:

You will wear a face mask in a chamber under exposures of 2 hours duration to clean air on the first day then to concentrated Chapel Hill air particles on the second day; the amount of particles you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. The exposure chamber is 4 ft wide by 6.25 ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. Your blood pressure will be measured intermittently.

If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Immediately following the exposure, you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have another BAU measurement at GCRC.
- Be assessed and discharged by the nursing staff.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest until you return on the follow-up day, for your safety you should not shower or bathe until after the monitor is removed. However, you will be given an instruction to follow if you need to have a shower or bath.

Eighteen hours after PM exposure follow up visit (about 3 hours)

You will return to the HSF the next morning (approximately 18 hours after PM exposure) and you will:

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and

Initial/Date _____

then blow it out as hard and fast as you can. We will ask you to do this several times.

- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have your blood pressure and heart rate measured intermittently by a blood pressure monitor and removed when you leave.
- You will have another BAU measurement at GCRC.
- Have blood drawn (about 80 ml or about 5 1/3 tablespoons).
- Fill out a symptom score questionnaire.
- Have the Holter monitor removed.

If there are any samples left over after all study information is collected, we will continue to store the samples for as yet undesignated studies. This allows us to make the best use of the samples we collect from subjects. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely in order to participate in this study.

What are the possible benefits from being in this study?

You will not benefit directly from being in this research study, though by participating in this study you will receive a medical examination that includes blood work, respiratory test, and ECG monitoring of heart at no charge. However, this is not a substitute for a routine doctor visit. The medical staff will explain to you any remarkable findings regarding your overall health status. In addition, if we observe changes in your health status as a consequence of exposure to air pollutants, you may elect to use this information to avoid exposure on high pollution days.

This research is designed to benefit society by gaining new knowledge. Given that every member of American society is currently exposed to these pollutants, this study has the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

What are the possible risks or discomforts involved with being in this study?

This study might involve the following risks and/or discomforts to you:
If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during this study. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like.

PM exposure: During the exposure to the concentrated air pollution particles, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. You will be monitored continuously during the exposure session through a window in the chamber or by closed-circuit television, and can communicate with a staff member via an intercom. Your heart rate and rhythm will also be constantly monitored for any adverse changes brought about by the exposure. A licensed physician is always on the premises during exposures, and is available to respond in an emergency. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the

Initial/Date _____

appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency.

Air pollution particles may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 4 ft in width, 6.25 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator via a microphone headset.

Supplementation: There is little risk associated with taking fish oil/olive oil supplements. You may experience allergic reaction if you have fish allergy. Fish oil/olive oil supplements will be provided by a certified vitamin company and have the same quality as the fish oil/olive oil supplements that you can buy from food store or pharmacy.

Heart rhythm monitoring: There is little risk associated with monitoring your heart by ECG or blood oxygen by pulse oximetry. However, preparing your skin for placement of adhesive ECG electrodes and removing the electrodes the next day may cause some irritation or skin discoloration, itching, or burning in some people. If this occurs you should call the nursing staff.

Venous blood sampling: The risks associated with taking blood samples are considered minimal. A well-trained member of the staff will draw the blood. Drawing blood could cause some bruising or minor pain, which usually resolves quickly. Also, a rare complication is skin infection or an infection of the vein in which the blood has been drawn. The risk of getting an infection is minimized by the use of sterile technique. If you do have signs of infection at the site (redness, warmth, painful skin, and swelling) after completion of the procedure, you will need to contact the EPA medical station.

Brachial artery ultrasound: There are no significant risks associated with ultrasound imaging of the brachial artery, or with brief episodes of forearm ischemia (reduced blood flow). Occlusion of blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness until the blood pressure cuff is released. A small number of patients (about 1 in 200) develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days. Sublingual nitroglycerin is a potent vasodilator, and may be associated with headache, flushing and transient lowering of your blood pressure. Since this drug is short-acting, however, these effects do not last long. To minimize the risk of low blood pressure, you will remain lying down for ten minutes after receiving nitroglycerin. As with any medication, nitroglycerin can cause an allergic reaction, such as a rash, in rare individuals. Some risks and discomforts may be unforeseeable.

Initial/Date _____

Breathing tests (spirometry): You may cough or become dizzy during these tests. You will be seated in a chair, and if these symptoms occur, they are usually only temporary. You will be exposed to low dose of acetylene for a brief period of time (single breath in and breath out), thus the risk will be quite low.

Blood pressure monitor: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

In addition, there may be uncommon or previously unrecognized risks that might occur. If you do notice any unusual symptoms occurring during the study you should call the EPA medical station or the on-call physician to report them.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number, however only study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Blood samples will be stored at the EPA Human Studies Facility.

Research studies may be done at many places at the same time. Your personal identifying information will not be sent to outside researchers.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any

Initial/Date _____

employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study and the total compensation for completion of this study will be approximate $1668.

If you are unable to complete the study for voluntary reasons or failure to comply with eligibility requirements you will receive full compensation for your participation up to that point.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. Payments totaling more than $600 in a year from a single or multiple EPA studies will be reported to the IRS. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. The following table details the expected compensation for completion of the entire study:

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
	Pre-study qualification total = $60
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
	Training day total =$61

Initial/Date _____

Exposures (2 x 8 hours)

Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Blood pressure monitor (2@$100 each)	$200
Time (16h @$12/h)	$192
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50

Total for completion of 2 exposures = $1306

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25

Total for completion of post-exposure =$141

Protocol Completion Bonus $100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and canceled. You will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus.

Initial/Date _____

Will it cost you anything to be in this study?
There will be no cost to you for participating in the study. However, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. EPA will not provide reimbursement for any follow-up care.**

All study procedures will be paid for by the study. We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by The U.S. Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions regarding this study, you should call one of the listed investigators on the first page of this form.

If you feel a research-related injury has occurred, please contact the HSF medical station or one of the investigators listed above. In addition, you should contact the Human Studies Division Human Research Officer and Director of the National Health Effects and Environmental Research Laboratory Human Research Protocol Office at 919-966-6217.

What if you have questions about your rights as a research subject?
All research on human subjects is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously, if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_ subjects@unc.edu and/or the EPA Director of the National Health and Environmental Effects Human Research Laboratory Protocol Office at 919-966-6217.

- -

Initial/Date ________________

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

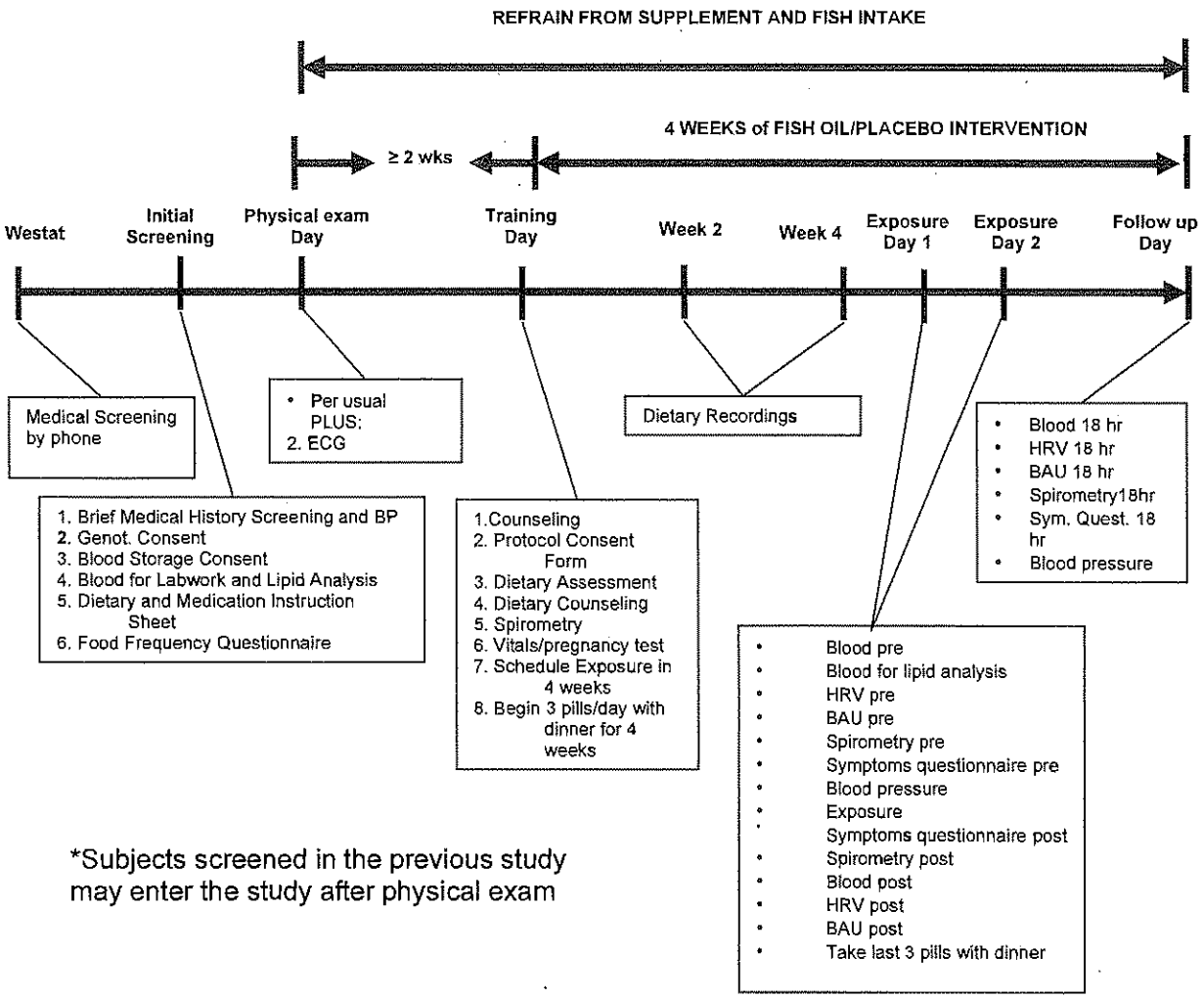
_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Initial/Date _____



