(CAPs; ~147 μg/m^3) and O3 (~0.12 ppm) simultaneously compared to clean air responses [39]. However, no separate exposure to O3 alone, or CAPs alone, were performed.

The purpose of this study is, first, to examine whether DE can alter lung and cardiovascular responses to O3 exposure when given a day before or during O3 exposure; second, to investigate if co-exposure of DE and O3 on day 1 augment the lung function decrements following a subsequent O3 exposure (day 2); third, to investigate if two consecutive days of O3 affect individual cardiovascular responses such as changes in heart rate variability (HRV) and blood pressure (BP), to ozone in young healthy adults. We will measure spirometric lung function in association with 2-hour exposures to O3. Results will be analyzed for effects of each or combinations of the above on changes in lung function. We hypothesize that an exposure to DE with O3 (day 1) or DE exposure (day 1) given prior to O3 exposure (day2) will not induce a significant decrement in lung function in healthy young adults as a group relative to O3 alone. We further hypothesize that an O3 exposure (on day 2) after the DE and O3 co-exposure (day 1) will cause significant cardiopulmonary responses in healthy young adults. Finally, we hypothesize that two consecutive days of O3 exposure will affect cardiovascular responses.

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. Researchers are reminded that additional approvals may be needed from relevant "gatekeepers" to access subjects (e.g., school principals, facility directors, hospital or healthcare system administrators).

A group of 15 healthy adults (men and women) between the ages of 18 and 55 years will participate and complete the study. However, because of potential dropouts and early terminations we may recruit as many as needed to have 15 individuals complete all 4 exposure regimens. Enrollment in this study will not be restricted to any specific races and ethnicities. All subjects will be non-smokers for at least a year, with no active cardiac or respiratory disease, or active medical problems of any kind. Pregnant women, those trying to become pregnant, and those breast-feeding, will not be accepted. All potential subjects will undergo screening procedures (previously approved 95-EPA-66 Phase I and II), including a medical history form, physical examination and routine chemical and hematologic screens. All subjects are required to be moderately active so that they could sustain a prolonged period of moderate exercise. All subjects will be recruited without regard to their genetic profiles; however, they will be genotyped and classified for presence or absence of polymorphisms of select genes.

Deleted: 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.¶

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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:

1. Healthy men and women between 18 and 55 years of age
2. Physical conditioning allowing intermittent, moderate exercise for 2.0 hours
3. Normal lung function:
   a. FVC > 75 % of that predicted for gender, ethnicity, age and height.
   b. FEV_1 > 75 % of that predicted for gender, ethnicity, age and height.
   c. FEV_1/FVC ratio > 75 % of predicted values.
4. Oxygen saturation > 96 %.

Exclusion Criteria:

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1. A history of acute and chronic cardiovascular disease, chronic respiratory disease, diabetes, rheumatologic diseases, immunodeficiency state, and acute respiratory illness within 4 weeks.
2. Subjects who are asthmatic or have a history of asthma.
3. Allergic to chemical vapors or gases.
4. Any allergic symptoms during the time of participation in the study
5. Female subjects who are currently pregnant, attempting to become pregnant, or breastfeeding
6. Subjects unwilling or unable to stop taking vitamin C or E or medications which may impact the results of the ozone challenge (such as, systemic steroids and beta blockers) at least 2 weeks prior to the study and for the duration of the study. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
7. Current and past smokers within 1 year.
8. Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
9. Subjects who do not understand or speak English
10. Subjects unable to perform the moderately active exercise required for the study.
11. Subjects with a history of skin allergies to adhesives used in securing heart rate monitor electrodes.
12. Unspecified diseases or conditions, which in the judgment of the investigator might influence the responses to the exposures, will be a basis for exclusion.
13. Subjects unwilling to stop taking over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications ("NSAIDS") for 48 hr prior to the exposures and post-exposure visits.

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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.

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This is a randomized crossover single blind study with 4 arms separated by at least 13 days. In this study we will measure cardiopulmonary responses to O3 (approximately mean concentration of 0.3 ppm over the 2 hr exposure period) and diesel exhaust (DE; ~300 $\mu g/m^3$) combined exposure and singularly, and clean air control in healthy adults undergoing moderate intermittent exercise (minute ventilation = ~ 25 liters/min/m^2 BSA). [See figure 1].

Each subject will be exposed randomly to all 4 exposure regimes separated by at least 13 days. For all regimes, the subject will be exposed to the pollutant or clean air for 2 hrs with moderate exercise during the exposures. Moderate exercise will involve riding a stationary bike in the chamber for 15 min intervals beginning after the first 15 min of exposure and resting for 15 min after each time for a total of 1 hr of moderate exercise per chamber exposure. Ventilation rate measurements will be taken at ~ 6 min into each exercise session to ensure ventilation rates are within the appropriate parameters [target ~25 L/min/ m2 BSA, which in most subjects is ~ 50 L/min.]. For the DE and O3 co-exposure, there is a potential for the O3 to interact with the DE, but the O3 concentration will be maintained at a final mean concentration of about 0.3 ppm.

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 times will be 2 hours. The exposure atmosphere will be at approximately 40 ±

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10% RH and approximately 22 ± 2 °C. Clean air will be passed through an air purification system to ensure no presence of pollutant gases (O_3, NO, NO_2, SO_2, CO) in the air. Ozone and DE concentrations will be monitored continuously. The DE will be generated from a diesel engine used to power an air compressor that is located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean HEPA and charcoal filtered and humidified air to give a chamber concentration of approximately 300 $\mu g/m^3$. The monitoring analyzer registers a concentration in real time. Exposures will be terminated at values $\geq$ 400 $\mu g/m^3$ during runs in which the exposure targets 300 $\mu g/m^3$ DE. This safety limit will prevent an inhalational exposure greater than 1,080 µg, which assumes that the subject, only resting for 1 hr, exercising for 1 hr, will inhale 2.7 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 approximately 0.05-0.2 µm. Preliminary testing has shown the mean particle size to be approximately 0.15 ± 0.05 µm. Levels of carbon monoxide (CO), and oxides of nitrogen (NOx; mainly NO, and some NO_2), will be kept under 10 ppm CO, 15 ppm NO, and 3.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average for these substances are: CO=50 ppm; NO=25 ppm; NO_2=5 ppm; Diesel fuel used for the study will be purchased as a commercial ultra low sulfur fuel. 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. Particle mass will be measured in real time and validated further by weighing filters after the 2 hr exposure is finished. Particle size distribution will be determined at regular intervals. Filter samples will also be analyzed for chemical composition of particles. Minute ventilation will be measured during the exercise periods of exposure.

Lung Function Test:

Primary endpoints for evaluating health effects are changes in lung functions, mainly FEV_1, FVC and FEV_1/FVC before, immediately after, and approximately every hour for up to 4 hrs post exposure.

Measurements and Collections:

Blood pressure, telemetry, and oxygen saturation may be monitored during the exposure period. Another primary endpoint for evaluating health effects, in addition to lung function, is heart rate variability (HRV). Secondary endpoints may include markers of inflammation recovered in blood and exhaled breath including total and differential cell counts as well as pro-inflammatory cytokines and immune system mediators and clotting factors. Additionally, blood, breath, and urine samples will serve as indicators of the exposure based on the levels of metals (eg, Zn) and organics (eg, PAHs) and other components that may be derived from DE deposition. From the collected blood samples, additional parameters and indices may be obtained as analysis tools become available. Genetic analysis may also be performed on collected blood samples for susceptibility factors. A questionnaire measuring perceived stress by the subject at the beginning of an exposure regimen will be performed. Some of the supplemental spirometric measurements and secondary endpoints may not be obtained if they are deemed unnecessary.

In order to participate in this study, subjects will be asked 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.
- Avoid the use of ozone-based home air purifiers during study participation.
- On the exposure days, eat a light breakfast.
- Refrain from all over the counter anti-inflammatory agents including those for allergies for a period of 48 hrs prior to exposure.

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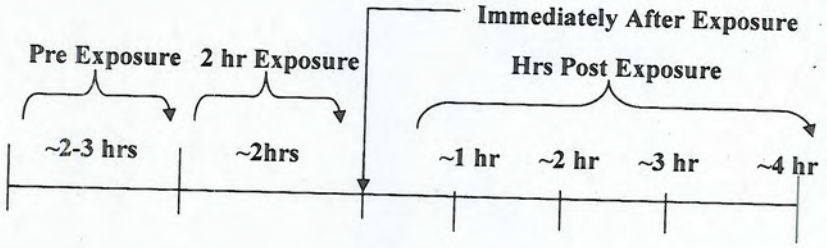
Figure 1

Experimental design:

	Day 1 2 hr exposure	Day 2 2hr exposure	Day 3
Exposure Regime 1	DE + O3	O3	follow up (no exposure)
Exposure Regime 2	O3	O3	follow up (no exposure)
Exposure Regime 3	DE	O3	follow up (no exposure)
Exposure Regime 4	Clean Air	O3	follow up (no exposure)

Figure 2
Endpoints:

Day 1



Measurements & Collections	*	*				
Lung Function	*	*	*	*	*	*

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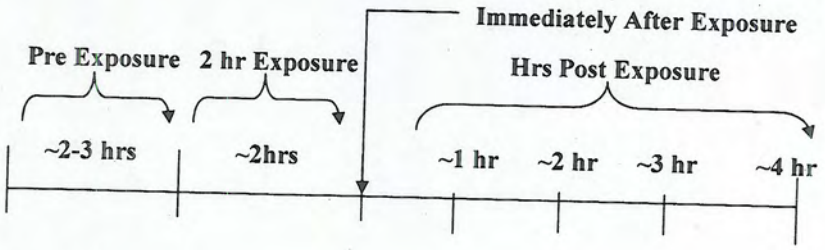
Day 2



Measurements & Collections	*	*				
Lung Function	*	*	*	*	*	*

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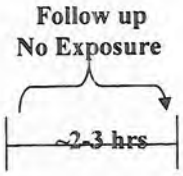
Day 3



Measurements & Collections *

Lung Function *

Protocol: Written consent will be obtained. The subjects will be trained (on D0) in techniques for obtaining lung function measurements. Subjects also will exercise on a bicycle, and appropriate settings (the speed and grade of incline) required to produce a desired value of minute ventilation will be determined. On the first day of exposure, subjects will be assessed for vital signs (blood pressure, pulse, temperature and respiratory rate) at the check-in. Subjects may perform pre-exposure baseline lung functions in clean air, have resting heart rate variability (HRV) assessed, and urine, blood, and exhaled breath collected for later analyses of soluble mediators such as clotting factors, inflammatory proteins, and lipids and blood cell types and numbers. These samples may also serve to determine biomarkers of exposure, such as the concentration of PAHs . A questionnaire measuring perceived stress by the subject at the beginning of an exposure regimen will be filled out; this is included as Attachment #1. [Information about grading the questionnaire (Sheldon Cohen Perceived Stress Scale) is described at www.mindgarden.com].The subject then will enter the chamber already set for the appropriate conditions (DE & O3, DE alone, O3 alone, or clean air), and after 15 min begin exercising on a stationary bicycle. Each exposure session will consist of a 4 periods of 15 rest/15 min exercise at a level of approximately 25 liters/min/m^2 body surface area (BSA) in minute ventilation followed. Measurements of lung function may be obtained immediately after, and every hour for up to 4 hr post exposure. Resting HRV may be recorded, and blood, urine, and breath collected immediately post exposure. Following the day 1 exposure, the subject may remain at the Medical Station for follow up measurements of lung function for approximately 2-4 hr. The subjects will go home with a heart rate monitor and blood pressure cuff. On Day 2, the subject will report to the medical station in the morning. The same measurements (resting HRV, lung function; symptoms; venipuncture performed) as Day 1 may be done pre- and post- exposure to O3. Subjects will return on Day 3 for follow up measurements but with no exposures.

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Experimental procedures:
Medical history will be collected, including current medications and any recent illness.

Vital signs will be measured to include height, weight, heart rate, respiratory rate, blood pressure, oxygen saturation, and temperature. In addition a pregnancy test will be given to all women before each exposure regime.

Spirometry may be performed on a dry seal digital spirometer interfaced to a computer; volumes are corrected to body temperature and pressure (BTPS). From the forced expiratory maneuver, forced vital capacity (FVC), forced expired volume in the first second (FEV_1), and FEV_1/FVC.

Exhaled Breath Collection: The participant will be asked to breathe into a cooled tube for up to 15 minutes. The breath condensate may be collected and analyzed for markers of airway inflammation and immune system alterations and exposure markers. Additionally a small Tedlar bag of uncooled, exhaled breath will be collected (up to 5 min) to examine gas phase exposure and inflammation/immune system constituents.

For exercise measurements the subjects may breathe for up to 3 min through a pneumotachograph while performing exercise and ventilation parameters including the minute ventilation, tidal volume and breathing frequency will be obtained. Heart rate will be monitored throughout exposure using a telemetry monitor.

Cardiac measurements: Heart Rate Variability monitoring (ambulatory ECG) monitoring: On the day 1 and day 2 of each of the exposure regimes we will mount a Holter monitor to the participants. The skin may be shaved (men only) and prepped. Ten ECG electrodes may be attached and connected to the beeper-sized Holter monitor, which participants may wear around their waist. After mounting the monitor onto participants, they will be asked to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. Participants may be released while wearing the Holter monitor and return to the HSF the next morning (day 2 of the study segment) to obtain the Holter monitor information and change the batteries, if necessary. The monitor will be worn for day 2 and removed on day 3. This procedure may be conducted for all 4 exposure regimes. We will analyze time and frequency as well as re-polarization parameters. Participants will be given instructions for the removal of the monitor in case it becomes necessary.

Blood pressure (BP) measurements: BP will be monitored during the study. A BP cuff may be worn to measure BP intermittently. 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. The subjects may wear the cuff for day 1 and 2 of the study session and have it removed on day 3. Participants will be given instructions for the removal of the monitor in case it becomes necessary.

Venipuncture: Up to approximately 500 ml of blood may be collected for the 8 exposure days over a minimum of 8 weeks. This translates to approximately 50 ml of blood for each exposure day and 25 ml on the follow up day. A portion of the sample will be used for genotyping to examine sensitivity in selected blood types. Blood will be analyzed for, but not limited to, clotting/coagulation and inflammation factors and biomarkers of immune response.
A portion of the peripheral blood sample will be used for genetic testing, to identify potential polymorphisms that may make subjects more susceptible to air pollutants. In addition, samples may be stored for as yet undesignated research. Unwillingness to have samples used for genotyping or stored will NOT exclude a subject from the panel study. Consent for genotyping will be included with the general consent form.

Urine: The entire volume of a urine void will be collected on the pre- and post- exposure on Days 1 and 2, and the morning of day 3. Samples may be analyzed for markers of cigarette smoke exposure (eg, cotinine), creatinine for value normalization, exposure markers (eg, PAHs), and mediators of effect related to inflammation and immune system functions (eg, peptidoleukotrienes, other lipids).

Likely Personnel assignment

Subject recruitment and payment	Westat Corporation (Protocol #950518)
Chamber setup and maintenance	TRC Environmental Corporation
Subject surveillance during exposure	Martin Case, BS, Mike Madden, PhD, Tina Stevens, PhD, Howard Kehrl, MD, David Diaz-Sanchez PhD, Medical Station personnel

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Lung function testing	Martin Case, BS, Mike Madden PhD, Tina Stevens, PhD Howard Kehrl, MD
Blood sampling	Human Studies Facility Medical Station personnel
Subject screening tests	Human Studies Facility Medical Station personnel
Genomic assay	Michael Schmitt, MS

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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.

The results of this study will contribute to the overall assessment of air pollution effects in the U.S. and thereby influence health policy. The subjects will not benefit personally from being in this research study other than by undergoing a free limited physical examination and screening.

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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.

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

1. Blood sampling will be performed by well-trained personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise. Subjects are closely monitored for any signs of faintness, and only allowed to leave the facility after a 15-min waiting period.
2. Breathing tests (spirometry): There pulmonary function tests are standard clinical tests that are commonly performed in hospitals and entail little or no risk to the subjects. However, cough or dizziness may occur during these tests. If these symptoms occur, they are usually temporary. Subjects will remain seated in a chair until symptoms disappear. There is an extremely small chance that the subject could have a bronchospasm or faint upon performing a forced breath expiratory maneuver for spirometry.
3. Exhaled breath condensate and exhaled breath gas: Minimal risk is associated with these procedures. The subject is seated in a chair during these collections and the technician is always available during this procedure in case the participant becomes light-headed due to hyperventilation.
4. Moderate exercise on a stationary bicycle entails the potential, although minimal, risk of occasional muscle soreness, cramps or general fatigue. These discomforts are temporary and not harmful. Heart rate and rhythm will be monitored continuously. Heart rate will be kept below age related maximum (i.e., 220 bpm – age in years). Exercise will be terminated at any

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time upon the request of the subject or if the investigator/medical staff observes problems while monitoring the ECG readout.

5. Ozone exposure: Potential risks may include mild airway obstruction, irritation to the nose, eyes, throat and airways, pain on deep inspiration and cough. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. Ozone may induce an inflammatory reaction that may last for about 24-48 hours after exposure and may increase the chance of catching a cold.
6. 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.
7. Blood pressure and heart rate measurement: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.
8. Diesel exhaust (DE) exposure: The amount of DE (target of 300 $\mu g/m^3$) used in this study would be equivalent to concentrations of DE particulate matter that would be encountered at busy intersections in large urban areas and in occupational exposures. Occupational levels for some truck drivers are generally about 100-300 $\mu g/m^3$ and average ~900 $\mu g/m^3$ for some mines where diesel powered machinery are used. A recent study demonstrated diesel exhaust particulate matter concentrations during drive-by incidents averaged about 125 and199 µg/m3 at the height of an adult pedestrian and a child in a stroller, respectively. Using a 2006 diesel engine, (generally recognized as emitting less PM mass than most older models currently on the road), it was demonstrated that an average DEP concentration up to 364 ug/m^3 (over ~9 sec) could be generated at near roadside monitoring stations at head level during drive by simulations with a peak concentration of 860 ug/m^3. [40].