STUDY FLOW DIAGRAM

Initial/Date _____

First Review of IRB Submission
Modification

Pending Reviews

Submission Type	Date Received	Approval Date	IRB Admin	Review Type
Adverse Event	5/05/2010	Not Approved	Ina Friedman	Expedited

Training Not Met (Haiyan Tong, James Scott, Ana Rappold, Tracey Montilla, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 5/06/2010 **Expiration Date :** 1/24/2011 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Admin :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Laura Munn

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☒ Approval letter: 5/12/10
  - ☐ Draft letter prepared
  - ☒ Approved by chair as attached (Initials/Date: 5/12/10)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☒ Email copy sent
  - ☒ Hard copy sent

Consent forms attached: 1

Other attachments: 0

FINAL ACTIONS:

- ☒ Approved 5/12/10
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Closure

RECEIVED
MAY 06 2010
IRB UNC-CH

To: IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

RECEIVED
5/6/10
CTRC

From: Haiyan Tong
Environment Protection Agency
CB# 7315
E-mail: tong.haiyan@epa.gov
Phone number: 966-4993

May 6, 2010

Study #: 07-0190
Other #: CTRC: 2579
Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Dear IRB review committee,

Thanks for the Board Review for the report of an unanticipated problem submitted to IRB on may 5, 2010. Enclosed are the revised sbumission of protocol and study consent form in response to the "Contingencies to be addressed on May 6, 2010".

An exclusion criterion of "A hisory of migraine headache" has been added in the protol and consent form which is highlighted in yellow.

Please let me know if you have any questions regarding this revision.

Sincerely,

Haiyan Tong

RECEIVED
MAY 06 2010
IRB UNC-CH

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

RECEIVED 5/6/10 CTRC

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
x	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
X	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. If adding personnel, include name, location (UNC or specific outside location), role, and email address for each person who should receive electronic copies of IRB correspondence to PI. For those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/) include documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1). Addition of "A history of migraine headache" as an exclusion criterion in the protocol and study consent form in response to an unanticipated problem occurred on May 4, 2010.

2. Is this modification being submitted in response to an unanticipated problem/adverse event or new findings? _x_ yes ___ no

If yes, explain, including whether these events or findings are relevant to participants' willingness to continue.
A female study subject (OMC-028) with a history of migraine headache had migraine headache remission after air pollution exposure on May 4, 2010. The symptom disappeared the next day. The subject was willing to continue the study and she completed the study the next day.

3. Do any of the proposed changes increase risk? ___ yes _x_ no If yes, explain.

IRB study #: 07-0190 CTRC #: 2579 **Date:** May 6, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor:** James Samet, PhD

(if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:

Investigator Brochure version #:

Any other details you need documented on IRB approval:

Version date:

Signature of Principal Investigator or designee

Date 5/6/10

Signature of Faculty Advisor (if applicable)

Date 5/6/10

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

RECEIVED
MAY 06 2010
IRB UNC-CH

Part A.1. Contact Information, Agreements, and Signatures

Date: May 6, 2010

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate _x_ postdoc __ resident __ other

Name of faculty advisor: James Samet, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD; Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):

__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify): EPA-Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
x	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
☐	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
☐	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
☐	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator — Date: 5/6/10

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor — Date: 5/6/10

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee — Date

Print Name of Department Chair or designee — Department

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? ***If yes***, *see instructions for* ***Consent for Stored Samples***.	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? ***If yes***, *approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? ***If yes***, *approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? ***If yes***, *submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? ***If yes***, *obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is ***yes***, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the desigu, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.
Participants Thirty-two healthy 50-75 year-old male and female subjects will be involved in the study.
Procedures (methods): The subjects will be exposed to clean air for 2 hours on the first day then concentrated ambient fine and ultrafine PM for 2 hours on the second day after being randomly supplemented with four weeks of omega-3 fatty acid or olive oil.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac

autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (15-17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (20). Therefore, the role of GSTs in mediating the cardiovascular effects following acute PM exposure will also be studied in the protocol in GSTM1 null and GSTM1 positive healthy older adults.

The respiratory effects of PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.

6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
15. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal is approximately 32 subjects for the study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially

by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of migraine headache.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This will be a randomized, double-blinded study. Thirty-two study subjects will be randomly given fish oil (15 subjects) or olive oil (15 subjects) supplements for 4 weeks. The subjects will be given a container filled with 4 weeks supply of enteric-coated either 3g/day of fish oil or 3g/day of olive oil capsules. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 410 mg of eicosapentaenoic acid (EPA) and 274 mg of docosahexaenoic acid (DHA). Each 1000 mg of this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. Each subject will be exposed to clean air for 2 hour on the first day then air containing fine and ultrafine PM (PM<2.5) for 2 hours on the second day in an exposure chamber after the supplementation. If the PM concentrations are too low on the scheduled exposure day, they may be rescheduled. (See the study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotyping consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 25, methods of recruiting) will not need genotyping. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 80 ml of blood collected. A portion of the blood will be used for genotyping of GSTM1 and another portion of the blood will be used for the cholesterol levels, phospholipid and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back to the medical station.

- ***GSTM1 genotyping***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null.
- ***Food frequency questionnaire in the study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency

Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. The height of subject's mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. The resting minute ventilation will be measured. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the study:*** Subjects will be randomly given an approximately 4 weeks supply of either fish oil or olive oil supplements. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record every other week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 4 weeks of supplementation period. We will give the subjects another 4 weeks supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the exposure, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU), pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 48 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.
- **Blood pressure and heart rate measurement** will be measured intermittently by a BP monitor. A blood pressure cuff and a monitor which is about the size of the Holter monitor will be placed and will remain in place until the subjects leave for the day. The subjects will be instructed to keep arm relaxed and still when the pressure cuff is inflated.
- ***Brachial artery ultrasound:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation.

The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Pulmonary function*** will be measured by spirometry.
- ***Blood sampling*:** approximately 80 ml of blood sample will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

Exposure:

The subjects will be exposed to clean air on the first day then concentrated ambient fine and ultrafine PM on the second day after the dietary supplementation. The PM exposure day may be rescheduled if the PM concentrations are too low on the scheduled day.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. During the exposure, subject will be monitored continuously by visual camera monitor, continuous pulse oximetry, blood pressure every 15 minutes, and telemetry. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours.

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.
The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will be up to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The particle mass of the air entering the chamber will be monitored continuously. If it is confirmed that particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, exposure will be terminated. The process for confirmation may take up to additional 10 minutes. The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to

removal of the subject from the chamber. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 80 ml of blood samples will be collected.

Eighteen Hours Post-PM exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function (BAU) measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 80 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for two times of 24 hours duration using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Blood Pressure and Heart Rate will be measured intermittently on the exposure days and follow up day by a BP monitor. Systemic, diastolic, and mean blood pressure and heart rate data will be recorded.

Flow-Mediated Dilatation (brachial artery ultrasound) Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before and immediately after (within 1 hr after exposure ends) each exposure, and approximately 18 hrs after PM exposure, blood will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In this study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Blood pressure and heart rate measurement**: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.
- **Brachial artery ultrasound:** There are no known risks associated with imaging of the brachial artery. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 3g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will be up to 600 ug/m^3 and the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are

occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test the hypothesis that PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.

We will measure a number of endpoints (e.g., neutrophils, inflammation markers, and lung function). Several markers of the cardiovascular response to PM will also be measured (e.g., blood pressure, HRV, brachial arterial diameter, change in blood vasoactivators, and coagulation factors). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect 0.13 units of change in BAU diameter as observed in the pilot study and estimated that 15 subjects per group are needed to achieve type I error rate 0.05 at power of 80%. We used estimates of variance in BAU diameter (0.27) and correlation among the repeated measurements (0.97) from the OMEGA pilot study. We used the equation for comparing two groups (placebo and treatment) with a single explanatory variables (PM concentration).

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4%

decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of PM on changes in: heart rate variability in both time and frequency domains, blood CBC, fibrinogen, D-dimer, and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data.** Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). ___ yes ___ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). ___ yes ___ no
Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the study will take approximately 12 months. Participant recruitment is expected to be continuous throughout the study until the intended number of participants is reached.

The individual will have 6 visits to the research facility over approximately 6-7 weeks. The pre-enrollment and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after the blood work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment will require approximately 3 hours. Each exposure day will last approximately 8 hours. Eighteen hours after the PM exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study. If the subject is qualified and finished the entire study, the total compensation for completion of this study will be approximate $1668.

Pre-study qualifications	
Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30
Pre-study qualification total = $60	
Training day (3 hours)	
Time (3h @$12/h)	$36
FFQ	$25
Training day total = $61	
Exposures (2 x 8 hours)	
Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Time (16h @$12/h)	$192
Blood pressure monitor	$200
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50
Total for completion of 2 exposures =$1306	
Eighteen hours after exposure (3 hour)	
Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25
Total for completion of post-exposure = $141	
Protocol Completion Bonus	$100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

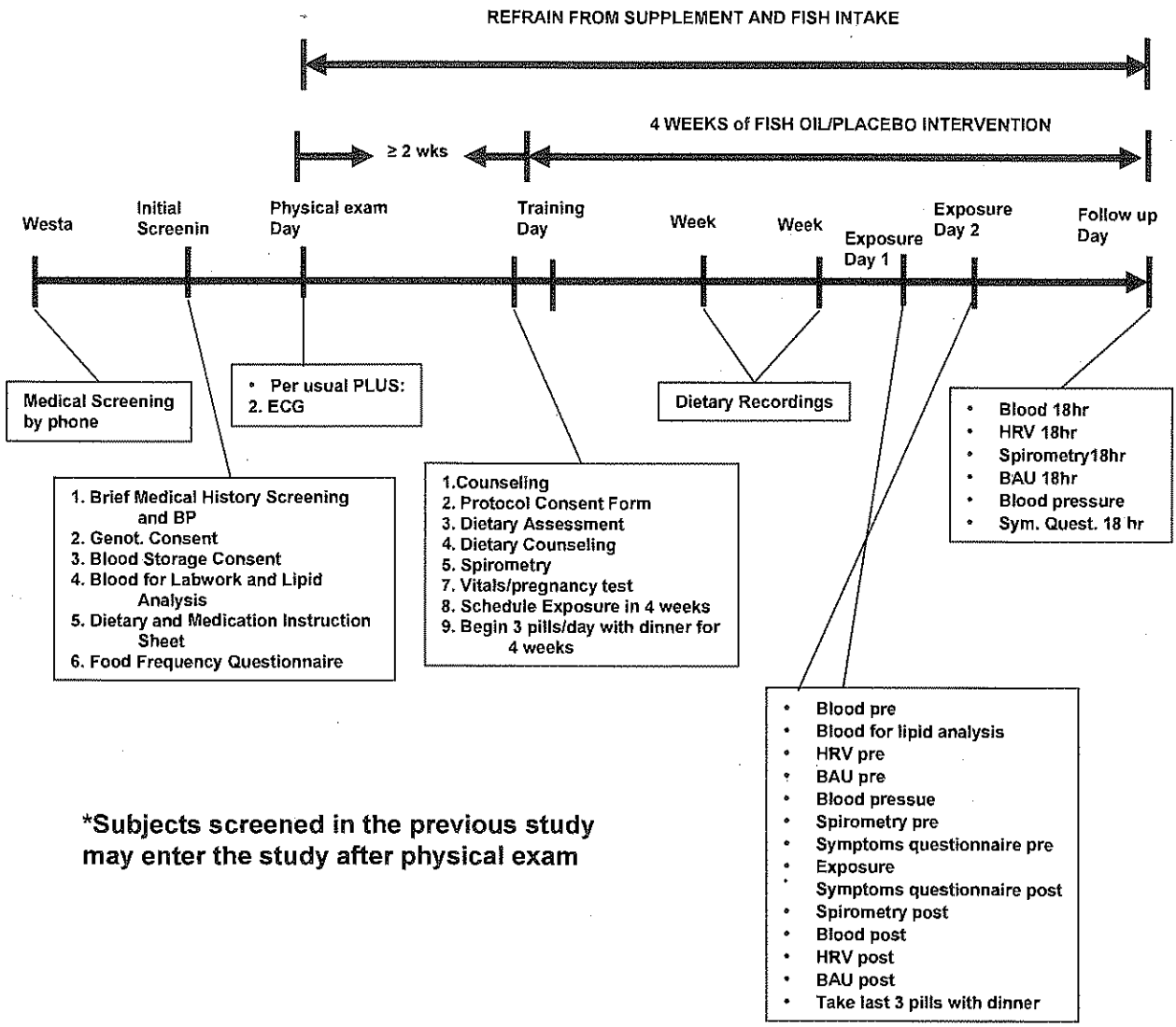
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



STUDY FLOW DIAGRAM

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 5/26/2010
Expiration Date of Approval: 1/24/2011

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Unanticipated Problem
Expedited Category: Minor Change to Previously Approved Research
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:

A study subject (OMC-029) had intermittent atrial and ventricular ectopy. No symptoms. Submission includes details of event, as requested by IRB. No further action is needed.

Investigator's Responsibilities:

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

Response to Minor Stipulations
Adverse Event

Receipt Date : 5/25/2010 Expiration Date : 1/24/2011 Previous Review Type: **Renewal Full Board**

IRB: Biomedical PI: Haiyan Tong IRB ID : 07-0190 IRB Admin : Ina Friedman

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

☐ Full Board Review ☑ Not Full Board Review

Study Description :

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description :

A study subject (OMC-029) had cardiac arrhythmia.

Thank + Approve.
No further action needed.
[illegible]

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☑ Approval letter:
  - ☐ Draft letter prepared
  - ☑ Approved by chair as attached (Initials/Date: [illegible] 5/26/10)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☑ Email copy sent [illegible]
  - ☑ Hard copy sent

Consent forms attached:
Other attachments:

FINAL ACTIONS:

- ☑ Approved [illegible] 5/26/10
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to Full IRB
- ☐ Return to sender
- ☐ Closure

RECEIVED
MAY 2 5 2010
IRB UNC-CH

To: IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

From: Haiyan Tong
Environment Protection Agency
CB# 7315
E-mail: tong.haiyan@epa.gov
Phone number: 966-4993

May 25, 2010

Study #: 07-0190
Other #: CTRC: 2579
Study Title: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Dear IRB review committee,

Thanks for the Board Review of an unanticipated problem report submitted to IRB on May 25, 2010. Below are a point-by-point responses to the items listed in the "Contingencies to be addressed following IRB review on May 25, 2010".

What was the duration of exposure at the time the arrhythmia was noted?
The subject was exposed to two-hour of filtered air which is the clean air control of the air pollution exposure study. The arrhythmia was noted on the telemetry right after the filtered air exposure began.

What was the nature and duration of the cardiac arrhythmia?
Intermittent premature ventricular contraction (PVC) and supraventricular ectopy were noticed during the 2 hrs of exposure session. The Holter monitor data collected from 8 am on May 24 to 8 am on May 25 showed that the PVC and supraventricular ectopy were presented throughout the 24 hr period.

Was the subject symptomatic?
The subject had no symptom with arrhythmia.

What treatment and follow up were recommended, if any?
In consideration of clean air exposure does not cause cardiac arrhythmia and the subject was symptom free, we did not treat the subject. However, the subject was counseled for his arrhythmia and provided a copy of the Holter monitor report and advised to see his own primary physician for further investigation.

Was there anything in the subject history to indicate risk or prior occurence?
The subject had no history of arrhythmia. The ECG was normal on the pre-study physical exam which was evaluated by a cardiologist.