Controlled, acute DE exposure studies in Sweden, using about 300 $\mu g/m^3$ of 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. DE particles contain some probable carcinogenic polycyclic aromatic hydrocarbons and other components, 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 time and concentrations to be used in this protocol are minimal relative to the durations required to induce lung cancer. Overall, there are no known long-term health risks in healthy individuals acutely exposed to DE at the PM concentrations given in this study. Short-term health effects include slight alterations in blood clotting, oxygen diffusion capacity and changes in heart rate variability. These symptoms typically disappear within 24 hrs of exposure. 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. No major adverse effects were observed in a completed diesel exhaust study of healthy adults (ages 18-40; entitled "Physiological, Cellular, and Biochemical Effects of Diesel Exhaust in Healthy Young Adults"; Mike Madden, PI; UNC IRB # # 99-0842) where the PM concentration averaged 106 ug/m3. No major adverse health effects were observed in a completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA

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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 and the subject withdrawn from the chamber if the O_2 saturation value is $\leq$92%. 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 and rhythm, 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.

9. 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 a locked cabinet in a locked room at the US EPA facility. Any abnormal medical findings (CBC, ECG, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician. 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.

In addition, there may be uncommon or previously unknown risks that might occur. During all testing, subjects will be monitored continuously by the EPA personnel. A duty physician will be available in response to an emergency. A group of physicians employed by EPA and UNC Center for Environmental Medicine, Asthma & Lung Biology have primary responsibility for medical coverage of studies conducted in the EPA Human Exposure Facility. This facility 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 for any challenge or exposure study.

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Deleted: The data storage plan ultimately proposed by the PI is reviewed by the US EPA Quality Assurance officer assigned to the project. The data storage plan is reviewed for storage safety, confidentiality, backup, longevity, validity, integrity, and other issues. We believe that the data storage plan proposed is commensurate with the study size and complexity (eg, not a multisite study).

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A.4.8. **Data monitoring and 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). Describe the provisions for monitoring the data to ensure the safety of participants. These plans could range from the investigator monitoring subject data for any safety concerns to a sponsor-based DSMB, depending on the study.

The research objective of this study is to determine if healthy volunteers safely exposed to low levels of O3 and DE alone or in combination, will show changes in cardiopulmonary endpoints including modest decrements in lung function. The study will follow a randomized, repeated measures design with each subject exposed to 4 different exposure regimens (see Figures 1 and 2 above). The data analysis will be focused on changes in lung function measurements, primarily FVC and FEV_1, between pre and post 2hr exposure for each exposure, but also blood endpoints include inflammatory factors (eg IL-6), blood clotting factors (eg fibrinogen), and susceptibility factors (eg specific genotypes such as glutathione-S-transferase M1 (GSTM1) null).

Deleted: A.4.8. **Data monitoring and 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). Describe the provisions for monitoring the data to ensure the safety of participants. These plans could range from the investigator monitoring subject data for any safety concerns to a sponsor-based DSMB, depending on the study. ¶

In evaluating exposure effects of O3 and DE, alone or in combination, upon lung function, the difference between the pre-exposure and post-exposure FEV1 will be calculated and the pre-post differences for the air and the exposures will be compared utilizing repeated measures ANOVA parametric test. Clinically significant change is determined to be a 10% decrease in FEV1 measured prior to and immediately after the exposure compared to the pre exposure value. For a conservative sample size calculation, we used one sided test for a single sample under the assumption of normal distribution with Type I error of 5% and Type II error of 20%. Therefore, a ρ value of 0.05 or less will be considered significant and the proposed sample size will provide adequate (80%) power for detecting a 10% decrease in ΔFEV1.

Sample size calculation was based on the Forced Expiratory Volume in 1 Second (FEV1) in response to the O3 exposure. Data for the power calculation were obtained from results of FEV_1 [41] for a constant acute exposure to 0.3 ppm O_3 versus clean air (CA). In 22 subjects, the pre exposure FEV_1 was 4.608 ± 0.514 (mean ± SEM) in CA exposure and 4.656 ± 0.592 in O_3. The post exposure FEV_1 was 4.64 ± 0.476 in CA exposure and 3.856± 0.687 in O_3. We used 0.687 as an estimate of standard deviation after the ozone exposure and estimated 10% decrease in pre-exposure FEV1 levels to be 0.46 ml. Based on these data and the test for the mean of a normal distribution with a one-sided alternative the required sample size is 14 subjects. We will recruit 15 subjects for this study to ensure the statistical power that can analyze small differences expected from low concentration exposures.

Although EPA does not have an official Data Safety Monitoring Board, we do have measures in place to insure the safety of our subjects. Adverse events will be reported to Bob Truckner, the EPA/NHEERL human research protection officer of the IRB.

Precautions taken to minimize risk to the subject include:

1. subject monitoring via direct observation or CCTV during exposure
2. Cardiac function will be assessed throughout exposures and development of excess dysrthymia, tachycardia, or bradycardia will prompt reevaluation of the study design (eg, pollutant concentrations, exposure duration, exercise levels, etc).
3. Lung function decrements below 30%, as measured by FEV1 or FVC, will also prompt reevaluation of the study design.
4. Pulse oximetry will be performed during the exposures and the subject withdrawn from the chamber if the oxygen saturation value is ≤ 92%.
5. 24 hr Holter monitor data show excess arrthymias, tachycardia, and/or bradycardia will prompt reevaluation of the study design.
6. Subjects will be aware of their right to terminate their participation in the study without prejudice.
7. Any exposure will be stopped upon evidence of any adverse effects suffered by the subject during the exposure

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8. Full resuscitation equipment will be available at all times during exposures and used if needed
9. In the event of an emergency, after initial medical assessment, patients will be transported to the UNC Hospitals Emergency Room for continued treatment
10. A formal pause to the study will be performed by the medical station staff after 4 subjects complete the 4 exposure regimes to assess whether highly adverse effects occurred with respect to lung and cardiac physiology responses. If no highly adverse effects are observed, the study will continue.

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A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No _x_ 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. **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).

Deleted: 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).¶

All individuals who have been granted access to the data to perform their research-related duties have received full ethics training. Computer data files are password protected and subjects are coded as an unrelated subject identifying number. The participant's actual identity cannot be ascertained exclusively from these data. The participant's name will not be used in any publications. Access to the records of this study will be provided by the identifying number only and given only to those individuals associated with the study who require access to the data to perform their duties. All such individuals will be bound by this agreement of confidentiality. All records are maintained in a locked room in the medical records office of the USEPA Human Studies Facility.

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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.**
Describe who will be obtaining consent (or permission) and from whom. Include discussion, as relevant, any waiting period between the initial consent discussion and obtaining consent, and steps that will be taken to minimize coercion or undue influence. 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. It is expected that the information in the consent document(s) will be communicated to participants or their LAR. *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.*

Prior to participation, all volunteers will be required to read and sign the consent form which asserts that they have read and understood the following: 1.) Subject participation is strictly voluntary; 2.) The purpose of the study; 3.) The nature and extent of subject participation; 4.) The subject's rights to withdraw at any time; 5.) The subject's right to privacy, 6.) The risks associated with participation; 7.) The method and schedule of compensation; and 8.) The limits of the EPA, University and PI's liability. The PI, co-PIs or study coordinator will briefly describe the study and answer any questions that each

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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.*

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subject might have about the study. Subjects will have the opportunity to ask questions at any time during the study by contacting the PI and/or the study coordinators. 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. *Choose 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). *Participants should be asked whether they want documentation linking them with the research and the participants' wishes will govern whether they sign the form.* Note: This justification cannot be used in FDA-regulated research. ___ yes ___ no ___ 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).

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 ___ yes ___ no

subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)*
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 may 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 to that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, "Recruitment and Screening of Potential Subjects for EPA Studies" (95-0518; Howard Kehrl, PI). Subjects may be identified from mass emailing for recruits, website [https://www.epastudies.org/ with contact phone numbers listed as 919-966-0604 and toll free 888-279-9353] and newspaper and brochure advertising by Westat Corporation [a recent, advertisement brochure for subject recruitment is included as Attachment #2], and from the Westat database for subjects. These documents (email recruitment wording to current subjects from other studies and potentially new subjects , newspaper advertisement, a study description handout, study reminder notices to subjects, are attached in the appendix section of this application as Attachments 2-7. Every effort will be made to recruit women and members of racial minority groups into 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 and complete Section C.

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?

Deleted: 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:

Deleted: 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).¶
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).¶
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? ... [3]

Deleted: 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 t ... [4]

Deleted: 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.¶

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.

This study will take approximately 18 months (actual study time) to complete. The study duration is based on 1-2 exposure days per week and 1 subject on each exposure day. This scheduling, however, is subject to change depending on the availability of study subjects and the number of concurrent studies requiring the same chamber facility.

All subjects will have at least 13 visits to the research facility over approximately 3 months. On the first visit, the subject will go through a consent process and then a training session for approximately 3 hours. During each of three subsequent visits (exposure days), subjects are required to check in by about 8:00 am and will be discharged approximately at 4:00 pm. Therefore, subjects will remain in the research facility for approximately 8 hours on exposure day 1. The following day, subjects are required to check in by 8:00 am and will be discharged approximately at 4:00 pm. Therefore, subjects will remain in the research facility for approximately 8 hours on exposure day 2. The 3rd day the subjects are required to check in by 8:00 am and will be discharged approximately at 11:00 am, for a total of approximately 3 hrs. Subjects will come back for the next 3 regimes following the same schedule for days 1-3 with 13 or more days between each regime. The total amount of time at this facility will be ~79 hr

Deleted: 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.¶

B.4. Where will the subjects be studied? Describe locations where subjects will be studied, both on and off the UNC-CH campus.

All subjects will be studied in the USEPA Human Studies Facility located at 104 Mason Farm Road in Chapel Hill on the UNC Campus.

Deleted: B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.¶

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 and phone conversations will be conducted from either the Westat Recruiting office or the Medical Station 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. Physical exams and other procedures will occur in appropriate clinical areas of the EPA. Occasionally 2 subjects may be seen in the clinical area at one time; however, sensitive information is only discussed in private (medication use, pregnancy test results). The subjects will be scheduled with the TRC (Chamber operators) group using a study number; however TRC only maintains a single separate log with the study number and the subject's number but not personal identifying information. Subjects may be contacted by email to schedule/remind them about study visits or to answer specific questions. Any information sent via US mail or campus mail will simply have a return address, no other study specific information.

Deleted: 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).¶

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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).

Deleted: 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). Be aware that payment over a certain amount may require the collection of 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 the subject's 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 ($12 per hour) and some procedures in the study (See below). In addition, subjects traveling from areas beyond Chapel Hill 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 compensated for his/her participation up to that point and will receive compensation at the hourly rate of $12 per hour for that particular scheduled 3 day study session.

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% of procedure fees 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.

Money received by participants in research studies is normally treated as ordinary income by taxing authorities and payments made to you to the Internal Revenue Service as required by law. The money is not intended to coerce completion or participation, but to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study.

Subject Compensation for Procedures during Diesel and Ozone Exposure Study

Training Day (assume 3 hr @$12/hr)	$36
First Exposure:	
Day 1	
Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Stress questionnaire	$2.50
Blood Draw (twice)	$30
Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60
Lunch	$5
Day 2	
Hourly payment (assume 8 hr @$12/hr)	$96

On-time bonus (8 a.m.)	
Blood Draw (twice)	$25
Urine Sample (3x)	$30
Exhaled breath collection (twice)	$15
24 hr Holter Monitoring	$10
24 hr Blood Pressure Monitoring	$100
Lung function (6x)	$50
Lunch	$60
	$5
Day 3	
Hourly payment (assume 3 hr @$12/hr)	
Blood Draw	$36
Exhaled breath collection	$15
Urine Sample	$5
Lung function	$5
	$10

[Total payment First Exposure]	$855.50
Second Exposure	$855.50
Third Exposure	$855.50
Fourth Exposure	$855.50
Completion Bonus (for completing all 4 exposure sessions)	$100
APPROXIMATE TOTAL (including training day)	$3,558.00

Other Possible Costs:
Extra Blood sticks ($10 each)
Extra hours ($12 per hour)
Travel from out of town (based on mileage)

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 excluding child and dependent care. Parking fees are covered by the study in the form of parking vouchers. All study related diagnostic tests such as pregnancy tests, pulmonary function test and labs are covered by the study. Westat subjects are primarily recruited from the Chapel Hill area. Volunteers who come from outside this area may be reimbursed for mileage at the current government rate.

Deleted: 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.¶

Attachment #1 The Sheldon Cohen Self-Perceived Stress Questionnaire (from www.mindgarden.com)

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name _____ Date _____

Age _____ Gender (*Circle*): M F Other _____

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
7. In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
8. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9. In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Protocol title: "Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults"; T. Stevens, PI

July 27, 2009 version

Deleted: 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.¶
¶ ... [5]

Deleted: M. Madden

Attachment# 2 Newspaper Advertisement from Westat Inc, the recruitment contractor for US EPA Studies

The US Environmental Protection Agency is seeking

Adult Volunteers

For Research Study

Now recruiting healthy non-smoking adults ages 18 to 55 for a study about ozone, diesel exhaust & exercise. Study requires screening plus 13 visits over about 10 weeks and pays up to $3,558.

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

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

Attachment# 3 Web Site Announcement of this specific Study (IRB# 09-1344) at the US EPA Studies Webpage (www.epastudies.org)

DEPOZ Study, IRB Study #09-1344
Web Site Announcement
www.epastudies.org

DEPOZ

What is the purpose of the research study?

The purpose of this research is to find out how the air pollution that causes the haze seen in some polluted cities affects the heart, blood vessels and lungs of healthy adults.

Can I take part in the study?
You may be able to be in the study if you

- Are between 18 and 55 and
- Do not smoke and
- Do not have any heart or lung problems

What will I be asked to do?

- Have a free physical exam
- Have blood drawn
- Take part in several different breathing tests, including spirometry (explanation link provided)
- Have your heart rate (explanation link provided) and blood pressure monitored
- Use an exercise bike during testing
- Come in for a training visit, and then come in 3 days in a row generally every other week for 4 times.
- On the first day of each 3-day study session you will have tests and
  - Breathe clean air, or
  - Breathe air polluted with a carefully controlled amount of diesel exhaust, or
  - Breathe air polluted with carefully controlled amount of ozone, or
  - Breathe a combination of diesel exhaust and ozone,
- On the second day of each 3-day study session you will have tests and breathe air with ozone only
- On the third day of each 3-day study session you will have more tests

The polluted air that you breathe will be a lot like air you might breathe in a city like Los Angeles, New York, or Mexico City on a smoggy day

How long will it take?

- After screening there will be 13 visits, including 1 training visit and 4 sets of 3-day exposure visits, each set separated by at least 13 days
- In total, the study will take about 79 hours over about 10 weeks.

What will I get for volunteering?

- If you complete all visits and procedures, we will pay you $3,558.

How can I sign up or get more information?
Call or send an email (link provided). 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**

Attachment# 4 Email Advertisement from Westat Inc, the recruitment contractor for US EPA Studies, of this study (IRB# 09-1344) to current participants in EPA studies

DEPOZ Study E-mail to Current Volunteers

(these are people who are already participating in other EPA studies)

IRB #09-1344

E-mail Subject Line: EPA Research Study about Ozone, Diesel Exhaust and Exercise.

Dear _____,

You have been pre-qualified for a study called "DEPOZ" which will begin [date to be decided]. This research study is for healthy non-smokers between 18 and 55. The purpose of this study is to find out how air pollution – the kind that often causes the haze seen on smoggy days in polluted cities – affects the heart, blood vessels and lungs of healthy young adults. This study involves up to 13 visits after screening over about 10 weeks. It requires a commitment of about 79 hours, including four 2 ½ consecutive day sessions generally every other week. If all visits and procedures are completed this study pays $3,558. If you are interested in learning more about the study and/or in scheduling a repeat physical exam (if needed) please call us at 919-966-0604 (or 888-279-9353). Please also visit **www.epastudies.org** for more information.

We look forward to hearing from you!
[Recruiter's name]

Attachment# 5 Email Advertisement from Westat Inc, the recruitment contractor for US EPA Studies, of this study (IRB# 09-1344) to potential participants (ie, not participating currently in EPA studies)

DEPOZ Study E-mail Announcement

(intended for targeted lists, such as that provided by UNC to subscribers)

SUBJECT: INFORMATIONAL: Research Study about Ozone, Diesel Exhaust and Exercise.

This research study is for healthy non-smokers between 18 and 55. The purpose of this study is to find out how air pollution – the kind that often causes the haze seen on smoggy days in polluted cities – affects the heart, blood vessels and lungs of healthy young adults. This study involves up to 13 visits after screening over about 10 weeks. It requires a commitment of about 79 hours, including four 2 ½ consecutive day sessions generally every other week. If all visits and procedures are completed this study pays $3,558. Please visit **www.epastudies.org** for more information or call the Westat EPA Recruiting Office at 919-966-0604.

Approved [date], by the Office of Human Research Ethics Biomedical Institutional Review Board. IRB # 09-1344: Cardiopulmonary responses to exposure to ozone and diesel exhaust with moderate exercise in healthy adults.

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

Attachment# 6 Recruitment Script describing this specific study (IRB# 09-1344) from Westat Inc, the recruitment contractor for US EPA Studies, to potential participants

Study Name: DEPOZ
IRB Study # 09-1344

Title
Cardiopulmonary responses to exposure to ozone and diesel exhaust with moderate exercise in healthy adults.

Purpose
The purpose of this research study is to find out how the air pollution that causes the haze seen in some polluted cities (like Los Ageles, New York, and Mexico City) affects the heart, blood vessels and lungs of healthy young adults. This study is for people ages 18 to 55 who are able to exercise.

Procedures and Payment

The study will require two screening visits to the EPA Human Studies Facility (one to complete medical history paperwork, and the other for a physical exam), followed by a 3-hour training session and four 3-day study sessions. Study sessions are generally about two weeks apart, so this study requires 13 visits after screening over about 10 weeks. On the first day of each segment you will be exposed for 2 hours to either clean air, or air polluted with a carefully controlled amount of diesel exhaust or ozone, or both. Then the next day you will come back for another 2-hour exposure just to ozone. Each exposure day will last 8 hours. The third day of each segment will last about 3 hours.

During exposure sessions you will be asked to give blood, urine and breath samples, ride an exercise bike at 15-minute intervals during exposures, and perform several different breathing tests. You will also wear heart and blood pressure monitors during the study sessions and over night. The total amount of time is about 79 hours over about 10 weeks. Since each set of exposures is separated generally by 2 weeks, you will spend 2½ days at the clinic every other week, 4 times. Does this seem like something that your schedule can handle?

If you complete the training visit and all four 2½ day sessions, you will be paid $3,558.00.