The unanticipated problem reported is specific to this subject. We do not revise the study protocol, consent forms, and recruitment materials at this time.

Please let me know if you have any further questions.

Sincerely,

Haiyan Tong

Haiyan Tong

IRB Notice
IRB
to:
Haiyan Tong
05/25/2010 02:02 PM
Cc:
James Samet, Robert Truckner, wsimmons+IRB
Show Details

To: Haiyan Tong
Environmentally Protection Agency
CB# 7315

From: Biomedical IRB

Date: 5/25/2010 **RE**: Contingencies to be addressed following IRB review of unanticipated problem report
Submission Type: Unanticipated Problem
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Submission Description:

A study subject (OMC-029) had cardiac arrhythmia.

The IRB has reviewed your unanticipated problem report and determined that changes to the study are warranted as described below. Please address these changes in a cover memo that includes a point-by-point response to the item(s) listed and attach any revised study documents, including protocol, consent forms, recruitment materials, etc.

When you respond, please be sure to cite the IRB study number noted above, and indicate that you are responding to an unanticipated problem report. Failure to link your response to this study, in general, and this memo, in particular, may result in delays. Any additional changes beyond what has been requested (including new materials) must also be listed and discussed in the memo. Please submit 2 copies of all revised materials, one in which all changes are underlined and the other a "clean" copy with no underlining.

Study Specific Details:

Please provide additional information. What was the duration of exposure at the time the arrhythmia was noted? What was the nature and duration of the cardiac arrhythmia? Was the subject symptomatic? What treatment and follow up were recommended, if any? Was there anything in the subject history to indicate risk or prior occurence?

CC:
James Samet, Environmentally Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

IRB Informational Message—please do not use email REPLY to this address

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Haiyan Tong
Environmental Protection Agency
CB# 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 1/05/2011
Expiration Date of Approval: 1/04/2012

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Renewal
Expedited Category: 8(c) Continuing Review - Data Analysis Only
Study #: 07-0190
Other #: CTRC : 2579
Study Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles
Sponsors: EPA Intramural Federal Research

This submission has been approved by the above IRB for the period indicated.

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Regulatory and other findings:

This research, which was originally approved by the Full Board, is being renewed by the IRB under Expedited Review, Category 8c. The research has been closed to the accrual of new subjects and all subjects have completed intervention/interaction. Renewal is granted for data analysis only.

Investigator's Responsibilities:

Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other

recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Any unanticipated problem involving risks to subjects or others (including adverse events reportable under UNC-Chapel Hill policy) should be reported to the IRB using the web portal at https://irbis.unc.edu/irb.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40CFR 26 (EPA), where applicable.

CC:
James Samet, Environmentally Protection Agency
Howard Kehrl, (EPA), Non-IRB Review Contact
Wanda Simmons, (CTRC), Non-IRB Review Contact

UNC
THE NORTH CAROLINA
TRANSLATIONAL & CLINICAL
SCIENCES INSTITUTE

RECEIVED
JAN 28 2011
IRB UNC-CH

TO: Dr. Haiyan Tong

FROM: Dr. Michael W. Fried, Director, CTRC

DATE: January 5, 2011

SUBJECT: Approval of Renewal for **GCRC-2579. Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Health Older Subjects Exposed to Air Pollution Particles IRB Study #: 07-0190**

Both the Clinical and Translational Research Center Oversight Committee and the Institutional Review Board (IRB) have reviewed this proposal and have approved it until **January 4, 2012.**

REMEMBER:
The NC TraCS Institute CTRC is evaluated based on how many published manuscripts cite our CTSA grant number. Please acknowledge award number UL1RR025747 from the National Center for Research Resources on any publication where this can be justified. In addition, all publications, press releases, or other documents that result from the utilization of any NC TraCS Institute resources are required to comply with NIH Public Access Policy (submission to PubMed Central).
https://tracs.unc.edu/?option=com_content&view=article&id=130&Itemid=193

Inpatient and outpatient visit requests should be scheduled at https://ctrc.med.unc.edu/scheduler/ or with Gloria Scott or Sherri McLaurin (966-1435). The approved consent form must be signed by each participant before any studies are done.

Should any significant side effects occur in any volunteer participating in this study, you are expected to inform the Director of the Clinical and Translational Research Center and the Chairman of the Human Rights Committee immediately.

Michael W. Fried, MD, Director

Clinical and Translational Research Center
Campus Box # 7600
T: 919-966-1435
F: 919-966-1576
tracs.unc.edu/ctrc

First Review of IRB Submission

Renewal

Training Not Met (Haiyan Tong, James Scott, Ana Rappold, David Demarini, Martin Case, Mary Bassett)

Receipt Date : 1/04/2011 **Expiration Date :** 1/24/2011 **Previous Review Type:** **Renewal Full Board**

IRB: Biomedical **PI:** Haiyan Tong **IRB ID :** 07-0190 **IRB Admin :**

Title: GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

☐ Not-HSR ☐ Exempt (Category:) ☒ Not Full IRB (Category:) ☐ Full IRB

Agenda Date Reviewer 1: Reviewer 2:

Entered by: Wyomie Milliken

Study Description:

The purpose of this study is to identify an optimal concentration of diesel exhaust which can be used to study the risks of cardiac changes in healthy older subjects. Healthy adults, ages 50 to 75 years, will complete seven visits over 18-20 weeks.

Submission Description:

PROCESSING STEPS (OFFICE USE ONLY):

- ☐ Reviewer Checklist completed
- ☐ Minor Stipulation letter:
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date:)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent
- ☐ Approval letter: 1/5/11
  - ☐ Draft letter prepared
  - ☐ Approved by chair as attached (Initials/Date: 1/5/11)
  - ☐ Approved by chair, see edits (Initials/Date:)
  - ☐ Email copy sent
  - ☐ Hard copy sent

Consent forms attached:

Other attachments:

FINAL ACTIONS:

- ☐ Approved 1/5/11
- ☐ Approved with Minor Stipulations
- ☐ NHSR
- ☐ Return to sender
- ☐ Closure

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board
REQUEST FOR RENEWAL OF IRB APPROVAL OR STUDY CLOSURE
Version August 26, 2009

RECEIVED
JAN 04 2011
IRB UNC-CH
RECEIVED
1/4/11
CTRC

If the research is continuing:

- Check the relevant items.
- Include two collated sets of copies (sorted in the order listed) of checked items.

→ **Submissions will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This form (renewal or closure).	2
x	2. Any items specifically requested in questions # 4 through 9 (in that order).	2
x	3. The most recent application submitted for IRB approval. This application should be updated to include any modifications since the study was initially approved or last renewed. **If there are any new modifications included with this renewal, highlight the proposed modifications by underlining.**	2
□	4. Clean copies of all consent document(s) to be used in the upcoming approval period, for stamping.	2
x	5. Only for those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1

IRB study #: 07-0190 **Date:** 1/4/2011

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Principal Investigator: Haiyan Tong, MD, PhD **Faculty advisor** (if applicable): James Samet, PhD

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

1. In a few sentences, describe the past year's work, and describe what you plan for the upcoming year, including data analysis, if relevant.

Ten subjects have been enrolled and finished the study since the last renewal of IRB protocol. We requested to add two more subjects which was approved by IRB since the last renewal but we did not recruit these two because we have achieved expected result. Therefore we have finished the data collection phase and no more subjects will be enrolled in the coming years. We will finish the blood assay and enter the data analysis phase.

2. Number of subjects involved through direct contact or use of their data (for multi-site studies, include only subjects covered by this IRB) (Note: **b+d** should not be larger than **a**)
   a. Total projected number as approved by IRB: 32
   b. Total number of subjects involved to date (for clinical trials include "screen failures"): 30
   c. Number of subjects added since last renewal: 10
   d. Number to be included in upcoming year: 0

Answer the following questions based on information since initial approval or last renewal. Only include subjects covered by this IRB.

3. Have there been any modifications approved since the last review? If your IRB application has not already been updated to reflect these changes, do so now and attach any revised documents, including application and/or consent documents.	_x yes __ no
4. Have any subjects withdrawn voluntarily or been withdrawn from the study? **If yes, explain**; give number and reasons for withdrawals. Three study subjects have been withdrawn from the study after they enrolled since the last renewal. One subject (subject ID number OMC-020) had paranoid personality disorder who did not want to continue the study after being exposed to filtered air on the first day of the study on January 11, 2010. The second subject (subject ID number OMC-029) had abnormal heart rate rhythm after the filtered clean air exposure on May 24, 2010 and the third subject (subject ID number OMC-032) had abnormal ECG after the filtered clean air exposure on June 21, 2010. We do not expect any adverse health effect from filtered clean air exposure so these subjects' symptoms were not related to the research. The subjects were discharged from EPA Medical Station with normal vital signs and reportedly feeling fine. It was recommended that the subjects follow up with their primary care physicians for better management.	_x yes __ no
5. Have there been any complaints about the research from subjects or others? **If yes, explain**	__ yes _x no
6. Have there been any findings (e.g., publications, new information) that alter the risk/benefit ratio or otherwise impact the study? **If yes, explain, including whether these new findings are relevant to participants' willingness to continue.**	__ yes _x no
7. Have there been any relevant multi-center reports? **If yes, provide a copy of the report.**	__ yes _x no
8. Does this study have a Data and Safety Monitoring Committee (DSMC or DSMB)? **If yes, provide a report** from the DSMC.	__ yes _x no
9. Have there been unanticipated problems or serious adverse events since the last renewal? **If yes, include all copies** of local Adverse Event reports with this submission.	x_ yes __ no
10. Has this study been audited by external sponsor or monitor since approved or last renewed? **If yes, include a copy** of the audit report.	__ yes _x no
11. Are you requesting any changes to the study or consent documents? **If yes, include the form** requesting *Modification of Approved Human Subjects Research* and underline the proposed change in the updated application and/or consent documents.	__ yes _x no
12. Will you be enrolling, consenting or re-consenting subjects in the upcoming approval period? **If yes, include clean copies** of consents/assents/fact sheets to be used to receive a new stamp. **Include any new recruitment materials** to be used with subjects.	__ yes _x no

Action requested by Principal Investigator (choose only one):

Renew approval:

__ Study has always involved *only* analysis of existing data or specimens. Continue as approved.

Study involves(ed) direct interaction/intervention or contact with subjects:

__ Continue as approved: Enrollment of new subjects continues.

__ Enrollment of new subjects closed; interaction/intervention with previously enrolled subjects continues.

__ Direct interaction with subjects completed but subsequent monitoring or follow up continues.

_x_ Subjects' involvement completed but renewal is requested for data analysis.

Closure of Study:

__ Research completed: Identifiable data or human biological specimens are stored according to plan already approved by the IRB.

__ Research completed: All data or human biological specimens are deidentified.

__ Lack of funding or other *(specify)*:

Signature of Principal Investigator	1/4/2011 Date
Signature of Faculty Advisor (if applicable)	Date

University of North Carolina - Chapel Hill

IRB Number:	07-0190	**Legacy ID:**	
PI:	Haiyan Tong	**IRB:**	Biomedical
Sponsor:	EPA Intramural Federal Research		
Study Title:	GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles		

Certified:	06/22/2010
Reference ID:	1653

>> Brief Description of Event

A study subject (OMC-032) had cardiac arrhythmia (ventricular tachycardia) after clean air exposure. The brief arrhythmia was noted on a 24 hr Holter monitor recording and the subject had no symptoms

A1) Did this event occur at a site for which a UNC-CH IRB has direct oversight responsibility or involve a research participant at one of those sites? Yes

A2) Was the event unexpected in nature, severity, or frequency? Yes

Please explain:

We do not expect the study subjects have any cardiac arrhythmia after controlled clean air exposure. The 24 hr Holter monitor recording showed that the subject had a 4-beats ventricular tachycardia about 6 hrs after clean air exposure which is the control exposure. The subject had no symptoms and she was not doing any strenuous activities. The subject was consoled and was given a copy of the ECG. She was advised to see her own cardiologist for further investigation of her arrhythmia.

A3) Do you think the event was related or possibly related to this research? No

Based on your response this event is not required to be reported to the IRB. According to federal guidance and University policy, only events that are unexpected and related to the research must be reported. You may still have obligations to report this event to the research sponsor, coordinating center, data safety monitoring board (DSMB), or other oversight committee.

>> Attachments

There are no attachments for this event

University of North Carolina - Chapel Hill

IRB Number:	07-0190	**Legacy ID:**	
PI:	Haiyan Tong	**IRB:**	Biomedical
Sponsor:	EPA Intramural Federal Research		
Study Title:	GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles		

Certified:	05/25/2010
Reference ID:	1523

>> Brief Description of Event

A study subject (OMC-029) had cardiac arrhythmia.

A1) Did this event occur at a site for which a UNC-CH IRB has direct oversight responsibility or involve a research participant at one of those sites? Yes

A2) Was the event unexpected in nature, severity, or frequency? Yes

Please explain:

The subject was exposed to filtered air and heart rate variability monitor showed that the subject had arrhythmia. We do not expect filtered air exposure causes heart arrhythmia. For this case, we think the subject has his own underline medical condition.

A3) Do you think the event was related or possibly related to this research? No

Based on your response this event is not required to be reported to the IRB. According to federal guidance and University policy, only events that are unexpected and related to the research must be reported. You may still have obligations to report this event to the research sponsor, coordinating center, data safety monitoring board (DSMB), or other oversight committee.

>> Attachments

There are no attachments for this event

University of North Carolina - Chapel Hill

IRB Number:	07-0190	**Legacy ID:**	
PI:	Haiyan Tong	**IRB:**	Biomedical
Sponsor:	EPA Intramural Federal Research		
Study Title:	GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles		

Certified: 05/05/2010

Reference ID: 1476

>> Brief Description of Event

A female study subject (OMC-028) with a history of migraine headache had migraine remission after air pollution exposure on May 4, 2010. The symptom disappeared the next day.

A1) Did this event occur at a site for which a UNC-CH IRB has direct oversight responsibility or involve a research participant at one of those sites? Yes

A2) Was the event unexpected in nature, severity, or frequency? Yes

Please explain:

We did not expect air pollution exposure cause migraine headache.

A3) Do you think the event was related or possibly related to this research? Yes

Please explain:

The subject has a history of migraine headache and she had a sinus surgery three years ago. She said the face mask she was wearing during air pollution exposure compressed on her nose and face which might trigger the episode. She had headache and vomited once about 2 hours after exposure. She was given Tylenol and rested in a dark room and the symptom much improved and was discharged. She had no headache the next day.

A4) Does the event suggest that the research places subjects or others at a greater risk or harm than was previously known or recognized?

Economic: No
Legal: No
Physical: Yes
Psychological: No
Social: No

Please explain all "yes" or "don't know" responses

The migraine headache resulted in discomfort to the subject.

>> Information About the Event

B1) Date of Event: 05/04/10

B2) Location of Event: Human studies facility of US EPA.

B3) Full Description of Event.

A 57 years old female study subject (OMC-028) with a history of migraine headache and sinus surgery three years ago had migraine remission after air pollution exposure on May 4, 2010. The subject was wearing a face mask during a 2 hours of air pollution exposure from 11 to 1 pm. She complained headache (rated 7 out of 10 in severity) after exposure. The subject thoght the face mask compressed on her nose and face (the sinus area) might cause the headache. Her blood pressure, heart rate, and oxygen saturatiion were normal. She vomitted once at 3 pm during a brachial artery ultrasound procedure in CTRC. She was taken back to the Human Studies Facility at EPA and her blood pressure, heart rate, and tempreture were normal. She was given a tablet of tylenol. The on-duty physician Dr. Ghio saw her and reassured her that perhaps this is a migraine episode. She rested in a dark room for about 30 minutes and felt much better. She was discharged at 4:30 pm

with Dr. Ghio's home phone number, pager number and Medical Station's telephone number in the event of any untoward symptoms.

>> Was this a serious adverse event?

C1) Did the event result in death? No

C2) Was the event life-threatening (i.e., placed the subject at immediate risk of death from the event, as it occurred)? No

C3) Did the event result in inpatient hospitalization or prolongation of existing hospitalization? No

C4) Did the event result in a persistent or significant disability/incapacity? No

C5) Did the event result in a congenital anomaly/birth defect? No

C6) Based upon appropriate medical judgment, did the event jeopardize the subject's health and/or require medial or surgical intervention to prevent one of the other outcomes listed above, e.g., allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse?
No

>> Protocol/Consent Forms

D1) Given this event's occurrence, are there revisions to the study or consent documents that you would like to submit at this time? No

>> Corrective Action

E1) Have you established a corrective action plan to prevent future occurrence of the event? Yes

Describe the corrective action plan:

Exclude the potential subject with a history of migraine headache.