Study Schedule

Screening, 2 visits, as needed: 3 hours
Training visit, *days & times to be decided*: 3 hours
Segment 1, Day 1, 8 AM, *days to be decided*: 8 hours
Segment 1, Day 2, 8 AM, day after Day 1, 8 hours
Segment 1, Day 3, 8 AM, day after Day 2, 3 hours

Study Restrictions
In addition to meeting all study eligibility criteria (*in the IRB application*), volunteers must be willing to adhere to the following restrictions as well:

- No over the counter pain medications such as aspirin, Advil, Aleve, or other non-steroidal anti-inflammatory medications including those used for allergies for 48 hours prior to the exposure and post-exposure visits

- Medications not specifically mentioned here may be reviewed by the investigators prior to your inclusion in the study
- No Vitamins C or E, or supplements containing C or E, for at least 2 weeks prior to and throughout 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
- Avoid the use of ozone-based home air purifiers during study participation.
- Eat a light breakfast on the exposure day
- Do not consume caffeine for 2 hours prior to the exposure on days 1 & 2 and post-exposure visits

Attachment# 7 Reminder sheet from Westat Inc, the recruitment contractor for US EPA Studies, of this study (IRB# 09-1344) to accepted participants

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TO:

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

STUDY: **DEPOZ STUDY, IRB # 09-1344**

DATE:

TIME:

PARKING is available on Mason Farm Rd directly in front of the EPA Human Studies building. Please park in Visitor Spaces only. Enter the building, give your name to the Guard, and request a Visitor Permit.

If the EPA lot is full you may also park in [provide current parking availability information]. Use Patient/Visitor spaces only. If you are a UNC Student or are UNC staff or faculty and must use the Dogwood Deck or ACC Lot 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.

May be folded here for display

Study Instructions

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.

Active Allergies or Hay Fever
If you have had seasonal allergy symptoms in the past week, please call Recruitment to reschedule.

Medications
- No over the counter pain medications such as aspirin, Advil, Aleve, or other non-steroidal anti-inflammatory medications, including those taken for allergies, for 48 hours prior to the exposure and post-exposure visits. Tylenol is permitted.

Diet and Exercise
- No Vitamins C or E, or supplements containing C or E, for at least 2 weeks prior to and throughout the study.
- No drinking alcohol 24 hours before all visits
- No strenuous exercise for 24 hours prior to and after all visits
- No caffeine for 2 hours before to the exposure on days 1 & 2 and post-exposure visits.
- Eat a light breakfast on exposure days.

Other
- No smoke and fumes for 24 hours before all visits
- No ozone-based home air purifiers throughout the study
- Wear comfortable clothes and shoes suitable for exercise and bring a change of clothes

- Shower only in the morning before exposures, following the medical staff's instructions for removing and reattaching monitors.

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

Thank You! Westat EPA Support Services

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. 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_

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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

I certify that I am employed as a research biologist by the U.S. Environmental Protection Agency (US EPA, the sponsor of this research) and as such collect salary and possible awards from the Agency.

Michael Madden

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.

Faculty Advisor Date

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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. __ yes __ no
Choose 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). *Participants should be asked whether they want documentation linking them with the research and the participants' wishes will govern whether they sign the form.* Note: This justification cannot be used in FDA-regulated research. __ 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).
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 <u>no greater than minimal risk</u> 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.

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).

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).

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.

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Page 28: [4] Deleted ctsuser **12/18/2009 3:41:00 PM**

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.

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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

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

IRB Study # 09-1344 GCRC #: N/A
Consent Form Version Date: December 23, 2009

Title of Study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults
Principal Investigator: Tina Stevens, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 843-8031
Email Address: madden.michael@epa.gov
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Faculty Advisor: N/A

Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-6257 (Michael Madden)
Study Contact email: madden.michael@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 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?

We breathe a complex mixture of ambient air pollution, and ozone and diesel exhaust from an engine are generally major and important components. Controlled exposures of volunteers to either pollutant have resulted in biological effects such as lung physiological changes. However it is not known if co-exposure to both pollutants, similar to inhaling polluted ambient air, can induce effects than either pollutant alone. Additionally it is also uncertain if exposure to diesel exhaust alone, or diesel exhaust mixed with ozone, can alter the body's responses to breathing ozone the following day. This study proposes to examine whether exposure to both ozone and diesel exhaust can cause more of an effect than from an either pollutant alone. This study will also determine if breathing diesel exhaust can change a response upon exposure to ozone the day after. The purpose of this study is to measure cardiopulmonary responses in healthy young adults before, during, and after exposure to combinations of ozone (O3) and diesel exhaust for 2 hours with moderate exercise and to primarily investigate whether diesel exhaust modulates the O3-induced effects on the lung and cardiovascular systems.

This study proposes to examine whether co-exposures to O3 and DE, at doses in the upper range of those encountered in urbanized settings, can induce additive or synergistic effects, and whether a previous DE exposure alters a response upon subsequent exposure to O3. The data obtained from this study will contribute to the overall assessment of air pollution effects in the U.S. and thereby may influence future health policy. The ambient permissible concentrations of both O3 and DE are currently regulated individually by the US EPA, but the Agency is moving to regulating pollutant mixtures. 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.

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, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You cannot perform moderate exercise
- You cannot remain in a small exposure chamber for about 2 hours
- 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 less than 18 years old or greater than 55 years old
- 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 ("NSAIDS") for 48 hr prior to the exposure and post-exposure visits. Acetaminophen, eg, Tylenol, is 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 days.
- Not consume caffeine for 1-2 hours prior to the exposures on days 1 & 2 and post-exposure visits.
- Stop taking vitamin C or E or medications which may impact the results of the exposures at least 2 weeks prior to the study and for the duration of the study. Medications not specifically mentioned here may be reviewed by the investigators prior to your inclusion in this study.
- Avoid the use of ozone-based home air purifiers during study participation

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 15 people who will complete this research study.

How long will your participation in this study last?

You will have up to 13 visits to the research facility over approximately about 10 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 4 exposure regimens, each of which will consist of 2 consecutive exposure days and 1 follow-up visit approximately 18 hrs after the last exposure. Each exposure day will last approximately 8 hours and the follow-up visit will be approximately 3 hrs long. The 4 exposure regimens will occur at least 13 days apart.

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

What will happen if you take part in the study?

During the course of this study, the following will occur:

Training:

You should have already undergone 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. Today you will familiarize yourself with some of the techniques you will perform for the study. These include instruction on the use of the stationary bicycle to be used during the study, how to perform spirometry and an exhaled breath collection, and heart rate variability (HRV) and blood pressure (BP) monitors.

After satisfactorily completing the training session, you will be scheduled for your first exposure through a company contracting with the US EPA (currently Westat).

You will be exposed to mixtures of air pollutants in 4 different regimens. Each regimen will last about 2 ½ days. During the regimens, a number of physiological and biochemical measurements will be made on you. You will have the opportunity to complete all 4 regimens separated by at least 2 weeks. The first day of each regimen you will be exposed to either clean air, diesel exhaust at about 300 micrograms particles/meter^3, ozone at about 0.3 parts per million (ppm), or diesel exhaust mixed with ozone. The second day of regimen, you will be exposed to approximately 0.3 ppm ozone. The third day of each regimen you will not be exposed to any air pollutants, but will have follow up measurements made.

You may terminate your participation from this study at any time. You will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms may include chest pain, difficulty breathing, 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 signs of cell damage, changes in clotting factors, as well as increases in inflammation. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Below is a description of what will be required from you and a summary of the measurements to be made on you on each day of each exposure regimen:

Day 1:

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, NSAID medications, 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 will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. There will be an on-time bonus of $25 for arriving by 8:05 a.m. You will need to wear comfortable clothes and shoes and bring a change of clothes.

Pre-exposure measurements:

Prior to the day 1 exposure, you will have you may:

- Answer a questionnaire on stress

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, and oxygen saturation level, and for women, a pregnancy test).
- 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 may be kept on you for the next two days and will be removed on Day 3 of the exposure session. 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 morning of the follow up visit (Day 3), there may be a 30 minute measurement of your heart rate and then the monitor will be removed.
- Blood pressure (BP) may be measured intermittently by a BP monitor. A blood pressure cuff and a monitor which is about the size of the Holter monitor may be fitted and will remain in place most of the time until Day 3. You will be asked to keep your arm relaxed and still when the pressure cuff is inflating.
- Have about 25 ml blood drawn.
- 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.
- Be asked to provide a urine sample; if you are female it will be tested to see if you are pregnant.
- We may collect your breath in two manners- one being to cool your exhaled breath to capture the water vapor, and the other just collecting the gas portion uncooled
- You will then enter an exposure chamber.

During the Day 1 exposure, you may:

- Be exposed to clean air or air pollutants for 2 hours. You will be asked to perform intermittent moderate exercise in an exposure chamber.
- At certain times during the exposure, you may be asked to breathe into a mouthpiece so that your rate of breathing can be measured. A staff member will be seated outside the chamber to observe you at all times and a physician will be available during the entire exposure session. During the exposure, your heart rhythm and rate, 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 a severe headache, nausea or vomiting the exposure will be terminated immediately.

Immediately following the Day 1 exposure, you may:

- Have your vital signs checked.
- Perform 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 about 1 assessment each hour
- We may collect your breath
- 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 25 ml).
- Provide a urine sample.

Later in the day, you may:

- Have spirometry assessed for up to 4 hr post exposure
- Provide a urine sample
- Be assessed and discharged by the nursing staff. You will spend about 8 hours at the EPA facility.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest and a blood pressure monitor cuff on your arm for the following two nights, we will give you instructions on how to care for and remove the monitors when necessary.

Day 2:

You will return to the HSF the next morning at 8:00 a.m. and you will perform testing similar to the first day.

Pre-exposure measurements and procedures will be performed as on Day 1, with the exception of the stress questionnaire. You will then enter the exposure chamber.

During the exposure on Day 2 you will be exposed to ozone at a concentration of approximately 0.3 ppm for 2 hours with intermittent moderate exercise in an exposure chamber. Similar measurements and monitoring will be performed as during the exposure on Day 1.

- **Similar measurements and procedures may be performed with you following the Day 2 exposure as on Day 1.** Your health will be assessed and you will be discharged by the nursing staff. You will spend about 8 hours at the EPA facility.

Day 3:

Follow up Visit:

Similar procedures and measurements may be made as the pre-exposure measurements of the two previous days. The Holter monitor attached to you will be removed. Your health will be assessed and you will be discharged by the nursing staff. You will spend about 8 hours at the EPA facility. You will spend about 3 hours at the EPA facility.

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 screening for this study you will have received a medical examination that included 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 in general 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. Eye wear protection goggles are available during the exposures to reduce possible 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 these exhausts, you will only be exposed to them for 2 hours. You could be potentially inhale a similar amount 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 (i.e., within the building facility) during exposures, and is available to respond in an emergency.

Ozone exposure: Potential risks may include mild airway obstruction, irritation to the nose, eyes, throat and airways, pain on deep inspiration and cough. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. O3 may induce an inflammatory reaction that may last for about 24 hours after exposure and may increase the chance of catching a cold.

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 during your visit, you should tell the nursing staff. If irritation occurs while you are home, you should remove the electrodes, wash gently with mild soap and water, and tell the study coordinator or nursing staff in the morning.

Blood pressure and heart rate measurement: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

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. Occasionally fainting or light-headedness occurs, and injury is minimized by having you seated. 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.

Exhaled breath collection: Minimal risk is associated with these procedures. You will be seated in a chair during collections and technicians are always available during this procedure in case you become light-headed.

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 a 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 human health research 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 an appointed US EPA physician to be due to your participation in this research, the US 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 States 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 approximately $3,558.00

We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill you will be reimbursed for mileage at the US Government mileage rate in effect at the time. 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:

Subject Compensation for Procedures during Diesel and Ozone Exposure Study

Training Day (assume 3 hr @$12/hr)	$36
First Exposure:	
Day 1	
Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Stress questionnaire	$2.50
Blood Draw (twice)	$30
Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60
Lunch	$5
Day 2	
Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Blood Draw (twice)	$30
Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60

Lunch	$5
Day 3	
Hourly payment (assume 3 hr @$12/hr)	$36
Blood Draw	$15
Exhaled breath collection	$5
Urine Sample	$5
Lung function	$10
[Total payment First Exposure]	$855.50
Second Exposure	$855.50
Third Exposure	$855.50
Fourth Exposure	$855.50
Completion Bonus (for completing all 4 exposure sessions)	$100
APPROXIMATE TOTAL (including training day)	$3,558.00

Other Possible Costs:
Extra Blood sticks ($10 each)
Extra hours ($12 per hour)
Travel from out of town (based on mileage) @ current federal travel rates

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. Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be compensated for his/her participation up to that point and will receive compensation at the hourly rate of $12 per hour for the scheduled 3 day study session.

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% of procedure fees up to a total maximum of $100 for canceled 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, or other unforeseen events. When feasible, the subject will be rescheduled.

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. The money is not intended to coerce completion or participation, but to

emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study.

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.

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 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:
Michael Madden, PhD 919-966-6257 Tina Stevens, PhD 919-843-8031

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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Title of Study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults

IRB Study # 09-1344

Principal Investigator: Tina Stevens, 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

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: Tina Stevens
EPA
CB:7315, MD58A USEPA, 104 Mason Farm Rd, Chapel Hill, NC 27599

From: Biomedical IRB

Date: 11/11/2009
RE: Notice of IRB Deferral following Full Board Review on 11/09/2009
Submission Type: Initial
Study #: 09-1344
Study Title: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust With Moderate Exercise in Healthy Adults

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 has deferred further consideration of this study to a future meeting to allow you to provide additional information and/or make substantive change(s), as described below:

Study Specific Details:
Thank you for coming to the full board meeting on November 9, 2009. The committee agreed that the information you brought to the meeting was helpful. However, there are two substantial points that need further clarification before approval is achieved.

1. Purpose: The overall purpose of the study in terms of benefits to society and science remains unclear in the application and the consent. From your comments, it appears that data from this study will contribute to the overall assessment of air pollution effects in the U.S. in different populations (young healthy, older with medical conditions, etc.) and thereby influence health policy. Additionally, you indicated that this range of ozone and diesel particulate dosing is, or will be, in or near the upper range encountered in urbanized settings. The committee determined that this information should be included in the background section of the IRB application and in the appropriate consent sections dealing with why the study is being done.
2. Application, Data Safety Monitoring, A.4.8: The second, and more important, issue relates to what is often termed a data safety monitoring board or committee (DSMB or DSMC) or Monitor (an individual with expertise not directly involved with the study). One of the purposes of such a board is to oversee the safety of subjects as they go through the research project. The structure of this review process often includes the naming of individual(s) who will review the project from the standpoint of adverse events reported by or about the subjects, and a periodic blinded data review looking for changes in the data related to procedures that might indicate a greater than anticipated risk to the subjects.

   This could, for instance, include a marked and consistent significant drop in FEV-1 after certain procedures that was unexpected in terms of magnitude, with or without clinical symptoms, and that might prompt a change in protocol or stopping the study. This is

particularly important when the levels of toxic exposure is relatively high, as it seems to be in your study.

Please revise this section to include a detailed description of safety monitoring procedures, including who will provide the oversight that will be in place for this study. Please include details of the levels of pulmonary and/or cardiac responses that would trigger stopping or changing procedures not just after the initial 4 subjects (which is almost 1/3 through the total number of subjects) but over the course of the research.

Please contact the OHRE at 966-3113 if you have any questions regarding the above.

The PI and researchers are reminded that upon submitting new applications, to go to the OHRE website and download the most recent version of the application rather than submitting older versions.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the items listed. Any additional changes (including new materials) must also be listed and discussed in the memo. You will need to return 3 copies of the revised application and all other study materials to the IRB, two in which all changes are underlined and the third a "clean" copy with no underlining.

CC:
Michael Madden, Environmental Protection Agency
Michael Schmitt
David Diaz-Sanchez
Howard Kehrl, Medicine
Robert Truckner, (EPA), Non-IRB Review Contact

RC 2

Full Committee Review
Initial - Reconsideration

Receipt Date : 7/27/2009 Expiration Date : Previous Review Type:

IRB: Biomedical PI: ~~Michael Madden~~ TINA STEVENS IRB ID : 09-1344 IRB Admin : Joy Bryde

Title: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust With Moderate Exercise in Healthy Adults

Agenda Date 11/09/2009 Reviewer 1: **Mann, Doug** (chair) Reviewer 2: **Yu, Bing**

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 :

DSMB ? not answered
- why - what hope to learn

CONSENTS ADULT
SPEC

QUESTIONNAIRE
AD
WEBSITE, EMAIL
SCRIPT
REMINDER

FINDING: HIPAA N/A

D

☑ Deferral JMB 11/11/09

INCLUDES COPY OF INITIAL DEFERRAL + MARKED CONSENTS

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 11/12/09 JBM
- ☐ Disapproved
- ☐ Return to sender
- ☐ Closure

IRB REVIEWER CHECKLIST FULL BOARD REVIEW

IRB#: 09-1344 PI: TINA STEVENS REVIEWER: MANN DATE: 11/9/09 mtg

	Questions and relevant section IRB Application	Y	N	N/A	Comments/Concerns
A.2	Is additional documentation required by items on Application Checklist included? **Specify**				
A.2	Is there a valid IND or IDE from the FDA (when applicable)?			✓	
A.3	Are there Conflicts of Interest with this study, disclosed by PI or perceived by reviewer?	~~N/A~~	✓		
A.4.1 A.4.2	Will the study result in generalizable knowledge that benefits society?	✓			Most points addressed.
A.4.2	Is the rationale (background) adequately described?				
A.4.2	Are the study purpose (hypothesis) and study groups clearly described?	✓			
A.4.2	Are the objectives and outcome measures consistent with the rationale?	✓			Shortened consent form. X3 pages
A.4.3	Is the selection of subjects equitable?	✓			language technical at times/p.2
A.4.4	Are inclusion /exclusion criteria appropriate and do they minimize risk to subjects?	✓	young people		but overall manageable by subjects.
A.4.5	Is the study design adequately justified? *(e.g., randomization, placebo, phase, deception)*	✓			point # 5. No DSMB.
A.4.5	Are the time demands, number of visits, procedures, and interventions clearly outlined?	✓			DSMB → data safety OK.
A.4.5 B.3	Is the study duration (and visit frequency) appropriate to achieve stated objectives?	✓	Good.		Data re: AEs + UPs. — Stopping rules.
A.4.5	Are experimental procedures distinguished from standard of care or treatment?			✓	
A.4.6	Are the potential benefits to subjects and/or society adequately described?	✓			
A.4.7	Are foreseeable risks clearly defined?		✓		
A.4.7	Are risks to subjects reasonable when compared to anticipated benefits?		✓		No benefits → min. risk
A.4.7	Are risks minimized by using procedures consistent with sound research design, avoiding unnecessary exposures(s), and/or using procedures already performed for diagnosis or treatment?	✓			
A.4.7	If vulnerable populations are involved, are adequate protections in place? **Specify**			✓	
A.4.7	Level of risk constituted by the research?				**Minimal** / **Greater than minimal**
A.4.7	If a medical device study, what is the risk?			✓	**Non-significant Risk** / **Significant Risk**
A.4.8	Are data monitoring provisions adequate for subject safety?	✓		~~N/A~~	