>> Attachments

There are no attachments for this event

University of North Carolina - Chapel Hill

IRB Number:	07-0190	**Legacy ID:**	
PI:	Haiyan Tong	**IRB:**	Biomedical
Sponsor:	EPA Intramural Federal Research		
Study Title:	GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles		

Certified: 06/22/2010

Reference ID: 1653

>> Brief Description of Event

A study subject (OMC-032) had cardiac arrhythmia (ventricular tachycardia) after clean air exposure. The brief arrhythmia was noted on a 24 hr Holter monitor recording and the subject had no symptoms

A1) Did this event occur at a site for which a UNC-CH IRB has direct oversight responsibility or involve a research participant at one of those sites? Yes

A2) Was the event unexpected in nature, severity, or frequency? Yes

Please explain:

We do not expect the study subjects have any cardiac arrhythmia after controlled clean air exposure. The 24 hr Holter monitor recording showed that the subject had a 4-beats ventricular tachycardia about 6 hrs after clean air exposure which is the control exposure. The subject had no symptoms and she was not doing any strenuous activities. The subject was consoled and was given a copy of the ECG. She was advised to see her own cardiologist for further investigation of her arrhythmia.

A3) Do you think the event was related or possibly related to this research? No

Based on your response this event is not required to be reported to the IRB. According to federal guidance and University policy, only events that are unexpected and related to the research must be reported. You may still have obligations to report this event to the research sponsor, coordinating center, data safety monitoring board (DSMB), or other oversight committee.

>> Attachments

There are no attachments for this event

University of North Carolina - Chapel Hill

IRB Number:	07-0190	**Legacy ID:**	
PI:	Haiyan Tong	**IRB:**	Biomedical
Sponsor:	EPA Intramural Federal Research		
Study Title:	GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles		

Certified: 05/25/2010

Reference ID: 1523

>> Brief Description of Event

A study subject (OMC-029) had cardiac arrhythmia.

A1) Did this event occur at a site for which a UNC-CH IRB has direct oversight responsibility or involve a research participant at one of those sites? Yes

A2) Was the event unexpected in nature, severity, or frequency? Yes

Please explain:

The subject was exposed to filtered air and heart rate variability monitor showed that the subject had arrhythmia. We do not expect filtered air exposure causes heart arrhythmia. For this case, we think the subject has his own underline medical condition.

A3) Do you think the event was related or possibly related to this research? No

Based on your response this event is not required to be reported to the IRB. According to federal guidance and University policy, only events that are unexpected and related to the research must be reported. You may still have obligations to report this event to the research sponsor, coordinating center, data safety monitoring board (DSMB), or other oversight committee.

>> Attachments

There are no attachments for this event

University of North Carolina - Chapel Hill

IRB Number:	07-0190	**Legacy ID:**	
PI:	Haiyan Tong	**IRB:**	Biomedical
Sponsor:	EPA Intramural Federal Research		
Study Title:	GCRC-2579: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles		

Certified: 05/05/2010

Reference ID: 1476

>> Brief Description of Event

A female study subject (OMC-028) with a history of migraine headache had migraine remission after air pollution exposure on May 4, 2010. The symptom disappeared the next day.

A1) Did this event occur at a site for which a UNC-CH IRB has direct oversight responsibility or involve a research participant at one of those sites? Yes

A2) Was the event unexpected in nature, severity, or frequency? Yes

Please explain:

We did not expect air pollution exposure cause migraine headache.

A3) Do you think the event was related or possibly related to this research? Yes

Please explain:

The subject has a history of migraine headache and she had a sinus surgery three years ago. She said the face mask she was wearing during air pollution exposure compressed on her nose and face which might trigger the episode. She had headache and vomited once about 2 hours after exposure. She was given Tylenol and rested in a dark room and the symptom much improved and was discharged. She had no headache the next day.

A4) Does the event suggest that the research places subjects or others at a greater risk or harm than was previously known or recognized?

Economic: No
Legal: No
Physical: Yes
Psychological: No
Social: No

Please explain all "yes" or "don't know" responses

The migraine headache resulted in discomfort to the subject.

>> Information About the Event

B1) Date of Event: 05/04/10

B2) Location of Event: Human studies facility of US EPA.

B3) Full Description of Event:

A 67 years old female study subject (OMC-028) with a history of migraine headache and sinus surgery three years ago had migraine remission after air pollution exposure on May 4, 2010. The subject was wearing a face mask during a 2 hours of air pollution exposure from 11 to 1 pm. She complained headache (rated 7 out of 10 in severity) after exposure. The subject thought the face mask compressed on her nose and face (the sinus area) might cause the headache. Her blood pressure, heart rate, and oxygen saturation were normal. She vomited once at 3 pm during a brachial artery ultrasound procedure in CTRC. She was taken back to the Human Studies Facility at EPA and her blood pressure, heart rate, and temperature were normal. She was given a tablet of tylenol. The on-duty physician (Dr. Ohio) saw her and reassured her that perhaps this is a migraine episode. She rested in a dark room for about 30 minutes and felt much better. She was discharged at 4:30 pm.

with Dr. Ghio's home phone number, pager number and Medical Station's telephone number in the event of any untoward symptoms.

>> Was this a serious adverse event?

C1) Did the event result in death? No

C2) Was the event life-threatening (i.e., placed the subject at immediate risk of death from the event, as it occurred)? No

C3) Did the event result in inpatient hospitalization or prolongation of existing hospitalization? No

C4) Did the event result in a persistent or significant disability/incapacity? No

C5) Did the event result in a congenital anomaly/birth defect? No

C6) Based upon appropriate medical judgment, did the event jeopardize the subject's health and/or require medial or surgical intervention to prevent one of the other outcomes listed above, e.g., allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse?
No

>> Protocol/Consent Forms

D1) Given this event's occurrence, are there revisions to the study or consent documents that you would like to submit at this time? No

>> Corrective Action

E1) Have you established a corrective action plan to prevent future occurrence of the event? Yes

Describe the corrective action plan:

Exclude the potential subject with a history of migraine headache.

>> Attachments

There are no attachments for this event

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 23-Apr-2008

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Part A.1. Contact Information, Agreements, and Signatures

Date: January 4, 2011

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

UNC-CH PID: Pager:
Phone #: (919) 966-4993 Fax #: (919) 966-6271 Email Address: tong.haiyan@epa.gov

For trainee-led projects: __ undergraduate __ graduate __ postdoc __ resident __ other

Name of faculty advisor: James Samet, PhD
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0665 Fax #: (919) 966-0676
Email Address: Samet.james@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Robert Devlin, PhD; David Diaz-Sanchez, PhD; Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Howard Kehrl, MD; Tracey Montilla, RN; Dave Peden, MD, MS; Ana Rappold, PhD; Michael Schmitt, MS; James Scott, BS; Susan Steck, PhD, RD; and Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _x_ Federal __ State __ industry __ foundation __ UNC-CH

__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
x	1. This application. One copy must have original PI signatures.	3
☐	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
☐	3. HIPAA authorization addendum to consent form.	3
☐	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
☐	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
☐	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
☐	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
☐	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
☐	9. Data use agreements (may be required for use of existing data from third parties).	1
☐	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
☐	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator — Date: 1/4/2011

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor — Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee — Date

Print Name of Department Chair or designee — Department

Part A.2. Summary Checklist *Are the following involved?*

	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	x__
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	x__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_x_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_x_	__
b. Non-English-speaking?	__	_x_
c. Decisionally impaired?	__	_x_
d. Patients?	_x_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_x_
f. Pregnant women?	__	_x_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_x_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_x_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_x_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_x_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_x_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_x_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_x_
b. Approved drugs for "non-FDA-approved" conditions?	__	_x_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	_x_	__
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_x_
A.2.11. Fetal tissue?	__	_x_
A.2.12. Genetic studies on subjects' specimens?	_x_	__
A.2.13. Storage of subjects' specimens for future research? ***If yes***, *see instructions for* ***Consent for Stored Samples***.	_x_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? ***If yes***, *approval by the* ***UNC-CH Radiation Safety Committee*** *is required.*	__	_x_
A.2.15. Recombinant DNA or gene transfer to human subjects? ***If yes***, *approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*	__	_x_
A.2.16. Does this study involve UNC-CH cancer patients? ***If yes***, *submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_x_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? ***If yes***, *obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_x_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_x_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_x_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_x_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_x_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

Signature of Principal Investigator — Date: 1/4/2011

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

Signature of Faculty Advisor — Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. This proposal is to examine the health effects of fine and ultrafine ambient particulate matter (PM) exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.
Participants Thirty-two healthy 50-75 year-old male and female subjects will be involved in the study.
Procedures (methods): The subjects will be exposed to clean air for 2 hours on the first day then concentrated ambient fine and ultrafine PM for 2 hours on the second day after being randomly supplemented with four weeks of omega-3 fatty acid or olive oil.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been

associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that fine and ultrafine ambient PM (PM<2.5) exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of PM exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (14)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (15-17). Fish oil supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (18). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (19). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (20). Therefore, the role of GSTs in mediating the cardiovascular effects following acute PM exposure will also be studied in the protocol in GSTM1 null and GSTM1 positive healthy older adults.

The respiratory effects of PM have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to PM in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to PM in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.

7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
15. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
16. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
17. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
18. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
19. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
20. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal is approximately 32 subjects for the study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, congestive heart failure, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer (possible exception for history of nonmelanoma skin cancer), rheumatologic diseases, immunodeficiency state, significant chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- A history of migraine headache.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Because of reported cardioprotective effects of red wine, subjects will be required to avoid drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or have a greater than/equal to a 5 pack year smoking history.
- No exposure will be conducted within 2 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Unspecified illnesses, which in the judgment of the investigators might increase the risk associated with PM inhalation will be a basis for exclusion.
- Have an allergy to latex.
- History of skin allergy to tape or electrodes.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This will be a randomized, double-blinded study. Thirty-two study subjects will be randomly given fish oil (16 subjects) or olive oil (16 subjects) supplements for 4 weeks. The subjects will be given a container filled with 4 weeks supply of enteric-coated either 3g/day of fish oil or 3g/day of olive oil capsules. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 410 mg of eicosapentaenoic acid (EPA) and 274 mg of docosahexaenoic acid (DHA). Each 1000 mg of this olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. Each subject will be exposed to clean air for 2 hour on the first day then air containing fine and ultrafine PM (PM<2.5) for 2 hours on the second day in an exposure chamber after the supplementation. If the PM concentrations are too low on the scheduled exposure day, they may be rescheduled. (See the study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotyping consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 25, methods of recruiting) will not need genotyping. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 80 ml of blood collected. A portion of the blood will be used for genotyping of GSTM1 and another portion of the blood will be used for the cholesterol levels, phospholipid and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back to the medical station.

- ***GSTM1 genotyping***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene. The genotype information will be used for comparison of cardiovascular effects from PM exposure in subjects with GSTM1 positive or null.
- ***Food frequency questionnaire in the study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the University of South Carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil,

rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the initial screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent for a physical. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. The height of subject's mouth while seated in the exposure chamber will be measured so that the particle tubing height can be adjusted. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. The resting minute ventilation will be measured. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the study:*** Subjects will be randomly given an approximately 4 weeks supply of either fish oil or olive oil supplements. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record every other week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 4 weeks of supplementation period. We will give the subjects another 4 weeks supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the

patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the exposure, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (vital signs, HRV, endothelial cell function by brachial artery ultrasound (BAU), pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 48 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. The subjects will be instructed to avoid strenuous activities while wearing the Holter monitor.
- **Blood pressure and heart rate measurement** will be measured intermittently by a BP monitor. A blood pressure cuff and a monitor which is about the size of the Holter monitor will be placed and will remain in place until the subjects leave for the day. The subjects will be instructed to keep arm relaxed and still when the pressure cuff is inflated.
- ***Brachial artery ultrasound:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.
- ***Pulmonary function*** will be measured by spirometry.
- ***Blood sampling***: approximately 80 ml of blood sample will be collected.

- ***Symptoms questionnaire*** before exposure will be collected.

Exposure:

The subjects will be exposed to clean air on the first day then concentrated ambient fine and ultrafine PM on the second day after the dietary supplementation. The PM exposure day may be rescheduled if the PM concentrations are too low on the scheduled day.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. During the exposure, subject will be monitored continuously by visual camera monitor, continuous pulse oximetry, blood pressure every 15 minutes, and telemetry. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours.

Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber is 100 liters per minute minus about 50 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. Approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.
The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will be up to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. Particle mass data for two minute averages will be recorded on a computer. The particle mass of the air entering the chamber will be monitored continuously. If it is confirmed that particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, exposure will be terminated. The process for confirmation may take up to additional 10 minutes. The shutdown procedure involves venting a valve on the exposure chamber which markedly reduces the particle exposure and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 80 ml of blood samples will be collected.

Eighteen Hours Post-PM exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function (BAU) measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 80 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for two times of 24 hours duration using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Blood Pressure and Heart Rate will be measured intermittently on the exposure days and follow up day by a BP monitor. Systemic, diastolic, and mean blood pressure and heart rate data will be recorded.

Flow-Mediated Dilatation (brachial artery ultrasound) Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before and immediately after (within 1 hr after exposure ends) each exposure, and approximately 18 hrs after PM exposure, blood will be drawn by the standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following: omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In this study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to PM exposure and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of PM on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.
- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Blood pressure and heart rate measurement**: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.
- **Brachial artery ultrasound:** There are no known risks associated with imaging of the brachial artery. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache,

flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.

- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 3g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Particle exposure:** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will be up to 600 ug/m^3 and the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes, as determined by reading of 900 ug/m^3 by DataRAM. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is exceedingly small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

Confidentiality Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's

name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects can not be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test the hypothesis that PM causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to PM.

We will measure a number of endpoints (e.g., neutrophils, inflammation markers, and lung function). Several markers of the cardiovascular response to PM will also be measured (e.g., blood pressure, HRV, brachial arterial diameter, change in blood vasoactivators, and coagulation factors). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect 0.13 units of change in BAU diameter as observed in the pilot study and estimated that 15 subjects per group are needed to achieve type I error rate 0.05 at power of 80%. We used estimates of variance in BAU diameter (0.27) and correlation among the repeated measurements (0.97) from the OMEGA pilot study. We used the equation for comparing two groups (placebo and treatment) with a single explanatory variables (PM concentration).

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to PM exposure compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial diameter, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial diameter. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of PM on changes in: heart rate variability in both time and frequency domains, blood CBC, fibrinogen, D-dimer, and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _x_ Names
b. _x_ Telephone numbers
c. _x_ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. _x_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _x_ Electronic mail addresses
g. __ Social security numbers
h. _x_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data**. Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _x_ no

A.4.11. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

__x No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data:

_x_ Secure network __ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_x Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no
Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the study will take approximately 12 months. Participant recruitment is expected to be continuous throughout the study until the intended number of participants is reached.

The individual will have 6 visits to the research facility over approximately 6-7 weeks. The pre-enrollment and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after the blood work and genotyping. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment will require approximately 3 hours. Each exposure day will last approximately 8 hours. Eighteen hours after the PM exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study. If the subject is qualified and finished the entire study, the total compensation for completion of this study will be approximate $1668.

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~80ml)	$30

Pre-study qualification total = $60

Training day (3 hours)

Time (3h @$12/h)	$36
FFQ	$25

Training day total = $61

Exposures (2 x 8 hours)

Venipuncture (~80ml, pre, 2@$30 each)	$60
Holter monitor(2@$100 each)	$200
Chamber exposure (2 x 2 hours, 1@$72 each)	$144
Venipuncture (~80ml, post, 2@$30 each)	$60
Time (16h @$12/h)	$192
Blood pressure monitor	$200
Brachial artery ultrasound (4@$50 each)	$200
Food records (2 times)	$150
Dietary supplementation completion bonus	$50
On-time bonus (2@$25 each)	$50

Total for completion of 2 exposures =$1306

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Brachial artery ultrasound	$50
Blood pressure monitor	$25