Comments/Concerns

IRB REVIEWER CHECKLIST FULL BOARD REVIEW

RC 2

IRB#: 09-1344 PI: Tina Stevens REVIEWER: Bing Yu DATE: 11/09/2009

Questions and relevant section IRB Application		Y	N	N/A	Comments/Concerns
A.2	Is additional documentation required by items on Application Checklist included? **Specify**		✓		
A.2	Is there a valid IND or IDE from the FDA (when applicable)?			✓	
A.3	Are there Conflicts of Interest with this study, disclosed by PI or perceived by reviewer?		✓		
A.4.1 A.4.2	Will the study result in generalizable knowledge that benefits society?				Not clear
A.4.2	Is the rationale (background) adequately described?		✓		
A.4.2	Are the study purpose (hypothesis) and study groups clearly described?	✓			
A.4.2	Are the objectives and outcome measures consistent with the rationale?				Not clear
A.4.3	Is the selection of subjects equitable?	✓			
A.4.4	Are inclusion /exclusion criteria appropriate and do they minimize risk to subjects?	✓			
A.4.5	Is the study design adequately justified? *(e.g., randomization, placebo, phase, deception)*	✓			
A.4.5	Are the time demands, number of visits, procedures, and interventions clearly outlined?	✓			
A.4.5 B.3	Is the study duration (and visit frequency) appropriate to achieve stated objectives?	✓			
A.4.5	Are experimental procedures distinguished from standard of care or treatment?			✓	
A.4.6	Are the potential benefits to subjects and/or society adequately described?		✓		
A.4.7	Are foreseeable risks clearly defined?	✓			
A.4.7	Are risks to subjects reasonable when compared to anticipated benefits?				Not clear.
A.4.7	Are risks minimized by using procedures consistent with sound research design, avoiding unnecessary exposures(s), and/or using procedures already performed for diagnosis or treatment?				Not clear
A.4.7	If vulnerable populations are involved, are adequate protections in place? **Specify**	✓			
A.4.7	Level of risk constituted by the research?				**Minimal** / **Greater than minimal**
A.4.7	If a medical device study, what is the risk?				**Non-significant Risk** / **Significant Risk**
A.4.8	Are data monitoring provisions adequate for subject safety?	✓	✓		

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH & ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
RESEARCH TRIANGLE PARK, NC 27711

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MD# 58B, Human Studies Division, Clinical Research Branch
104 Mason Farm Road, CB# 7315
US EPA Human Studies Facility
Chapel Hill, NC 27599-7315
919-843-8031 (Office) 919-966-6271(FAX)
stevens.tina@epa.gov

September 29, 2009

Biomedical Research IRB Committee Members
UNC Office of Human Research Ethics
CB# 7097, Med. Building 52
Carolina Campus

RE: Protocol Application # 09-1344

Dear Committee Members:

I am submitting a revised protocol (original and two copies) to the Biomedical Research IRB Committee for review. The PI on this protocol has been switched from Michael Madden to myself; Dr Madden will remain as a Co-PI and he and Dr. Diaz-Sanchez will serve as mentors for me on this study. The protocol ("Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults"; Tina Stevens, PI) proposes controlled human subject exposures to diesel exhaust at the U.S. EPA facility on the UNC Campus. The Principal and Co-principal Investigators are U.S. EPA employees, with three (Drs. Madden, Diaz-Sanchez, and Kehrl) of them holding adjunct appointments with UNC.

My colleagues and I have revised the protocol substantially in response to the reviewer's comments. These revisions are indicated by underlining as requested. The responses are summarized below in a point by point manner. We additionally reworded the documents in response to edits and markups on the original hardcopy, and removed Figure 1 with the flow chart as suggested. Additionally the documents for recruitment of potential subjects, and reminders to accepted subjects of what is needed of them on a study day, are included in an Appendix. A few minor changes were made to the wording and grammar of some sentences. I

Date: 8/11/2009

RE: Notice of IRB Deferral following Full Board Review on 8/10/2009

Submission Type: Initial

Study #: 09-1344

Study Title: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults

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 has deferred further consideration of this study to a future meeting to allow you to provide additional information and/or make substantive change(s), as described below:

Study Specific Details:

1. IRB application A.4.3: Please indicate whether or not all races and ethnic groups will be considered for this study. We have now indicated that all races/ethnicities are allowed in this study.
2. IRB application, A.4.2, rationale: the text provided is highly detailed. Please provide an introductory paragraph which clearly and succinctly addresses the need for this research and why healthy subjects should be exposed to O3 and DE. Please also summarize this information under purpose of study in the consent form. We have better explained the study rationale in protocol and with similar wording in the consent form.
3. IRB application A.4.5: please complete the waiver of written consent as applicable to the telephone screen. Telephone interviews will not be used, but rather emails and newspaper advertisements will be utilized by the US EPA contractor, as shown in attachments 2-6.
4. IRB application B1: Please provide a copy of the telephone script to be used for the screening procedure described Telephone interviews will not be used, but rather emails and newspaper advertisements will be utilized, as shown in attachments 2-6.
5. IRB application, A.4.7, risks: Please justify not using a DSMB. [Data Safety Monitoring Board]. We believe that the security of the proposed data storage plan of both hard copies and electronic data is commensurate with the study complexity, and will be reviewed by the US EPA Quality Control officer before implementation. The QA officer will review the data storage proposal as to assure not only quality, but also storage safety in order to meet federal guidelines for record maintenance. This propsed system is utilized for most, if not all, EPA studies at this facility.

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

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APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 8-May 19th

Part A.1. Contact Information, Agreements, and Signatures

Title of Study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults

Date: September 17, 2009

Name and degrees of Principal Investigator: Tina Stevens, Ph.D.
Department: US EPA
Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 843-8031 Fax #: (919) 966-6367 Email Address: stevens.tina@epa.gov

Name and degrees of Co-Investigator: Michael Madden, Ph.D, David Diaz-Sanchez, Ph.D., Howard Kehrl, MD

For trainee-led projects: __ undergraduate __ graduate __ postdoc __ resident __ other
Name of faculty advisors: N/A

Department: Mailing address/CB #:

Phone #: Fax #: Email Address:

Department: Mailing address/CB #:

Phone #: Fax #: Email Address:

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: Michael Schmitt
Mailing address/CB #: 104 Mason Farm Rd.
Chapel Hill, NC
27599-7315

Phone #: (919) 966-0647 Email Address: schmitt.mike@epa.gov

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:**
Co-PI: David Diaz-Sanchez, PhD (diaz-sanchez.david@epa.gov); Tina Stevens, PhD (stevens.tina@epa.gov); Howard Kehrl, MD (kehrl.howard@epa.gov)
Robert Devlin, Ph.D; Maryann Bassett, RN; Martha Sue Carraway, MD; Martin Case, BS; Andrew Ghio MD; Tracey Montilla, RN; Ana Rappold, PhD; Joachim Pleil, Ph.D; Michael Schmitt, MS; and Heidi Hiers, RN;

Name of funding source or sponsor (*please do not abbreviate*): Environmental Protection Agency
__ not funded x_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify):
Sponsor or award number: N/A

RAMSeS number (from Office of Sponsored Research):
For industry sponsored research (if applicable):
Sponsor's master protocol version #: Date:
Investigator Brochure version: Date:
Any details you need documented on IRB approval:

Checklist of Items to Include with Your Submission

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
☐	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; (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal).	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 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.

Tina Stevens, Signature of Principal Investigator — Date: 9/29/09

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.

David Diaz-Sanchez, Signature of Faculty Co-Advisor — Date: 9/29/09

Michael Madden, Signature of Faculty Co-Advisor — Date: 9/29/09

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. 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?	__	_x_
If yes, approval by the UNC-CH Radiation Safety Committee is required.		
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

I certify that I am employed as a research biologist by the U.S. Environmental Protection Agency (US EPA, the sponsor of this research) and as such collect salary and possible awards from the Agency.

Tina Stevens

~~Michael Madden~~ Tina Stevens

<u>Certification by Principal Investigator</u>: 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.

Tina Stevens — 9/29/09

Signature of Principal Investigator — Date

<u>Faculty Advisor if PI is a Student or Trainee Investigator</u>: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

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: The purpose of this study is to measure cardiopulmonary responses in healthy young adults before, during, and after exposure to combinations of ozone (O3) and diesel exhaust (DE) for 2 hours with moderate exercise and to primarily investigate whether DE modulates the O3-induced effects on the lung and cardiovascular systems.

Participants: Fifteen (15) healthy young men and women in the age of 18 – 55 years

Procedures (methods): Each subject will be exposed in environmentally controlled exposure chambers to 4 exposure regimens. Each regimen will require a 2 hr exposure with intermittent, moderate exercise on 2 consecutive days. Day 1 exposure will consist of a combined exposure to DE and O3, O3 only (i.e., no DE), or DE only, or clean air (CA) only. For all 4 regimens, subjects will return the next day (Day 2) to be exposed to O3 alone and again on Day 3 for a follow up visit. Each regimen will be separated by at least 13 days. Techniques measuring lung and cardiac physiology will be performed pre, during, and post exposure. Blood, urine, and breath samples will also be obtained and analyzed for immune and inflammatory markers, clotting factors, susceptibility factors, and exposure markers.

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 air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infections, exacerbation of asthma symptoms, chronic bronchitis, ischemic heart disease, and stroke [1] and other health effects. Understanding the components responsible for these effects is difficult because ambient air pollution is a complex mixture of gases and particulate matter (PM). In this complex mixture of ambient air pollution, ozone (O3) and diesel exhaust (DE) are generally major and important components. Controlled exposure of volunteers to either pollutant have resulted in biological effects such as lung physiological changes. However it is not known if co-exposure to both pollutants, similar to polluted ambient air, can induce additive or synergistic effects. Additionally it is also uncertain if exposure to DE, or DE with O3, can alter a subsequent exposure to O3. This study proposes to examine whether co-exposures to O3 and DE can induce additive or synergistic effects, and whether a previous DE exposure alters a response upon subsequent exposure to O3.

One of the best studied gas phase pollutants is ozone (O3). Effects of O3 exposure in a controlled exposure setting have been well documented particularly for decrements of lung function [2-12] and an influx of neutrophils and other markers of inflammation [13-17] at concentrations as low as 0.08 ppm in a dose-response manner. Repeated exposure of healthy adolescents to about 0.5 ppm O3 for 2 hrs on 5 consecutive days has shown a decrement in lung function after the first exposure, an even greater decrement after a second day exposure, but then increasing attenuated lung function decrements for the next 3 exposure days [18-21]. Hence the greatest lung function decrement is after 2 days exposure, with

an adaptation occurring after the first two exposures. The adaptation is associated with attenuation of some soluble biochemical mediators collected by lung lavage (eg, PGE2), but not others (eg, IL-8), after 5 consecutive days of exposure [22].

A wide variation in lung function and lung cellular response to O3 exposure has been observed among individuals with no clear understanding in the susceptibility factors involved. NSAID usage has been shown to attenuate O3-induced lung function decrements [23]. Recent reports have linked certain genetic polymorphisms to possible sensitivities to O3. For example, exercising individuals had greater lung function decrements as a group when they possessed a glutathione-S-transferase M1 (GSTM1) null genotype. Field studies over 3 years have shown increased respiratory breathing problems in children living in Mexico City who were GSTM1 null [24, 25], but no increased respiratory problems were associated with those children who were GSTP1 null.

PM exposure has been associated primarily with premature mortality [26, 27], but also with morbidity such as increased hospitalizations for cardiopulmonary problems including lung infection, asthma symptoms, and heart attacks [28-30]. Controlled ambient PM exposure studies of humans have been used to examine biological responses as surrogates for understanding the health effects and mechanisms involved in the responses. These controlled exposures have utilized inhalation of ambient PM that was concentrated to achieve increased mass concentrations (average 120 ± 14 $\mu g/m^3$ for PM2.5, with a maximum of 207 $\mu g/m^3$) at our EPA facility in Chapel Hill [31]. The controlled exposure studies have demonstrated no changes in lung physiological measurements. However, a dose-dependent increase in total number of cells, neutrophils, and monocytes was observed in the lavage fluid. Cardiac measurements showed no changes (standard deviation of normal to normal beat intervals (SDNN), percentage of normal to normal beat interval differences > 50 ms (PNN50), the high frequency domain (HF), the low frequency domain (LF), or the ratio of HF/LF) either immediately following PM2.5 exposure and 24 hrs after exposure. Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation, and potentiates atherosclerosis [32].

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 contain primarily fine (≤ 2.5 μm) and ultrafine (≤ 0.1 μm) carbonaceous particulates generated by incomplete combustion of fuel. The composition of the DE PM and the gases can vary depending on the type and age of engine, quality of fuel and additives, emission controls, load characteristics, and after treatment. Few controlled human exposures to diesel exhaust have been performed. Healthy human volunteers exposed to 300 $\mu g/m^3$ diesel exhaust for one hour with intermittent exercise resulted in marked systemic and redox-sensitive pulmonary inflammatory responses [33, 34], and vascular dysfunction and impaired endogenous fibrinolysis that links diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction [35, 36]. Despite a decade of intensive studies, much about the PM health effects problem, especially the cardiovascular effect, is still not well understood.

There are few studies looking at lung responses with combined O3 and DE in a controlled exposure setting. One study using healthy, young adults showed that a sequential exposure to 300 $\mu g/m^3$ DE for 1 hr followed 5 hr later by exposure to 0.2 ppm O3 induced more lung inflammation (eg. neutrophils collected by induced sputum) than DE alone with no O3 exposure [37]. The same research group, using similar exposure regimen exposure levels and timing, showed that diesel exhaust increased O3-induced lung inflammation (i.e., neutrophils collected by bronchoscopy) relative to O3 exposure alone [38]. Few studies with controlled O3 exposures have examined changes induced in extrapulmonary responses, including cardiac physiology and blood clotting and inflammatory factors. One study showed an increased diastolic blood pressure in healthy adults exposed to concentrated ambient particular matter (CAPs; ~147 $\mu g/m^3$) and O3 (~0.12 ppm) simultaneously compared to clean air responses [39]. However, no separate exposure to O3 alone, or CAPs alone, were performed.

The purpose of this study is, first, to examine whether DE can alter lung and cardiovascular responses to O3 exposure when given a day before or during O3 exposure; second, to investigate if co-exposure of DE and O3 on day 1 augment the lung function decrements following a subsequent O3 exposure (day 2); third, to investigate if two consecutive days of O3 affect individual cardiovascular responses such as changes in heart rate variability (HRV) and blood pressure (BP), to ozone in young healthy adults. We will measure spirometric lung function in association with 2-hour exposures to O3. Results will be analyzed for effects of each or combinations of the above on changes in lung function. We hypothesize that an exposure to DE with O3 (day 1) or DE exposure (day 1) given prior to O3 exposure (day2) will not induce a significant decrement in lung function in healthy young adults as a group relative to O3 alone. We further hypothesize that an O3 exposure (on day 2) after the DE and O3 co-exposure (day 1) will cause significant cardiopulmonary responses in healthy young adults. Finally, we hypothesize that two consecutive days of O3 exposure will affect cardiovascular responses.

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.

A group of 15 healthy adults (men and women) between the ages of 18 and 55 years will participate and complete the study. However, because of potential dropouts and early terminations we may recruit as many as many as needed to have 15 individuals complete all 4 exposure regimens. Enrollment in this study will not be restricted to any specific races and ethnicities. All subjects will be non-smokers for at least a year, with no active cardiac or respiratory disease, or active medical problems of any kind. Pregnant women, those trying to become pregnant, and those breast-feeding, will not be accepted. All potential subjects will undergo screening procedures (previously approved 95-EPA-66 Phase I and II), including a medical history form, physical examination and routine chemical and hematologic screens. All subjects are required to be moderately active so that they could sustain a prolonged period of moderate exercise. All subjects will be recruited without regard to their genetic profiles; however, they will be genotyped and classified for presence or absence of polymorphisms of select genes.

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:

1. Healthy men and women between 18 and 55 years of age
2. Physical conditioning allowing intermittent, moderate exercise for 2.0 hours
3. Normal lung function:
   a. FVC $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   b. $FEV_1 \geq$ 75 % of that predicted for gender, ethnicity, age and height.
   c. FEV_1/FVC ratio $\geq$ 75 % of predicted values.
4. Oxygen saturation $\geq$ 96 %.

Exclusion Criteria:

1. A history of acute and chronic cardiovascular disease, chronic respiratory disease, diabetes, rheumatologic diseases, immunodeficiency state, and acute respiratory illness within 4 weeks.
2. Subjects who are asthmatic or have a history of asthma.
3. Allergic to chemical vapors or gases.
4. Any allergic symptoms during the time of participation in the study
5. Female subjects who are currently pregnant, attempting to become pregnant, or breastfeeding

6. Subjects unwilling or unable to stop taking vitamin C or E or medications which may impact the results of the ozone challenge (such as, systemic steroids and beta blockers) at least 2 weeks prior to the study and for the duration of the study. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
7. Current and past smokers within 1 years.
8. Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
9. Subjects who do not understand or speak English
10. Subjects unable to perform the moderately active exercise required for the study.
11. Subjects with a history of skin allergies to adhesives used in securing heart rate monitor electrodes.
12. Unspecified diseases or conditions, which in the judgment of the investigator might influence the responses to the exposures, will be a basis for exclusion.
13. Subjects unwilling to stop taking over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications ("NSAIDS") for 48 hr prior to the exposures and post-exposure visits.

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 is a randomized crossover single blind study with 4 arms separated by at least 13 days. In this study we will measure cardiopulmonary responses to O3 (approximately mean concentration of 0.3 ppm over the 2 hr exposure period) and diesel exhaust (DE; ~300 $\mu g/m^3$) combined exposure and singularly, and clean air control in healthy adults undergoing moderate intermittent exercise (minute ventilation = ~ 25 liters/min/m^2 BSA). [See figure 1].

Each subject will be exposed randomly to all 4 exposure regimes separated by at least 13 days. For all regimes, the subject will be exposed to the pollutant or clean air for 2 hrs with moderate exercise during the exposures. Moderate exercise will involve riding a stationary bike in the chamber for 15 min intervals beginning after the first 15 min of exposure and resting for 15 min after each time for a total of 1 hr of moderate exercise per chamber exposure. Ventilation rate measurements will be taken at ~ 6 min into each exercise session to ensure ventilation rates are within the appropriate parameters [target ~25 L/min/ m2 BSA, which in most subjects is ~ 50 L/min.]. For the DE and O3 co-exposure, there is a potential for the O3 to interact with the DE, but the O3 concentration will be maintained at a final mean concentration of about 0.3 ppm.

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 times will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. Clean air will be passed through an air purification system to ensure no presence of pollutant gases (O_3, NO, NO_2, SO_2, CO) in the air. Ozone and DE concentrations will be monitored continuously. The DE will be generated from a diesel engine used to power an air compressor that is located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean HEPA and charcoal filtered and humidified air to give a chamber concentration of approximately 300 $\mu g/m^3$. The monitoring analyzer registers a

concentration in real time. Exposures will be terminated at values ≥ 400 μg/m^3 during runs in which the exposure targets 300 μg/m^3 DE. This safety limit will prevent an inhalational exposure greater than 600 μg, 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 approximately 0.05-0.2 μm. Preliminary testing has shown the mean particle size to be approximately 0.15 ± 0.05 μm. Levels of carbon monoxide (CO), and oxides of nitrogen (NOx; mainly NO, and some NO_2), will be kept under 10 ppm CO, 15 ppm NO, and 3.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average for these substances are: CO=50 ppm; NO=25 ppm; NO_2=5 ppm; Diesel fuel used for the study will be purchased as a commercial ultra low sulfur fuel. 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. Particle mass will be measured in real time and validated further by weighing filters after the 2 hr exposure is finished. Particle size distribution will be determined at regular intervals. Filter samples will also be analyzed for chemical composition of particles. Minute ventilation will be measured during the exercise periods of exposure.

Lung Function Test:

Primary endpoints for evaluating health effects are changes lung functions, mainly FEV_1, FVC and FEV_1/FVC before, immediately after, and approximately every hour for up to 4 hrs post exposure.

Measurements and Collections:

Blood pressure, telemetry, and oxygen saturation may be monitored during the exposure period. Primary endpoints for evaluating health effects, in addition to lung function, are heart rate variability (HRV). Secondary endpoints may include markers of inflammation recovered in blood and exhaled breath including total and differential cell counts as well as pro-inflammatory cytokines and immune system mediators and clotting factors. Additionally, blood, breath, and urine samples will serve as indicators of the exposure based on the levels of metals (eg, Zn) and organics (eg, PAHs) and other components that maybe derived from DE deposition. From the collected blood samples, additional parameters and indices may be obtained as analysis tools become available. Genetic analysis may also be performed on collected blood samples for susceptibility factors. A questionnaire measuring perceived stress by the subject at the beginning of an exposure regimen will be performed. Some of the supplemental spirometric measurements and secondary endpoints may not be obtained if they are deemed unnecessary.

In order to participate in this study, subjects will be asked 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.
- Avoid the use of ozone-based home air purifiers during study participation.
- On the exposure days, eat a light breakfast.
- Refrain from all over the counter anti-inflammatory agents including those for allergies for a period of 48 hrs prior to exposure.

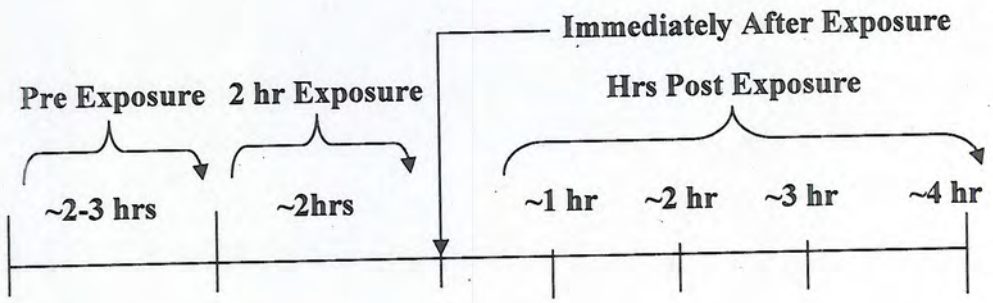
Figure 1

Experimental design:

	Day 1 **2 hr exposure**	**Day 2** **2hr exposure**	**Day 3**
Exposure Regime 1	DE + O3	O3	follow up (no exposure)
Exposure Regime 2	O3	O3	follow up (no exposure)
Exposure Regime 3	DE	O3	follow up (no exposure)
Exposure Regime 4	Clean Air	O3	follow up (no exposure)

Figure 2
Endpoints:

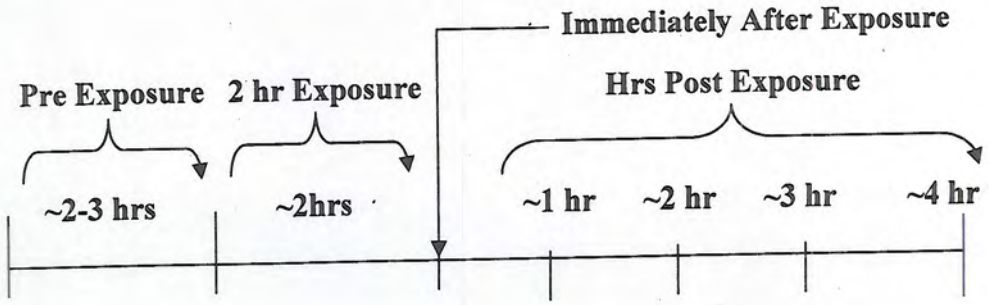
Day 1



Measurements & Collections	*	*				
Lung Function	*	*	*	*	*	*

****

Day 2



Measurements & Collections	*	*				
Lung Function	*	*	*	*	*	*

****

Day 3

Follow up
No Exposure

~2-3 hrs

Measurements & Collections	*
Lung Function	*

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Protocol: Written consent will be obtained. The subjects will be trained (on D0) in techniques for obtaining lung function measurements. Subjects also will exercise on a bicycle, and appropriate settings (the speed and grade of incline) required to produce a desired value of minute ventilation will be determined. On the first day of exposure, subjects will be assessed for vital signs (blood pressure, pulse, temperature and respiratory rate) at the check-in. Subjects may perform pre-exposure baseline lung functions in clean air, have resting heart rate variability (HRV) assessed, and urine, blood, and exhaled breath collected for later analyses of soluble mediators such as clotting factors, inflammatory proteins, and lipids and blood cell types and numbers. These samples may also serve to determine biomarkers of exposure, such as the concentration of PAHs . A questionnaire measuring perceived stress by the subject at the beginning of an exposure regimen will be filled out; this is included as Attachment #1. [Information about grading the questionnaire (Sheldon Cohen Perceived Stress Scale) is described at www.mindgarden.com].The subject then will enter the chamber already set for the appropriate conditions (DE & O3, DE alone, O3 alone, or clean air), and after 15 min begin exercising on a stationary bicycle. Each exposure session will consist of a 4 periods of 15 rest/15 min exercise at a level of approximately 25 liters/min/m^2 body surface area (BSA) in minute ventilation followed. Measurements of lung function may be obtained immediately after, and every hour for up to 4 hr post exposure. Resting HRV may be recorded, and blood, urine, and breath collected immediately post exposure. Following the day 1 exposure, the subject may remain at the Medical Station for a follow up measurements of lung function for approximately 2-4 hr. The subjects will go home with a heart rate monitor and blood pressure cuff. On Day 2, the subject will report to the medical station in the morning. The same measurements (resting HRV, lung function; symptoms; venipuncture performed) as Day 1 may be done pre- and post- exposure to O3. Subjects will return on Day 3 for follow up measurements but with no exposures.

Experimental procedures:
Medical history will be collected, including current medications and any recent illness.

Vital signs will be measured to include height, weight, heart rate, respiratory rate, blood pressure, oxygen saturation, and temperature. In addition a pregnancy test will be given to all women before each exposure regime.

Spirometry may be performed on a dry seal digital spirometer interfaced to a computer; volumes are corrected to body temperature and pressure (BTPS). From the forced expiratory maneuver, forced vital capacity (FVC), forced expired volume in the first second (FEV_1), and FEV_1/FVC.

Exhaled Breath Collection: The participant will be asked to breathe into a cooled tube for up to 15 minutes. The breath condensate may be collected and analyzed for markers of airway inflammation and immune system alterations and exposure markers. Additionally a small Tedlar bag of uncooled, exhaled breath will be collected (up to 5 min) to examine gas phase exposure and inflammation/immune system constituents.

For exercise measurements the subjects may breathe for up to 3 min through a pneumotachograph while performing exercise and ventilation parameters including the minute ventilation, tidal volume and breathing frequency will be obtained. Heart rate will be monitored throughout exposure using a telemetry monitor.

Cardiac measurements: Heart Rate Variability monitoring (ambulatory ECG) monitoring: On the day 1 and day 2 of each of the exposure regimes we will mount a Holter monitor to the participants. The skin may be shaved (men only) and prepped. Ten ECG electrodes may be attached and connected to the beeper-sized Holter monitor, which participants may wear around their waist. After mounting the monitor onto participants, they will be asked to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. Participants may be released while wearing the Holter monitor and return to the HSF the next morning (day 2 of the study segment) to obtain the Holter monitor information and change the batteries, if necessary. The monitor will be worn for day 2 and removed on day 3. This procedure may be conducted for all 4 exposure regimes. We will analyze time and frequency as well as re-polarization parameters. Participants will be given instructions for the removal of the monitor in case it becomes necessary.

Blood pressure (BP) measurements: BP will be monitored during the study. A BP cuff may be worn to measure BP intermittently. 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. The subjects may wear the cuff for day 1 and 2 of the study session and have it removed on day 3. Participants will be given instructions for the removal of the monitor in case it becomes necessary.

Venipuncture: Up to approximately 500 ml of blood may be collected for the 8 exposure days over a minimum of 8 weeks. This translates to approximately 50 ml of blood for each exposure day and 25 ml on the follow up day. A portion of the sample will be used for genotyping to examine sensitivity in selected blood types. Blood will be analyzed for, but not limited to, clotting/coagulation and inflammation factors and biomarkers of immune response.
A portion of the peripheral blood sample will be used for genetic testing, to identify potential polymorphisms that may make subjects more susceptible to air pollutants. In addition, samples may be stored for as yet undesignated research. Unwillingness to have samples used for genotyping or stored will NOT exclude a subject from the panel study. Consent for genotyping will be included with the general consent form.

Urine: The entire volume of a urine void will be collected on the pre- and post- exposure on Days 1 and 2, and the morning of day 3. Samples may be analyzed for markers of cigarette smoke exposure (eg, cotinine), creatinine for value normalization, exposure markers (eg, PAHs), and mediators of effect related to inflammation and immune system functions (eg, peptidoleukotrienes, other lipids).

Likely Personnel assignment

Subject recruitment and payment	Westat Corporation (Protocol #)

Chamber setup and maintenance Subject surveillance during exposure	TRC Environmental Corporation Martin Case, BS, Mike Madden, PhD, Tina Stevens, PhD, Howard Kehrl, MD, David Diaz-Sanchez PhD, Medical Station personnel
Lung function testing	Martin Case, BS, Mike Madden PhD, Tina Stevens, PhD Howard Kehrl, MD,
Blood sampling Subject screening tests Genomic assay	Human Studies Facility Medical Station personnel Human Studies Facility Medical Station personnel Michael Schmitt, MS,

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.

This research is designed to benefit society by gaining new knowledge. The subjects will not benefit personally from being in this research study other than by undergoing a free limited physical examination and screening.

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.

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

1. Blood sampling will be performed by well-trained personnel, and entails only a risk of mild discomfort with the infrequent possibility of lightheadedness, fainting, infection or developing a bruise. Subjects are closely monitored for any signs of faintness, and only allowed to leave the facility after a 15-min waiting period.
2. Breathing tests (spirometry): This pulmonary function tests are standard clinical tests that are commonly performed in hospitals and entail little or no risk to the subjects. However, cough or dizziness may occur during these tests. If these symptoms occur, they are usually temporary. Subjects will remain seated in a chair until symptoms disappear. There is an extremely small chance that the subject could have a bronchospasm or faint upon performing a forced breath expiratory maneuver for spirometry.
3. Exhaled breath condensate and exhaled breath gas: Minimal risk is associated with these procedures. The subject is seated in a chair during these collections and the technician is always available during this procedure in case the participant becomes light-headed due to hyperventilation.

4. Moderate exercise on a stationary bicycle entails the potential, although minimal, risk of occasional muscle soreness, cramps or general fatigue. These discomforts are temporary and not harmful. Heart rate and rhythm will be monitored continuously. Heart rate will be kept below age related maximum (i.e., 220 bpm – age in years). Exercise will be terminated at any time upon the request of the subject or if the investigator/medical staff observes problems while monitoring the ECG readout.
5. Ozone exposure: Potential risks, however, may include mild airway obstruction, irritation to the nose, eyes, throat and airways, pain on deep inspiration and cough. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. Ozone may induce an inflammatory reaction that may last for about 24-48 hours after exposure and may increase the chance of catching a cold.
6. 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.
7. Blood pressure and heart rate measurement: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal. Diesel exhaust (DE) exposure: The amount of DE (target of 300 $\mu g/m^3$) used in this study would be equivalent to concentrations of DE particulate matter that would be encountered at busy intersections in large urban areas and in occupational exposures. Occupational levels for some truck drivers are generally about 100-300 $\mu g/m^3$ and average ~900 $\mu g/m^3$ for some mines where diesel powered machinery are used. A recent study demonstrated diesel exhaust particulate matter concentrations during drive-by incidents averaged about 125 and199 μg/m3 at the height of an adult pedestrian and a child in a stroller, respectively [40].
8. Controlled, acute DE exposure studies in Sweden, using about 300 $\mu g/m^3$ of 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. DE particles contain some probable carcinogenic polycyclic aromatic hydrocarbons and other components, 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 time and concentrations to be used in this protocol are minimal relative to the durations required to induce lung cancer. Overall, there are no known long-term health risks in healthy individuals acutely exposed to DE at the PM concentrations given in this study. Short-term health effects include slight alterations in blood clotting, oxygen diffusion capacity and changes in heart rate variability. These symptoms typically disappear within 24 hrs of exposure. 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. No major adverse effects were observed in a completed diesel exhaust study of healthy adults (ages 18-40; entitled "Physiological, Cellular, and Biochemical Effects of Diesel Exhaust in Healthy Young Adults"; Mike Madden, PI; UNC IRB # # 99-0842) where the PM concentration averaged 106 ug/m3. 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 and the subject withdrawn from the chamber if the O_2 saturation value is $\leq$92%. 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 and rhythm, 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.

9. 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 a locked cabinet in a locked room at the US EPA facility. 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. 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. The data storage plan ultimately proposed by the PI is reviewed by the US EPA Quality Assurance officer assigned to the project. The data storage plan is reviewed for storage safety, confidentiality, backup, longevity, validity, integrity, and other issues. We believe that the data storage plan proposed is commensurate with the study size and complexity (eg, not a multisite study).

10. A formal stop to the study will be performed by the medical station staff after 4 subjects complete the 4 exposure regimes to assess whether highly adverse effects occurred with respect to lung and cardiac physiology responses. If not, the study will continue.

In addition, there may be uncommon or previously unknown risks that might occur. During all testing, subjects will be monitored continuously by the EPA personnel. A duty physician will be available in response to an emergency. A group of physicians employed by EPA and UNC Center for Environmental Medicine, Asthma & Lung Biology have primary responsibility for medical coverage of studies conducted in the EPA Clinical Research Laboratory. 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 for any challenge or exposure study.

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).

The research objective of this study is to determine if healthy volunteers safely exposed to low levels of O3 and DE alone or in combination, will show changes in cardiopulmonary endpoints including modest decrements in lung function. The study will follow a randomized, repeated measures design with each subject exposed to 4 different exposure regimens (see Figures 1 and 2 above). The data analysis will be focused on changes in lung function measurements, primarily FVC and FEV_1, between pre and post 2hr exposure for each exposure, but also blood endpoints include inflammatory factors (eg IL-6), blood clotting factors (eg fibrinogen), and susceptibility factors (eg specific genotypes such as glutathione-S-transferase M1 (GSTM1) null).

In evaluating exposure effects of O3 and DE, alone or in combination, upon lung function, the difference between the pre-exposure and post-exposure FEV1 will be calculated and the pre-post differences for the air and the exposures will be compared utilizing repeated measures ANOVA parametric test. Clinically significant change is determined to be a 10% decrease in FEV1 measured prior to and immediately after the exposure compared to the pre exposure value. For a conservative sample size calculation, we used one sided test for a single sample under the assumption of normal distribution with Type I error of 5% and Type II error of 20%. Therefore, a ρ value of 0.05 or less will be considered significant and the proposed sample size will provide adequate (80%) power for detecting a 10% decrease in ΔFEV1.

Sample size calculation was based on the Forced Expiratory Volume in 1 Second (FEV1) in response to the O3 exposure. Data for the power calculation were obtained from results of FEV_1 [41] for a constant acute exposure to 0.3 ppm O_3 versus clean air (CA). In 22 subjects, the pre exposure FEV_1 was 4.608 ± 0.514 (mean ± SEM) in CA exposure and 4.656 ± 0.592 in O_3. The post exposure FEV_1 was 4.64 ± 0.476 in CA exposure and 3.856± 0.687 in O_3. We used 0.687 as an estimate of standard deviation after the ozone exposure and estimated 10% decrease in pre-exposure FEV1 levels to be 0.46 ml. Based on these data and the test for the mean of a normal distribution with a one-sided alternative the required sample size is 14 subjects. We will recruit 15 subjects for this study to ensure the statistical power that can analyze small differences expected from low concentration exposures.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No _x_ 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).

All individuals who have been granted access to the data to perform their research-related duties have received full ethics training. Computer data files are password protected and subjects are coded as an unrelated subject identifying number. The participant's actual identity cannot be ascertained exclusively from these data. The participant's name will not be used in any publications. Access to the records of this study will be provided by the identifying number only and given only to those individuals associated with the study who require access to the data to perform their duties. All such individuals will be bound by this agreement of confidentiality. All records are maintained in a locked room in the medical records office of the USEPA 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 _x_ Encryption
__ Other (describe):
_x_ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device: password, encryption, and security pass needed to leave building with laptop.

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
_x_ 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.

The study data will be archived with identifiers by storage in a locked closet in the secured USEPA HSF building. If offsite storage space is ever required for the data, the data will be transferred to offsite storage according to the USEPA's record keeping guideline.