Total for completion of post-exposure = $141
Protocol Completion Bonus $100

Approximate TOTAL for completion of study = $1668

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

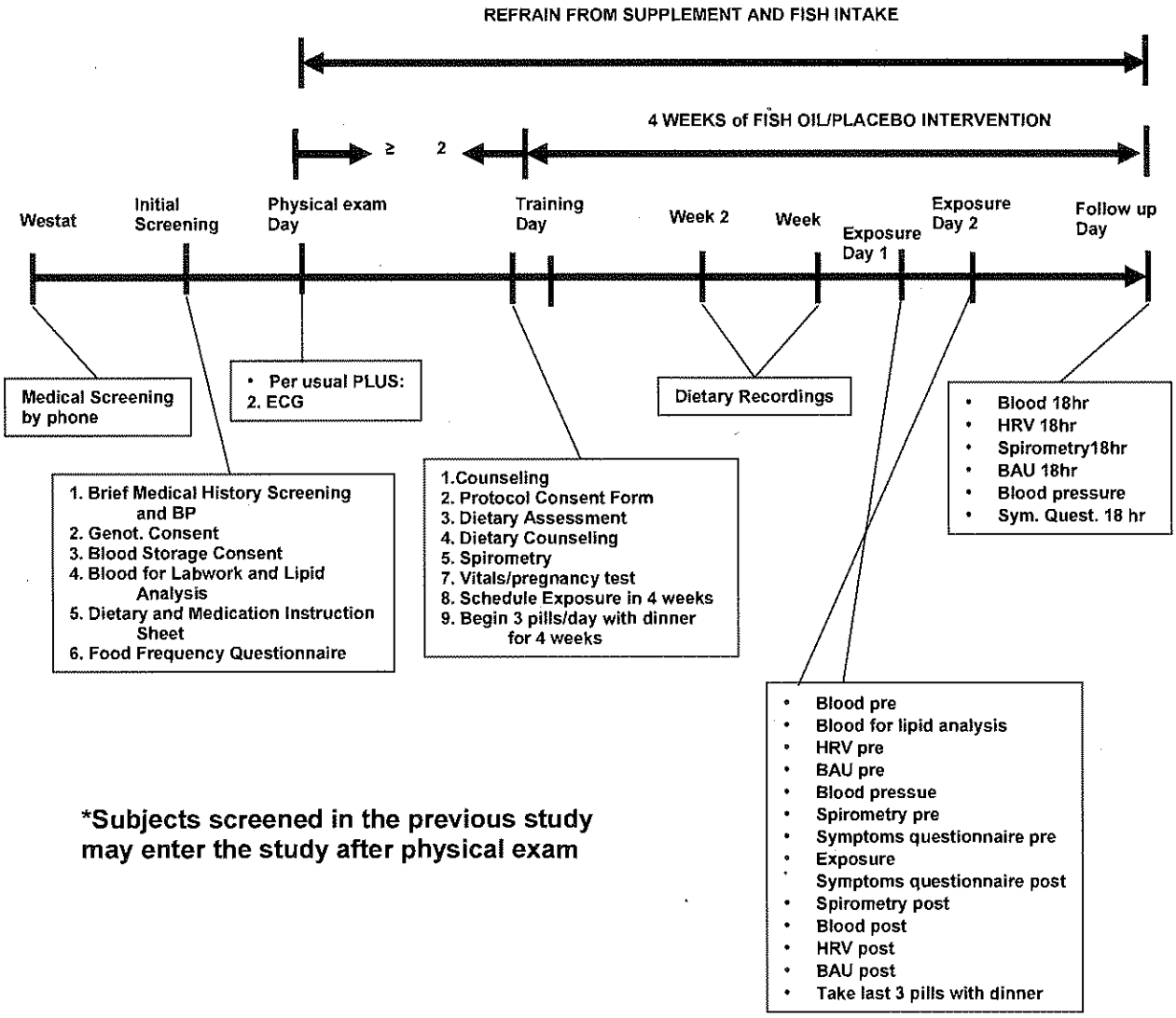
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	**NONE**	**TRACE**	**MILD**	**MODERATE**	**SEVERE**
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



STUDY FLOW DIAGRAM

CITI Course in The Protection of Human Research Subjects

Friday, April 29, 2005

CITI Course Completion Record
for Wayne Cascio

To whom it may concern:

On *4/29/2005*, *Wayne Cascio* (username=casciow; Employee Number=) completed all *CITI Program* requirements for the *Basic CITI* Course in The Protection of Human Research Subjects.

Learner Institution: *East Carolina University*

Learner Group: *Group 1*,

Learner Group Description: *All Biomedical Investigators and Key Personnel*

Contact Information:

Gender: Male
Department: Medicine
Which course do you plan to take?: Biomedical Investigator Course Only
Role in human subjects research: Clinical Reseacher
May we re-contact you to complete a course survey?: Yes
Mailing Address:
Brody School of Medicine at ECU
PCMH 378-TA
Greenville
NC
27834
USA
Email: casciow@mail.ecu.edu
Office Phone: 252-744-0083
Home Phone: 252-3212-0847

The Required Modules for *Group 1*. are:	**Date completed**
Introduction	04/29/05
History and Ethical Principles	04/29/05
Basic Institutional Review Board (IRB) Regulations and Review Process	04/29/05

Informed Consent	04/29/05
Social and Behavioral Research for Biomedical Researchers	04/29/05
Records-Based Research	04/29/05
Genetic Research in Human Populations	04/29/05
Research With Protected Populations - Vulnerable Subjects: An Overview	04/29/05
Vulnerable Subjects- Research With Prisoners	04/29/05
Vulnerable Subjects- Research Involving Minors	04/29/05
Vulnerable Subjects- Research Involving Pregnant Women and Fetuses in Utero	04/29/05
Group Harms:Research With Culturally or Medically Vulnerable Groups	04/29/05
FDA-Regulated Research	04/29/05
HIPAA and Human Subjects Research	04/29/05
Workers as Research Subjects-A Vulnerable Population	04/29/05
Hot Topics	04/29/05
Conflicts of Interest in Research Involving Human Subjects	04/29/05

Additional optional modules completed:	**Date completed**

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI participating institution. Falsified information and unauthorized use of the CITI course site is unethical, and may be considered scientific misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Course Coordinator

02/07/2008 14:45 FAX 919 966 6271 US EPA CRB 003

CITI Course in The Protection of Human Research Subjects

Monday, November 20, 2006

CITI Course Completion Record # 225240
for Fernando Holguin

To whom it may concern:

On *3/17/2006*, *Fernando Holguin* (username=fholgui; Emory ID Number=0014081Employee Number=0014081) completed all *CITI Program* requirements for the *Basic CITI* Course in The Protection of Human Research Subjects.

Learner Institution: *Emory University*

Learner Group: *Group 1. Biomedical Focus*

Learner Group Description: *This course is suitable for investigators and staff conducting BIOMEDICAL RESEARCH with human subjects. The IRB can assist you in determining whether these additional modules are necessary.*

Contact Information:

Gender: Male
Department: Medicine
Which course do you plan to take?: Biomedical Investigator Course Only
Role in human subjects research: Principal Investigator
Mailing Address:
NCEH
1600 Clifton RD NE MS E-17
Atlanta
GA
30307
USA
Email: fch5@cdc.gov
Office Phone: 404-498-1027
Home Phone: 404-377-5672

The Required Modules for *Group 1. Biomedical Focus* are:	Date completed
Introduction	01/27/06
History and Ethical Principles	03/17/06

02/07/2008 14:45 FAX 919 966 6271 US EPA CRB 004

Basic Institutional Review Board (IRB) Regulations and Review Process	03/17/06
Informed Consent	03/17/06
Research With Protected Populations - Vulnerable Subjects: An Overview	03/17/06
FDA-Regulated Research	03/17/06
HIPAA and Human Subjects Research	03/17/06
Conflicts of Interest in Research Involving Human Subjects	03/17/06
Emory University	03/17/06

Additional optional modules completed:	**Date completed**

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI participating institution. Falsified information and unauthorized use of the CITI course site is unethical, and may be considered scientific misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Course Coordinator

02/07/2008 14:45 FAX 919 966 6271 US EPA CRB 005

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
Office of the Vice Chancellor for Research and Economic Development

Campus Box 4100, 301 Bryan Hall
Chapel Hill, NC 27599-4100
(919) 962-7757 FAX: (919) 962-6769
http://research.unc.edu

February 7, 2008

To Whom It May Concern:

In accordance with the National Institutes of Health (NIH) notices OD-00-039 of August 25, 2000 and OD-01-061 of September 5, 2001, The University of North Carolina at Chapel Hill submits the following information concerning the education in the protection of human research participants undertaken by the key personnel involved with this project. The following persons have been trained in the ethical and regulatory requirements for protection of human research participants in compliance with NIH requirements and The University of North Carolina at Chapel Hill "Policy on Education and Certification of Investigators Involved in Human Subjects Research." http://ohre.unc.edu/irbtraining/

Name	Dept	Training
Haiyan Tong		CITI- Biomedical
Michael Schmitt	Inactive	CITI- Biomedical
Michael Schmitt	Inactive	CITI- Data only
Lynne Newlin	Enviro Med Asthma & Lung Bio	CITI- Biomedical
Tracey Montilla		CITI- Biomedical
Debra Levin		CITI- Biomedical
Margaret Herbst	Pediatrics	CITI- Biomedical
David DeMarini		CITI- Data only
Martin Case		CITI- Biomedical
Martha Carraway		CITI- Biomedical
Maryann Bassett		CITI- Biomedical

Should any additional persons come to work on this project and meet the definition of key personnel, they will be trained similarly; their names and certification of their training in the protection of human research participants will be forwarded.

Sincerely,

James E. Peterson

James E. Peterson, Ph.D.
Associate Vice Chancellor and Director,
Office of Sponsored Research