Specimens from subjects who consent for his/her samples to be stored will remain stored in a repository and will be released to investigators for use. Specimens from subjects who opt not to allow for storage will be destroyed at the end of the study.

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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.*

Prior to participation, all volunteers will be required to read and sign the consent form which asserts that they have read and understood the following: 1.) Subject participation is strictly voluntary; 2.) The purpose of the study; 3.) The nature and extent of subject participation; 4.) The subject's rights to withdraw at any time; 5.) The subject's right to privacy, 6.) The risks associated with participation; 7.) The method and schedule of compensation; and 8.) The limits of the EPA, University and PI's liability. The PI, co-PIs or study coordinator will briefly describe the study and answer any questions that each subject might have about the study. Subjects will have the opportunity to ask questions at any time during the study by contacting the PI and/or the study coordinators. 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 may 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 to that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, "Recruitment and Screening of Potential Subjects for EPA Studies" (95-0518; Howard Kehrl, PI). Subjects may be identified from mass emailing for recruits, website [https://www.epastudies.org/ with contact phone numbers listed as 919-966-0604 and toll free 888-279-9353] and newspaper and brochure advertising by Westat Corporation [a recent, advertisement brochure for subject recruitment is included as Attachment #2], and from the Westat database for subjects. These documents (email recruitment wording to current subjects from other studies and potentially new subjects , newspaper advertisement, a study description handout, study reminder notices to subjects, are attached in the appendix section of this application as Attachments 2-7. Every effort will be made to recruit women and members of racial minority groups into 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.

This study will take approximately 18 months (actual study time) to complete. The study duration is based on 1-2 exposure days per week and 1 subject on each exposure day. This scheduling, however, is subject to change depending on the availability of study subjects and the number of concurrent studies requiring the same chamber facility.

All subjects will have at least 13 visits to the research facility over approximately 3 months. On the first visit, the subject will go through a consent process and then a training session for approximately 3 hours. During each of three subsequent visits (exposure days), subjects are required to check in by about 8:00 am and will be discharged approximately at 4:00 pm. Therefore, subjects will remain in the research facility for approximately 8 hours on exposure day 1. The following day, subjects are required to check in by 8:00 am and will be discharged approximately at 4:00 pm. Therefore, subjects will remain in the research facility for approximately 8 hours on exposure day 2. The 3rd day the subjects are required to check in by 8:00 am and will be discharged approximately at 11:00 am, for a total of approximately 3 hrs. Subjects will come back for the next 3 regimes following the same schedule for days 1-3 with 13 or more days between each regime. The total amount of time at this facility will be ~79 hr

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

All subjects will be studied in the USEPA Human Studies Facility located at 104 Mason Farm Road in Chapel Hill on the UNC Campus.

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 and phone conversations will be conducted from either the Westat Recruiting office or the Medical Station 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. Physical exams and other procedures will occur in appropriate clinical areas of the EPA. Occasionally 2 subjects may be seen in the clinical area at one time; however, sensitive information is only discussed in private (medication use, pregnancy test results). The subjects will be scheduled with the TRC (Chamber operators) group using a study number; however TRC only maintains a single separate log with the study number and the subject's number but not personal identifying information. Subjects may be contacted by email to schedule/remind them about study visits or to answer specific questions. Any information sent via US mail or campus mail will simply have a return address, no other study specific information.

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). Be aware that payment over a certain amount may require the collection of 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 the subject's 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 ($12 per hour) and some procedures in the study (See below). In addition, subjects traveling from areas beyond Chapel Hill 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 compensated for his/her participation up to that point and will receive compensation at the hourly rate of $12 per hour for that particular scheduled 3 day study session.

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% of procedure fees 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.

Money received by participants in research studies is normally treated as ordinary income by taxing authorities and payments made to you to the Internal Revenue Service as required by law. The money is not intended to coerce completion or participation, but to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study.

Subject Compensation for Procedures during Diesel and Ozone Exposure Study

Training Day (assume 3 hr @$12/hr)	$36
First Exposure:	
Day 1	
Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Stress questionnaire	$2.50
Blood Draw (twice)	$30
Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60
Lunch	$5
Day 2	
Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Blood Draw (twice)	$30

Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60
Lunch	$5
Day 3	
Hourly payment (assume 3 hr @$12/hr)	$36
Blood Draw	$15
Exhaled breath collection	$5
Urine Sample	$5
Lung function	$10

[Total payment First Exposure]	$855.50
Second Exposure	$855.50
Third Exposure	$855.50
Fourth Exposure	$855.50
Completion Bonus (for completing all 4 exposure sessions)	$100
APPROXIMATE TOTAL (including training day)	$3,558.00

Other Possible Costs:
Extra Blood sticks ($10 each)
Extra hours ($12 per hour)
Travel from out of town (based on mileage)

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 excluding child and dependent care. Parking fees are covered by the study in the form of parking vouchers. All study related diagnostic tests such as pregnancy tests, pulmonary function test and labs are covered by the study. Westat subjects are primarily recruited from the Chapel Hill area. Volunteers who come from outside this area may be reimbursed for mileage at the current government rate.

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

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

IRB Study # 09-1344 GCRC #: N/A
Consent Form Version Date: September 29, 2009

Title of Study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults
Principal Investigator: Tina Stevens, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 843-8031
Email Address: madden.michael@epa.gov
Co-Investigators: Michael Madden, PhD; David Diaz-Sanchez, PhD; Howard Kehrl, MD
UNC-Chapel Hill Phone number: (919)966-6257, (919) 966-0676, (919) 966-6208
Email Address: stevens.tina@epa.gov; diaz-sanchez.david@.epa.gov; kehrl.howard@epa.gov;
Faculty Advisor: N/A

Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-6257 (Michael Madden)
Study Contact email: madden.michael@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 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?

We breathe a complex mixture of ambient air pollution, and ozone and diesel exhaust from an engine are generally major and important components. Controlled exposures of volunteers to either pollutant have resulted in biological effects such as lung physiological changes. However it is not known if co-exposure to both pollutants, similar to inhaling polluted ambient air, can induce effects than either pollutant alone. Additionally it is also uncertain if exposure to diesel exhaust alone, or diesel exhaust mixed with ozone, can alter the body's responses to breathing ozone the following day. This study proposes to examine whether exposure to both ozone and diesel exhaust can cause more of an effect than from an either pollutant alone. This study will also determine if breathing diesel exhaust can change a response upon exposure to ozone the day after. The purpose of this study is to measure cardiopulmonary responses in healthy young adults before, during, and after exposure to combinations of ozone (O3) and diesel exhaust for 2 hours with moderate exercise and to primarily investigate whether diesel exhaust modulates the O3-induced effects on the lung and cardiovascular systems.
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.

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, chronic obstructive pulmonary disease, tuberculosis, coughing up blood, recurrent pneumonia, chronic or allergic rhinitis or acute or chronic sinusitis.
- You cannot perform moderate exercise
- You cannot remain in a small exposure chamber for about 2 hours
- 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 less than 18 years old or greater than 55 years old
- 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 ("NSAIDS") for 48 hr prior to the exposure and post-exposure visits. Acetaminophen, eg, Tylenol, is 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 days.
- Not consume caffeine for 1-2 hours prior to the exposures on days 1 & 2 and post-exposure visits.
- Stop taking vitamin C or E or medications which may impact the results of the exposures at least 2 weeks prior to the study and for the duration of the study. Medications not specifically mentioned here may be reviewed by the investigators prior to your inclusion in this study.
- Avoid the use of ozone-based home air purifiers during study participation

How many people will take part in this study?
If you decide to be in this study, you will be one of approximately 15 people who will complete this research study.

How long will your participation in this study last?
You will have up to 13 visits to the research facility over approximately about 10 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 4 exposure regimens, each of which will consist of 2 consecutive exposure days and 1 follow-up visit approximately 18 hrs after the last exposure. Each exposure day will last approximately 8 hours and the follow-up visit will be approximately 3 hrs long. The 4 exposure regimens will occur at least 13 days apart.

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

What will happen if you take part in the study?
During the course of this study, the following will occur:

Training:
You should have already undergone 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. Today you will familiarize yourself with some of the techniques you will perform for the study. These include instruction on the use of the stationary bicycle to be used during the study, how to perform spirometry and an exhaled breath collection, and heart rate variability (HRV) and blood pressure (BP) monitors.

After satisfactorily completing the training session, you will be scheduled for your first exposure through a company contracting with the US EPA (currently Westat).

You will be exposed to mixtures of air pollutants in 4 different regimens. Each regimen will last about 2 ½ days. During the regimens, a number of physiological and biochemical measurements will be made on you. You will have the opportunity to complete all 4 regimens separated by at least 2 weeks. The first day of each regimen you will be exposed to either clean air, diesel exhaust at about 300 micrograms particles/meter^3, ozone at about 0.3 parts per million (ppm), or diesel exhaust mixed with ozone. The second day of regimen, you will be exposed to approximately 0.3 ppm ozone. The third day of each regimen you will not be exposed to any air pollutants, but will have follow up measurements made.

You may terminate your participation from this study at any time. You will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms may include chest pain, difficulty breathing, 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 signs of cell damage, changes in clotting factors, as well as increases in inflammation. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Below is a description of what will be required from you and a summary of the measurements to be made on you on each day of each exposure regimen:

Day 1:

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, NSAID medications, 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 will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. There will be an on-time bonus of $25 for arriving by 8:05 a.m. You will need to wear comfortable clothes and shoes and bring a change of clothes.

Pre-exposure measurements:

Prior to the day 1 exposure, you will have you may:

- Answer a questionnaire on stress.
- Have your vital signs checked (heart rate, respiratory rate, blood pressure, and oxygen saturation level, and for women, a pregnancy test).
- 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 may be kept on you for the next two days and will be removed on Day 3 of the exposure session. 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 morning of the follow up visit (Day 3), there may be a 30 minute measurement of your heart rate and then the monitor will be removed.

- Blood pressure (BP) may be measured intermittently by a BP monitor. A blood pressure cuff and a monitor which is about the size of the Holter monitor may be fitted and will remain in place most of the time until Day 3. You will be asked to keep your arm relaxed and still when the pressure cuff is inflating.
- Have about 25 ml blood drawn.
- 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.
- Be asked to provide a urine sample; if you are female it will be tested to see if you are pregnant.
- We may collect your breath in two manners- one being to cool your exhaled breath to capture the water vapor, and the other just collecting the gas portion uncooled
- You will then enter an exposure chamber.

During the Day 1 exposure, you may:

- Be exposed to clean air or air pollutants for 2 hours. You will be asked to perform intermittent moderate exercise in an exposure chamber.
- At certain times during the exposure, you may be asked to breathe into a mouthpiece so that your rate of breathing can be measured. A staff member will be seated outside the chamber to observe you at all times and a physician will be available during the entire exposure session. During the exposure, your heart rhythm and rate, 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 a severe headache, nausea or vomiting the exposure will be terminated immediately.

Immediately following the Day 1 exposure, you may:

- Have your vital signs checked.
- Perform 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 about 1 assessment each hour
- We may collect your breath

- 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 25 ml).
- Provide a urine sample.

Later in the day, you may:

- Have spirometry assessed for up to 4 hr post exposure
- Provide a urine sample
- Be assessed and discharged by the nursing staff. You will spend about 8 hours at the EPA facility.

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest and a blood pressure monitor cuff on your arm for the following two nights, we will give you instructions on how to care for and remove the monitors when necessary.

Day 2:

You will return to the HSF the next morning at 8:00 a.m. and you will perform testing similar to the first day.

Pre-exposure measurements and procedures will be performed as on Day 1, with the exception of the stress questionnaire. You will then enter the exposure chamber.

During the exposure on Day 2 you will be exposed to ozone at a concentration of approximately 0.3 ppm for 2 hours with intermittent moderate exercise in an exposure chamber. Similar measurements and monitoring will be performed as during the exposure on Day 1.

- **Similar measurements and procedures may be performed with you following the Day 2 exposure as on Day 1.** Your health will be assessed and you will be discharged by the nursing staff. You will spend about 8 hours at the EPA facility.

Day 3:

Follow up Visit:
Similar procedures and measurements may be made as the pre-exposure measurements of the two previous days. The Holter monitor attached to you will be removed. Your health will be assessed and you will be discharged by the nursing staff. You will spend about 8 hours at the EPA facility. You will spend about 3 hours at the EPA facility.

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 screening for this study you will have received a medical examination that included 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 in general 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. Eye wear protection goggles are available during the exposures to reduce possible 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 these exhausts, you will only be exposed to them for 2 hours. You could be potentially inhale a similar amount 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 (i.e., within the building facility) during exposures, and is available to respond in an emergency.

Ozone exposure: Potential risks may include mild airway obstruction, irritation to the nose, eyes, throat and airways, pain on deep inspiration and cough. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. O3 may induce an inflammatory reaction that may last for about 24 hours after exposure and may increase the chance of catching a cold.

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 during your visit, you should tell the nursing staff. If irritation occurs while you are home, you should remove the electrodes, wash gently with mild soap and water, and tell the study coordinator or nursing staff in the morning.

Blood pressure and heart rate measurement: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

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. Occasionally fainting or light-headedness occurs, and injury is minimized by having you seated. 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.

Exhaled breath collection: Minimal risk is associated with these procedures. You will be seated in a chair during collections and technicians are always available during this procedure in case you become light-headed.

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 a 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 human health research 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 an appointed US EPA physician to be due to your participation in this research, the US 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 States 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 approximately $3,558.00

We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill you will be reimbursed for mileage at the US Government mileage rate in effect at the time. 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:

Subject Compensation for Procedures during Diesel and Ozone Exposure Study

Training Day (assume 3 hr @$12/hr)	$36
First Exposure:	
Day 1	
Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Stress questionnaire	$2.50
Blood Draw (twice)	$30
Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60
Lunch	$5
Day 2	
Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Blood Draw (twice)	$30
Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60
Lunch	$5
Day 3	
Hourly payment (assume 3 hr @$12/hr)	$36
Blood Draw	$15
Exhaled breath collection	$5

Urine Sample	$5
Lung function	$10
[Total payment First Exposure]	$855.50
Second Exposure	$855.50
Third Exposure	$855.50
Fourth Exposure	$855.50
Completion Bonus (for completing all 4 exposure sessions)	$100
APPROXIMATE TOTAL (including training day)	$3,558.00

Other Possible Costs:
Extra Blood sticks ($10 each)
Extra hours ($12 per hour)
Travel from out of town (based on mileage) @ current federal travel rates

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. Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be compensated for his/her participation up to that point and will receive compensation at the hourly rate of $12 per hour for the scheduled 3 day study session.

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% of procedure fees up to a total maximum of $100 for canceled 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, or other unforeseen events. When feasible, the subject will be rescheduled.

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. The money is not intended to coerce completion or participation, but to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study.

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.

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 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:
Michael Madden, PhD 919-966-6257 Tina Stevens, PhD 919-843-8031

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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Title of Study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults

IRB Study # 09-1344

Principal Investigator: Tina Stevens, 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

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

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IRB Study # 09-1344
Consent Form Version Date: 9/29/09

Title of Study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults

Principal Investigator: Tina Stevens, PhD

UNC-Chapel Hill Department: N/A
UNC-Chapel Hill Phone number: (919)843-8031, **Fax:** 919-966-6367
Email Address: stevens.tina@epa.gov

Co-Investigators: Michael Madden, PhD; David Diaz-Sanchez, PhD Howard Kehrl, MD
stevens.tina@epa.gov;; Diaz-Sanchez.David@.epa.gov; kehrl.howard@epa.gov;

Funding Source and/or Sponsor: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-6257 (Michael Madden)
Study Contact email: madden.michael@epa.gov

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

You may refuse to take part in research.

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 specimen repository or "biobank?"
Research with blood, tissue, cells or body fluids (specimens) can help researchers understand how the human body works. Research can also answer other questions by using specimens. Researchers may develop new tests to find diseases, or new ways to treat diseases. In the future,

research may help to develop new products, such as drugs. Specimens are commonly used for genetic research. Sometimes researchers collect and store many specimens together and use them for different kinds of research, or share them with other scientists; this is called a specimen repository or "biobank."

All specimens for this study will be labeled with your study subject number that does not include personal identification information and will be stored in a repository where only project members will have access to the specimens. There is a need to store specimens in such a repository because this will be an ongoing study where specimens from subjects will be collected over an extended period of time. Storing of specimens allows for all specimens to be processed at the same time.

It also makes it possible to keep any remaining specimens in our biobank indefinitely and allows our scientists the opportunity to further study these specimens with as yet unknown questions and techniques. Research studies and questions in which the specimens may be used have not yet been determined. These studies may involve genetic research. Genetic research is about finding the specific location of genes, learning how genes work, and investigating relationships between a certain gene and the environment or people's habits and diets, and different diseases.

How will the specimens be collected?

Your specimens will be collected during the research study listed on the first page of this consent. No additional specimens will be collected from you.

What will happen to the specimens?

Study specimens will be stored in a secure room with restricted access at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill, North Carolina. The specimen will be prepared, labeled with the study subject identification number, and stored indefinitely in a freezer for future testing under *IRB# 07-1768, Repository for Storage of Human Specimens*. Names of subjects associated with ID numbers will be archived and locked; only medical and scientific personnel directly associated with this study will have access to this information. No personal identifying information will be attached to the biologic fluid specimen. Portions of the specimen may be shared with researchers at other scientific institutions, however, only coded specimens will be sent and the investigator will employ a data use agreement. Under no circumstances will any identifying information be sent along with specimens. The decision to destroy the specimens may be made by the investigator or by you if you notify the investigator in writing that you no longer want the specimens stored.

What are the possible benefits to you?

Benefits to you are unlikely. Research is designed to benefit society by gaining new knowledge. You will not benefit personally from having your specimens stored in this biobank.

What are the possible risks or discomforts involved with the use of your specimens?

This research is funded by the United States Environmental Protection Agency. Several of the investigators including the Principal Investigator are federal employees. The researchers do not, however, have a direct financial interest in the final results of the study.

What if you have questions about this research?
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.

What if you have questions about your rights as a research subject?
All research on human volunteers 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 e-mail to IRB_ subjects@unc.edu. You may also contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

Title of study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults

Principle investigator: Tina Stevens, 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 Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

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: Michael Madden
Environmental Protection Agency

From: Biomedical IRB

Date: 8/11/2009
RE: Notice of IRB Deferral following Full Board Review on 8/10/2009
Submission Type: Initial
Study #: 09-1344
Study Title: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust With Moderate Exercise in Healthy Adults

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 has deferred further consideration of this study to a future meeting to allow you to provide additional information and/or make substantive change(s), as described below:

Study Specific Details:

1. IRB application A.4.3: Please indicate whether or not all races and ethnic groups will be considered for this study.
2. IRB application, A.4.2, rationale: the text provided is highly detailed. Please provide an introductory paragraph which clearly and succinctly addresses the need for this research and why healthy subjects should be exposed to O3 and DE. Please also summarize this information under purpose of study in the consent form.
3. IRB application A.4.5: please complete the waiver of written consent as applicable to the telephone screen.
4. IRB application B1: Please provide a copy of the telephone script to be used for the screening procedure described.
5. IRB application, A.4.7, risks: Please justify not using a DSMB.
6. IRB application, A.4.7, risks, pg 16: please note if there are any possible long term effects from the DE exposure.
7. Consent p. 2. Smoking within 1 year is listed as exclusionary. In the IRB application it is smoking within two years (p. 9). Please reconcile.
8. Consent, what will happen: Please consider revising this section as it quite long and difficult to follow. The time required for each visit will need to be included.
9. Consent, pg 7, benefits: please revise that there are no direct benefits to subjects but possible benefits to society. Research procedures cannot be listed as "benefits."
10. Consent p. 8. Please describe the location and availability of the licensed "duty physician" on call for this protocol.
11. Advertisement: the compensation differs from the IRB application and consent. Please reconcile.
12. Specimen consent: please re-submit with study number at top. Please insert the subject injury

language as per template.

13. Please see marked consent for edits and suggested changes.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the items listed. Any additional changes (including new materials) must also be listed and discussed in the memo. You will need to return 3 copies of the revised application and all other study materials to the IRB, two in which all changes are underlined and the third a "clean" copy with no underlining.

CC:
Michael Schmitt
David Diaz-Sanchez
Tina Stevens, Epa
Howard Kehrl, Medicine
Robert Truckner, (EPA), Non-IRB Review Contact

MARKED

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

IRB Study # 09-1344 **~~GCRC #~~:**
Consent Form Version Date: July 27, 2009

Title of Study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults
Principal Investigator: Michael Madden, PhD
UNC-Chapel Hill Department: US Environmental Protection Agency
UNC-Chapel Hill Phone number: (919) 966-6257
Email Address: madden.michael@epa.gov
Co-Investigators: Tina Stevens, PhD; David Diaz-Sanchez, PhD; Howard Kehrl, MD
UNC-Chapel Hill Phone number: (919)966-6257, (919) 966-0676, (919) 966-6208
Email Address: stevens.tina@epa.gov; diaz-sanchez.david@.epa.gov; kehrl.howard@epa.gov;
Faculty Advisor: N/A

Funding Source: US Environmental Protection Agency Intramural Federal Research

Study Contact telephone number: (919) 966-6257 (Michael Madden)
Study Contact email: madden.michael@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 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 purpose of this study is to measure cardiopulmonary responses in healthy young adults before, during, and after exposure to combinations of ozone (O3) and diesel exhaust for 2 hours with moderate exercise and to primarily investigate whether diesel exhaust modulates the O3-induced effects on the lung and cardiovascular systems.
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.

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 cannot perform moderate exercise
- 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 over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications ("NSAIDS") for 48 hr prior to the exposure and post-exposure visits.
- 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 consume caffeine for 1-2 hours prior to the exposures on days 1 & 2 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 15 people who will complete this research study.

How long will your participation in this study last?
You will have up to 13 visits to the research facility over approximately 18-20 weeks if you are eligible for the study (see attached study design flow chart).

Your participation in this pilot study will include one training session (today) for about 3 hours, 4 exposure regimens, each of which will consist of 2 consecutive exposure days and 1 follow-up visit approximately 18 hrs after the last exposure. Each exposure day will last approximately 8 hours and the follow-up visit will be approximately 3 hrs long. The 4 exposure regimens will occur at least 2 weeks apart.

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

What will happen if you take part in the study?
During the course of this study, the following will occur:

Too long Entire section hard to understand

Screening and Testing:
You should have already undergone 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.

Today you will familiarize yourself with some of the techniques you will perform for the study. These include instruction on the use of the stationary bicycle to be used during the study, how to perform spirometry and an exhaled breath collection, and heart rate variability (HRV) and blood pressure (BP) monitors.

After screening and testing 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 of either filtered air, diesel exhaust at a concentration of approximately 300 $\mu g/m^3$, O3 at a concentration of approximately 0.3 ppm, or a combination of diesel exhaust and O3, for 2 hours with intermittent moderate exercise on a stationary bicycle in a small exposure chamber. The following day you will be exposed to O3 for 2 hours with intermittent exercise. The next day will be a follow-up visit. You will have the opportunity to complete all 4 regimens separated by at least 2 weeks.

You may terminate your participation from this study at any time. You will be monitored for symptoms that you may develop during the exposure and over the following 24 hour period. The symptoms may include chest pain, difficulty breathing, 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 signs of cell damage, changes in clotting factors, as well as increases in inflammation. The study physicians will stop the study if symptoms and/or changes detected in the blood samples that are considered clinically significant.

Day 1:

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, NSAID medications, 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 will be asked to eat a light breakfast and arrive at the EPA medical station at approximately 8 am. There will be an on-time bonus of $25 for arriving by 8:05 a.m. You will need to wear comfortable clothes and shoes and bring a change of clothes.

Prior to the day 1 exposure, you may:

- Answer a questionnaire on stress
- 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 may be kept on you for the next two days and will be removed on Day 3 of the exposure session. 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 morning of the follow up visit (Day 3), there may be a 30 minute measurement of your heart rate and then the monitor will be removed.
- Blood pressure (BP) may be measured intermittently by a BP monitor. A blood pressure cuff and a monitor which is about the size of the Holter monitor may be

fitted and will remain in place until Day 3. You will be asked to keep your arm relaxed and still when the pressure cuff is inflating.

- Have about 25 ml blood drawn.
- 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.
- Provide a urine sample.
- We may collect your breath in two manners- one being to cool your exhaled breath to capture the water vapor, and the other just collecting the gas portion uncooled
- You will then enter an exposure chamber.

During the Day 1 exposure, you may:

- Be exposed to either filtered air, diesel exhaust at a concentration of approximately 300 $\mu g/m^3$, ozone at a concentration of approximately 0.3 ppm, or a combination of DE and O3, for 2 hours with intermittent moderate exercise in a small exposure chamber.
- At certain times during the exposure, you may be asked to breathe into a mouthpiece so that your rate of breathing can be measured. A staff member will be seated outside the chamber to observe you at all times and a physician will be available during the entire exposure session. During the exposure, your heart rhythm and rate, 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 a severe 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 Day 1 exposure, you may:

- Have your vital signs checked.
- Perform 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 about 1 assessment each hour
- We may collect your breath
- 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 25 ml).
- Provide a urine sample.

Later in the day, you may:

- Have spirometry assessed for up to 4 hr post exposure
- Be assessed and discharged by the nursing staff.
- Provide a urine sample

Importantly, because you will be asked to wear the portable ECG monitor attached to your chest and a blood pressure monitor cuff on your arm for the following two nights, we will give you instructions on how to care for and remove the monitors when necessary.

Day 2:

You will return to the HSF the next morning at 8:00 a.m. and you will perform testing similar to the first day:

Prior to the day 2 exposure, you may:

- Have your vital signs checked
- Have your baseline heart rate viability (HRV) measured by a Holter monitor
- Blood pressure (BP) will be measured intermittently by a BP monitor
- Have about 25 ml blood drawn.
- Have a breathing test (spirometry).
- Provide a urine sample.
- We may collect your breath
- You will then enter the exposure chamber.

During the exposure on day, you may:

- You will be exposed to ozone at a concentration of approximately 0.3 ppm for 2 hours with intermittent moderate exercise in an exposure chamber
- At certain times during the exposure, you may be asked to breathe into a mouthpiece so that your rate of breathing can be measured.

Immediately following the Day 2 exposure, you may:

- Have your vital signs checked.
- Perform spirometry.
- We may collect your breath
- 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 25 ml).
- Provide a urine sample.

Later in the day, you may:

- Have spirometry assessed for up to 4 hr post exposure
- Be assessed and discharged by the nursing staff.
- Provide a urine sample

Day 3:

Follow up Visit, you may:

- Have your vital signs checked.
- Have a breathing test (spirometry).
- You will recline quietly for 20 minutes, after which the ECG monitor will take a 10-minute measurement of your heart rhythm.
- Have your breath collected
- Provide a urine sample
- Have blood drawn (about 25 ml).
- Have the Holter monitor and blood pressure 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? benefit society

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.

These are not benefit

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. Eye wear protection goggles are available during the exposures to reduce possible 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 these exhausts, you will only be exposed to them for 2 hours. You could be potentially inhale a similar amount 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.

Ozone exposure: Potential risks may include mild airway obstruction, irritation to the nose, eyes, throat and airways, pain on deep inspiration and cough. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. O3 may induce an inflammatory reaction that may last for about 24 hours after exposure and may increase the chance of catching a cold.

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 during your visit, you should tell the nursing staff. If irritation occurs while you are home, you should remove the electrodes, wash gently with mild soap and water, and tell the study coordinator or nursing staff in the morning.

Blood pressure and heart rate measurement: Similar to the regular blood pressure measurement, the risk associated with blood pressure monitor is considered minimal.

Venous blood sampling: Th~~e risks associated with taking blood samples are considered minima~~l. 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. What about fainting?

Exhaled breath collection: Mi~~nimal risk is associated with these procedures.~~ You will be seated in a chair during collections and technicians are always available during this procedure in case you become light-headed.

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 a 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 human health research 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 an appointed US EPA physician to be due to your participation in this research, the US 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 States 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.

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 $3,558.00

We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill you will be reimbursed for mileage at the US Government mileage rate in effect at the time. 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:

Subject Compensation for Procedures during Diesel and Ozone Exposure Study

Training Day (assume 3 hr @$12/hr)	$36
First Exposure:	
Day 1	
Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Stress questionnaire	$2.50
Blood Draw (twice)	$30

Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60
Lunch	$5

Day 2

Hourly payment (assume 8 hr @$12/hr)	$96
On-time bonus (8 a.m.)	$25
Blood Draw (twice)	$30
Urine Sample (3x)	$15
Exhaled breath collection (twice)	$10
24 hr Holter Monitoring	$100
24 hr Blood Pressure Monitoring	$50
Lung function (6x)	$60
Lunch	$5

Day 3

Hourly payment (assume 3 hr @$12/hr)	$36
Blood Draw	$15
Exhaled breath collection	$5
Urine Sample	$5
Lung function	$10

[Total payment First Exposure]	$855.50
Second Exposure	$855.50
Third Exposure	$855.50
Fourth Exposure	$855.50
Completion Bonus (for completing all 4 exposure sessions)	$100
APPROXIMATE TOTAL (including training day)	$3,558.00

Other Possible Costs:
Extra Blood sticks ($10 each)
Extra hours ($12 per hour)
Travel from out of town (based on mileage) @ current federal travel rates

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. Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be compensated for his/her participation up to that point and will receive compensation at the hourly rate of $12 per hour for the scheduled 3 day study session.

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% of procedure fees up to a total maximum of $100 for canceled 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, or other unforeseen events. When feasible, the subject will be rescheduled.

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. The money is not intended to coerce completion or participation, but to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study.

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.

Please revise font all same size

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 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.

<u>What if you have questions about this study?</u>
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:
Michael Madden, PhD 919-966-6257 Tina Stevens, PhD 919-843-8031

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.

<u>What if you have questions about your rights as a research subject?</u>
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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Title of Study:

Principal Investigator: Michael Madden, 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

Confusing – too scientific
re format for easier understanding

Figure 1

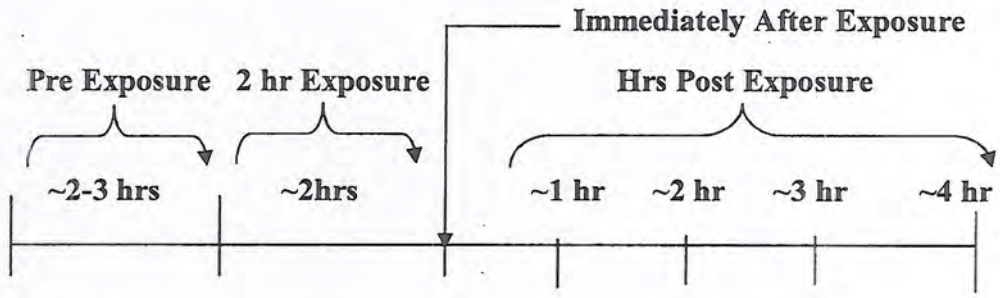
Experimental design:

	Day 1	Day 2	Day 3
Exposure Regime 1	DE + O3	O3	follow up (no exposure)
Exposure Regime 2	O3	O3	follow up (no exposure)
Exposure Regime 3	DE	O3	follow up (no exposure)
Exposure Regime 4	Filtered Air	O3	follow up (no exposure)

Figure 2

Endpoints:

Day 1



Measurements & Collections	*	*				
Lung Function	*	*	*	*	*	*

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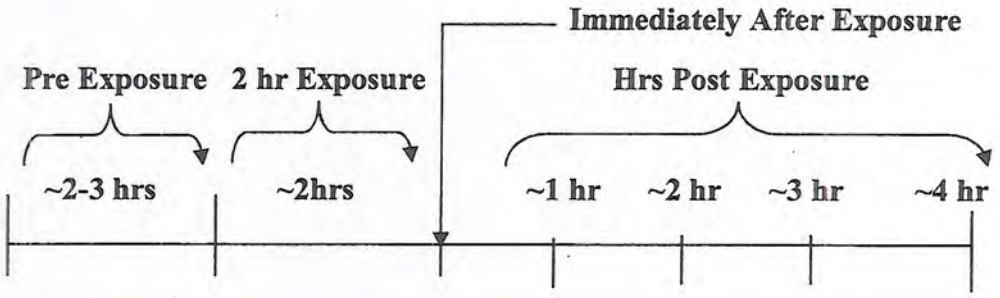
Day 2



Measurements & Collections	*	*				
Lung Function	*	*	*	*	*	*

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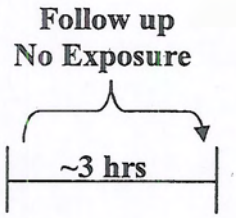
Day 3



Measurements & Collections	*
Lung Function	*

N4

Full Committee Review
Initial

Receipt Date : 7/27/2009 Expiration Date : Previous Review Type:

IRB: Biomedical PI: Michael Madden IRB ID : 09-1344 IRB Admin :

Title: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust With Moderate Exercise in Healthy Adults

Agenda Date 8/10/2009 Reviewer 1: **Mann, Doug** (chair) Reviewer 2: **Yu, Bing**

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 :

CONSENTS: ADULT
SPEC W/ID

MISSING AS OF 7/28/09:

? scale - have study ID#

QUESTIONNARE, ADS

consent - ~~[illegible]~~ confusing text - font

— FINDINGS - HIPAA N/A

- tables not clear - uses scientific name "endpoints"

- consent - benefits - delete what will happen - reformat

D

PROCESSING STEPS (OFFICE USE ONLY):

☐ Reviewer Checklist completed
☑ Minor Stipulation letter:
- ☐ Draft letter prepared GMB 08/11/09
- ☑ Approved by chair as attached (Initials/Date: 8/11/09 JAM)
- ☐ 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: 1 - MARKED
Other attachments: Ø

FINAL ACTIONS:

☐ Approved
☐ Approved with Minor Stipulations
☐ NHSR
☐ Deferred
☐ Disapproved
☐ Return to sender
☐ Closure

09-1344

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH & ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
RESEARCH TRIANGLE PARK, NC 27711

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IRB UNC-CH

MD# 58B, Human Studies Division, Clinical Research Branch
104 Mason Farm Road, CB# 7315
US EPA Human Studies Facility
Chapel Hill, NC 27599-7315
919-966-6257 (Office) 919-966-6367 (FAX)
madden.michael@epa.gov

July 27, 2009

Biomedical Research IRB Committee Members
UNC Office of Human Research Ethics
CB# 7097, Med. Building 52
Carolina Campus

Dear Committee Members:

I am submitting a protocol (original and two copies) to the Biomedical Research IRB Committee for review. The protocol ("Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults"; Michael Madden, PI) proposes controlled human subject exposures to diesel exhaust at the U.S. EPA facility on the UNC Campus. The Principal and Co-principal Investigators are U.S. EPA employees, with three of them (including myself) holding adjunct appointments with UNC.

I would be glad to answer any question you or the Committee has about the protocol. Thank you for your attention in the review of this document.

Respectfully,

Michael Madden

Michael Madden, Ph.D.

Research Biologist, U.S. Environmental Protection Agency, EPH Division & Clinical Research Branch

09-1344

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JUL 27 2009

IRB UNC-CH

OFFICE OF HUMAN RESEARCH ETHICS

Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 8-May 19th

Part A.1. Contact Information, Agreements, and Signatures

Title of Study: Cardiopulmonary Responses to Exposure to Ozone and Diesel Exhaust with Moderate Exercise in Healthy Adults

Date: July 27, 2009

Name and degrees of Principal Investigator: Michael Madden, Ph.D
Department: US EPA Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315

Phone #: (919) 966-6257 Fax #: (919) 966-6367 Email Address: madden.michael@epa.gov

Name and degrees of Co-Investigator: Tina Stevens, Ph.D., David Diaz-Sanchez, Ph.D., Howard Kehrl, MD

For trainee-led projects: __ undergraduate __ graduate __ postdoc __ resident __ other
Name of faculty advisors: N/A

Department: Mailing address/CB #:

Phone #: Fax #: Email Address:

Department: Mailing address/CB #:

Phone #: Fax #: Email Address:

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: Michael Schmitt Mailing address/CB #: 104 Mason Farm Rd. Chapel Hill, NC 27599-7315

Phone #: (919) 966-0647 Email Address: schmitt.mike@epa.gov

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:**
Co-PI: David Diaz-Sanchez, PhD (diaz-sanchez.david@epa.gov); Tina Stevens, PhD (stevens.tina@epa.gov); Howard Kehrl, MD (kehrl.howard@epa.gov)
Robert Devlin, Ph.D; Maryann Bassett, RN; Martha Sue Carraway, MD; Martin Case, BS; Andrew Ghio MD; Tracey Montilla, RN; Ana Rappold, PhD; Joachim Pleil, Ph.D; Michael Schmitt, MS; and Heidi Hiers, RN;

Name of funding source or sponsor (*please do not abbreviate*): Environmental Protection Agency
__ not funded x_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify):
Sponsor or award number: N/A

RAMSeS number (from Office of Sponsored Research):
For industry sponsored research (if applicable):
Sponsor's master protocol version #: Date:
Investigator Brochure version: Date:
Any details you need documented on IRB approval:

Checklist of Items to Include with Your Submission

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
☑	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; (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal).	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 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.

Michael C. Madden 7/27/09

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

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. 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_

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

I certify that I am employed as a research biologist by the U.S. Environmental Protection Agency (US EPA, the sponsor of this research) and as such collect salary and possible awards from the Agency.

Michael Madden 7/27/09

Michael Madden

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.

Michael Madden 7/27/09

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.

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: The purpose of this study is to measure cardiopulmonary responses in healthy young adults before, during, and after exposure to combinations of ozone (O3) and diesel exhaust (DE) for 2 hours with moderate exercise and to primarily investigate whether DE modulates the O3-induced effects on the lung and cardiovascular systems.

Participants: Fifteen (15) healthy young men and women in the age of 18 – 55 years

Procedures (methods): Each subject will be exposed in environmentally controlled exposure chambers to 4 exposure regimens. Each regimen will require a 2 hr exposure with intermittent, moderate exercise on 2 consecutive days. Day 1 exposure will consist of a combined exposure to DE and O3, O3 only (i.e., no DE), or DE only, or clean air (CA) only. For all 4 regimens, subjects will return the next day (Day 2) to be exposed to O3 alone and again on Day 3 for a follow up visit. Each regimen will be separated by at least 2 weeks. Techniques measuring lung and cardiac physiology will be performed pre, during, and post exposure. Blood, urine, and breath samples will also be obtained and analyzed for immune and inflammatory markers, clotting factors, susceptibility factors, and exposure markers.

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 air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infections, exacerbation of asthma symptoms, chronic bronchitis, ischemic heart disease, and stroke [1] and other health effects. Understanding the components responsible for these effects is difficult because ambient air pollution is a complex mixture of gases and particulate matter (PM).

One of the best studied gas phase pollutants is ozone (O3). Effects of O3 exposure in a controlled exposure setting have been well documented particularly for decrements of lung function [2-12] and an influx of neutrophils and other markers of inflammation [13-17] at concentrations as low as 0.08 ppm in a dose-response manner. Repeated exposure of healthy adolescents to about 0.5 ppm O3 for 2 hrs on 5 consecutive days has shown a decrement in lung function after the first exposure, an even greater decrement after a second day exposure, but then increasing attenuated lung function decrements for the next 3 exposure days [18-21]. Hence the greatest lung function decrement is after 2 days exposure, with an adaptation occurring after the first two exposures. The adaptation is associated with attenuation of some soluble biochemical mediators collected by lung lavage (eg, PGE2), but not others (eg, IL-8), after 5 consecutive days of exposure [22].

A wide variation in lung function and lung cellular response to O3 exposure has been observed among individuals with no clear understanding in the susceptibility factors involved. NSAID usage has been shown to attenuate O3-induced lung function decrements [23]. Recent reports have linked certain genetic

polymorphisms to possible sensitivities to O3. For example, exercising individuals had greater lung function decrements as a group when they possessed a glutathione-S-transferase M1 (GSTM1) null genotype. Field studies over 3 years have shown increased respiratory breathing problems in children living in Mexico City who were GSTM1 null [24, 25], but no increased respiratory problems were associated with those children who were GSTP1 null.

PM exposure has been associated primarily with premature mortality [26, 27], but also with morbidity such as increased hospitalizations for cardiopulmonary problems including lung infection, asthma symptoms, and heart attacks [28-30]. Controlled ambient PM exposure studies of humans have been used to examine biological responses as surrogates for understanding the health effects and mechanisms involved in the responses. These controlled exposures have utilized inhalation of ambient PM that was concentrated to achieve increased mass concentrations (average 120 ± 14 $\mu g/m^3$ for PM2.5, with a maximum of 207 $\mu g/m^3$) at our EPA facility in Chapel Hill [31]. The controlled exposure studies have demonstrated no changes in lung physiological measurements. However, a dose-dependent increase in total number of cells, neutrophils, and monocytes was observed in the lavage fluid. Cardiac measurements showed no changes (SDNN, PNN50, the high frequency domain, the low frequency domain or the ratio of HF/LF) either immediately following PM2.5 exposure and 24 hrs after exposure. Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation, and potentiates atherosclerosis [32].

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 contain primarily fine and ultrafine carbonaceous particulates generated by incomplete combustion of fuel. The composition of the DE PM and the gases can vary depending on the type and age of engine, quality of fuel and additives, emission controls, load characteristics, and after treatment. Few controlled human exposures to diesel exhaust have been performed. Healthy human volunteers exposed to 300 $\mu g/m^3$ diesel exhaust for one hour with intermittent exercise resulted in marked systemic and redox-sensitive pulmonary inflammatory responses [33, 34], and vascular dysfunction and impaired endogenous fibrinolysis that links diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction [35, 36]. Despite a decade of intensive studies, much about the PM health effects problem, especially the cardiovascular effect, is still not well understood.

There are few studies looking at lung responses with combined O3 and DE in a controlled exposure setting. One study using healthy, young adults showed that a sequential exposure to 300 $\mu g/m^3$ DE for 1 hr followed 5 hr later by exposure to 0.2 ppm O3 induced more lung inflammation (eg. neutrophils collected by induced sputum) than DE alone with no O3 exposure [37]. The same research group, using similar exposure regimen exposure levels and timing, showed that diesel exhaust increased O3-induced lung inflammation (i.e., neutrophils collected by bronchoscopy) relative to O3 exposure alone [38]. Few studies with controlled O3 exposures have examined changes induced in extrapulmonary responses, including cardiac physiology and blood clotting and inflammatory factors. One study showed an increased diastolic blood pressure in healthy adults exposed to concentrated ambient particular matter (CAPs; ~147 $\mu g/m^3$) and O3 (~0.12 ppm) simultaneously compared to clean air responses [39]. However, no separate exposure to O3 alone, or CAPs alone, were performed.

The purpose of this study is, first, to examine whether DE can alter lung and cardiovascular responses to O3 exposure when given a day before or during O3 exposure; second, to investigate if co-exposure of DE and O3 on day 1 augment the lung function decrements following a subsequent O3 exposure (day 2); third, to investigate if two consecutive days of O3 affect individual cardiovascular responses to ozone in young healthy adults. We will measure spirometric lung function in association with 2-hour exposures to O3. Results will be analyzed for effects of each or combinations of the above on changes in lung function. We hypothesize that an exposure to DE with O3 (day 1) or DE exposure (day 1) given prior to O3 exposure (day2) will not induce a significant decrement in lung function in healthy young adults as a group relative to O3 alone. We further hypothesize that an O3 exposure (on day 2) after the DE and O3

co-exposure (day 1) will cause significant cardiopulmonary responses in healthy young adults. Finally, we hypothesize that two consecutive days of O3 exposure will affect cardiovascular responses.

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.

A group of about 15 healthy adults (men and women) between the ages of 18 and 55 years will participate and complete the study. However, because of potential dropouts and early terminations we may recruit as many as 25 subjects to complete all 4 exposure regimens. All subjects will be non-smokers for at least 2 years, with no active cardiac or respiratory disease, or active medical problems of any kind. Pregnant women, those trying to become pregnant, and those breast-feeding, will not be accepted. All potential subjects will undergo screening procedures (previously approved 95-EPA-66 Phase I and II), including a medical history form, physical examination and routine chemical and hematologic screens. All subjects are required to be moderately active so that they could sustain a prolonged period of moderate exercise. All subjects will be recruited without regard to their genetic profiles. But they will be genotyped and classified for presence or absence of polymorphisms of select genes.

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:

1. Healthy men and women between 18 and 55 years of age
2. Physical conditioning allowing intermittent, moderate exercise for 2.0 hours
3. Normal lung function:
   a. FVC $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   b. $FEV_1 \geq 75$ % of that predicted for gender, ethnicity, age and height.
   c. FEV_1/FVC ratio $\geq$ 75 % of predicted values.
4. Oxygen saturation $\geq$ 96 %.

Exclusion Criteria:

1. A history of acute and chronic cardiovascular disease, chronic respiratory disease, diabetes, rheumatologic diseases, immunodeficiency state, and acute respiratory illness within 4 weeks.
2. Subjects who are asthmatic or have a history of asthma.
3. Allergic to chemical vapors or gases.
4. Any allergic symptoms during the time of participation in the study
5. Female subjects who are currently pregnant, attempting to become pregnant, or breastfeeding
6. Subjects unwilling or unable to stop taking antioxidant vitamins and supplements or medications which may impact the results of the ozone challenge (such as, systemic steroids and beta blockers) at least 2 weeks prior to the study and for the duration of the study. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
7. Current and past smokers within 2 years.
8. Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
9. Subjects who do not understand or speak English
10. Subjects unable to perform the moderately active exercise required for the study.

11. Subjects with a history of skin allergies to adhesives used in securing heart rate monitor electrodes.
12. Unspecified diseases or conditions, which in the judgment of the investigator might influence the responses to the exposures, will be a basis for exclusion.
13. Subjects unwilling to stop taking over-the-counter pain medications such as aspirin, Advil, Aleve or other non-steroidal anti-inflammatory medications ("NSAIDS") for 48 hr prior to the exposures and post-exposure visits.

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 is a randomized crossover single blind study with 4 arms separated by at least 2 weeks. In this study we will measure cardiopulmonary responses to O3 (approximately mean concentration of 0.3 ppm over the 2 hr exposure period) and diesel exhaust (DE; ~300 $\mu g/m^3$) combined exposure and singularly, and clean air control in healthy adults undergoing moderate intermittent exercise (minute ventilation = ~ 25 liters/min/m^2 BSA). [See figure below].

Each subject will be exposed randomly to all 4 exposure regimes separated by at least 2 weeks. For all regimes, the subject will be exposed to the pollutant or clean air for 2 hrs with moderate exercise during the exposures. Moderate exercise will involve riding a stationary bike in the chamber for 15 min intervals beginning after the first 15 min of exposure and resting for 15 min after each time for a total of 1 hr of moderate exercise per chamber exposure. Ventilation rate measurements will be taken at ~ 6 min into each exercise session to ensure ventilation rates are within the appropriate parameters [target ~25 L/min/ m2 BSA, which in most subjects is ~ 50 L/min.]. For the DE and O3 co-exposure, there is a potential for the O3 to interact with the DE, but the O3 concentration will be maintained at a final mean concentration of about 0.3 ppm.

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 times will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. Clean air will be passed through an air purification system to ensure no presence of pollutant gases (O_3, NO, NO_2, SO_2, CO) in the air. Ozone and DE concentrations will be monitored continuously. The DE will be generated from a diesel engine used to power an air compressor that is located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean HEPA and charcoal filtered and humidified air to give a chamber concentration of approximately 300 $\mu g/m^3$. The monitoring analyzer registers a concentration in real time. Exposures will be terminated at values $\geq$ 400 $\mu g/m^3$ during runs in which the exposure targets 300 $\mu g/m^3$ DE. This safety limit will prevent an inhalational exposure greater than 600 μg, 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 approximately 0.05-0.2 μm. Preliminary testing has shown the mean particle size to be approximately 0.15 $\pm$ 0.05 μm. Levels of carbon monoxide (CO), and oxides of nitrogen (NOx; mainly NO, and some NO_2), will be kept under 10 ppm CO, 15 ppm NO, and 3.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average for these substances are: CO=50 ppm; NO=25 ppm; NO_2=5 ppm; Diesel fuel used for

the study will be purchased as a commercial ultra low sulfur fuel. 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. Particle mass will be measured in real time and validated further by weighing filters after the 2 hr exposure is finished. Particle size distribution will be determined at regular intervals. Filter samples will also be analyzed for chemical composition of particles. Minute ventilation will be measured during the exercise periods of exposure.

Lung Function Test:

Primary endpoints for evaluating health effects are changes lung functions, mainly FEV_1, FVC and FEV_1/FVC before, immediately after, and approximately every hour for up to 4 hrs post exposure.

Measurements and Collections:

Blood pressure, telemetry, and oxygen saturation may be monitored during the exposure period. Primary endpoints for evaluating health effects, in addition to lung function, are heart rate variability (HRV). Secondary endpoints may include markers of inflammation recovered in blood and exhaled breath including total and differential cell counts as well as pro-inflammatory cytokines and immune system mediators and clotting factors. Additionally, blood, breath, and urine samples will serve as indicators of the exposure based on the levels of metals (eg, Zn) and organics (eg, PAHs) and other components that maybe derived from DE deposition. From the collected blood samples, additional parameters and indices may be obtained as analysis tools become available. Genetic analysis may also be performed on collected blood samples for susceptibility factors. A questionnaire measuring perceived stress by the subject at the beginning of an exposure regimen will be performed. Some of the supplemental spirometric measurements and secondary endpoints may not be obtained or analyzed if they are deemed unnecessary.

In order to participate in this study, subjects will be asked 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.
- Avoid the use of ozone-based home air purifiers during study participation.
- On the exposure days, eat a light breakfast.
- Refrain from all over the counter anti-inflammatory agents including those for allergies for a period of 48 hrs prior to exposure.

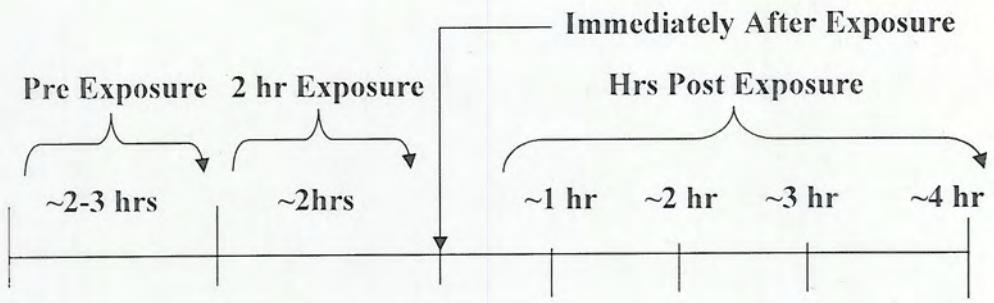
Figure 1

Experimental design:

	Day 1 2 hr exposure	Day 2 2hr exposure	Day 3
Exposure Regime 1	DE + O3	O3	follow up (no exposure)
Exposure Regime 2	O3	O3	follow up (no exposure)
Exposure Regime 3	DE	O3	follow up (no exposure)
Exposure Regime 4	Clean Air	O3	follow up (no exposure)

Figure 2
Endpoints:

Day 1



Measurements & Collections	*	*				
Lung Function	*	*	*	*	*	*

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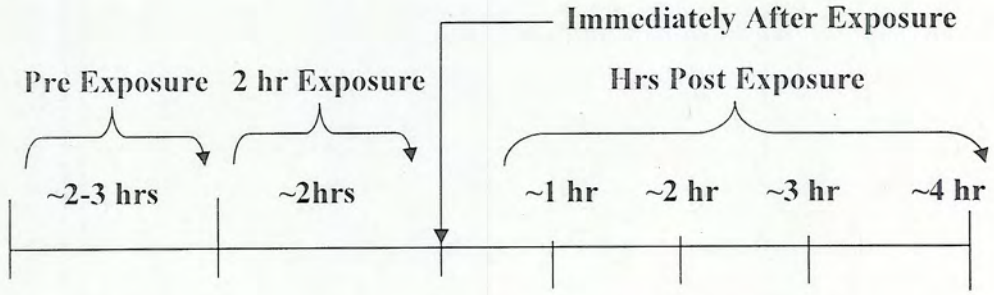
Day 2



Measurements & Collections	*	*				
Lung Function	*	*	*	*	*	*

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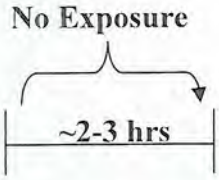
Day 3

Follow up



Measurements & Collections *

Lung Function *

Protocol: Written consent will be obtained. The subjects will be trained (on D0) in techniques for obtaining lung function measurements. Subjects also will exercise on a bicycle, and appropriate settings (the speed and grade of incline) required to produce a desired value of minute ventilation will be determined. On the first day of exposure, subjects will be assessed for vital signs (blood pressure, pulse, temperature and respiratory rate) at the check-in. Subjects may perform pre-exposure baseline lung functions in clean air, have resting heart rate variability (HRV) assessed, and urine, blood, and exhaled breath collected for later analyses of soluble mediators such as clotting factors, inflammatory proteins, and lipids and blood cell types and numbers. These samples may also serve to determine biomarkers of exposure, such as the concentration of PAHs . A questionnaire measuring perceived stress by the subject at the beginning of an exposure regimen will be filled out; this is included as Attachment #1. [Information about grading the questionnaire (Sheldon Cohen Perceived Stress Scale) is described at www.mindgarden.com].The subject then will enter the chamber already set for the appropriate conditions (DE & O3, DE alone, O3 alone, or clean air), and after 15 min begin exercising on a stationary bicycle. Each exposure session will consist of a 4 periods of 15 rest/15 min exercise at a level of approximately 25 liters/min/m^2 body surface area (BSA) in minute ventilation followed. Measurements of lung function may be obtained immediately after, and every hour for up to 4 hr post exposure. Resting HRV may be recorded, and blood, urine, and breath collected immediately post exposure. Following the day 1 exposure, the subject may remain at the Medical Station for a follow up measurements of lung function for approximately 2-4 hr. The subjects may go home with a heart rate monitor and blood pressure cuff. On Day 2, the subject will report to the medical station in the morning. The same measurements (resting HRV, lung function; symptoms; venipuncture performed) as Day 1 may be done pre- and post- exposure to O3. Subjects will return on Day 3 for follow up measurements but with no exposures.

Experimental procedures:
Medical history will be collected, including current medications and any recent illness.

Vital signs will be measured to include height, weight, heart rate, respiratory rate, blood pressure, oxygen saturation, and temperature.

Spirometry may be performed on a dry seal digital spirometer interfaced to a computer; volumes are corrected to body temperature and pressure (BTPS). From the forced expiratory maneuver, forced vital capacity (FVC), forced expired volume in the first second (FEV_1), and FEV_1/FVC.

Exhaled Breath Collection: The participant will be asked to breathe into a cooled tube for up to 15 minutes. The breath condensate may be collected and analyzed for markers of airway inflammation and immune system alterations and exposure markers. Additionally a small Tedlar bag of uncooled, exhaled breath will be collected (up to 5 min) to examine gas phase exposure and inflammation/immune system constituents.