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: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 11/20/2009
Expiration Date of Approval: 9/27/2010

RE: Notice of IRB Approval by Full Board Review
Submission Type: Initial
Study #: 09-1625

Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure
Sponsors: US Environmental Protection Agency - GRANTS

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

Study Description:

Purpose: The purpose of this protocol is to compare the genetic and epigenetic effects between diesel exhaust and ozone exposure in healthy individuals and in mid/moderate asthmatics. Participants: The PI will recruit up to 30 mild to moderate asthmatics and up to 30 healthy adults to participate in this study. Procedures (methods): Subjects will be exposed to clean air, to 300 ug/m3 of diesel exhaust for 2 hours and to 0.3 ppm of ozone for 2 hours with intermittent exercise in a controlled environment chamber. Primary endpoints will include spirometry and lung cell changes post-exposure. Secondary, endpoints will include analysis of soluble factors present in plasma and bronchial lavage and analysis of intracellular factors present in lung tissue obtained from a brush biopsy.

Investigator's Responsibilities:

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

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

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Any unanticipated problem involving risks to subjects or others (including adverse events reportable under UNC-Chapel Hill policy) should be reported to the IRB using the web portal at https://irbis.unc.edu/irb.

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

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
David Diaz Sanchez, Environmental Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

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To: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 4/22/2010
Expiration Date of Approval: 9/27/2010
RE: Notice of IRB Approval by Full Board Review
Submission Type: Modification
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure
Sponsors: US Environmental Protection Agency - GRANTS

This submission has been approved by the above IRB for the period indicated. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment, dated 01/20/2010, addresses:

1. new calculated exposure values for exercising subjects, correction of name, clarification that atropine may be given (not will be given), clarification in consent to be consistent with application that payment to subjects will be pro-rated and that subjects will complete a symptom score sheet.
2. submission of recruitment materials.
3. updated IRB application
4. updated consent

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

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
David Diaz Sanchez, Environmental Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

Dear Dr. Diaz-Sanchez:

I have now carefully reviewed the materials you sent regarding the protocol "Epigenetic effects of diesel exhaust and ozone".

I have only one clear suggested modification. Since you are studying "inflammatory effects", I would exclude individuals on "Statin" drugs as these have variable anti-inflammatory effects and would confound the study. The age group you propose (up to 55) certainly is such that statin drug use will not be rare.

Other than that, I have no safety of scientific issues with the Protocol

Sincerely yours,

Andrew Saxon, MD
Professor of Medicine
UCLA School of Medicine.

Although it is true that use of Statin drugs may be common in the age group we are targeting, previous studies conducted in our facilities have observed that their use does not affect our inflammatory endpoints. For this reason, we do not see it necessary to exclude individuals on statin drugs from this study.

David;

How about adding buccal cell collection for RNA to this protocol for expression?

I see no major safety or scientific concerns. The exposure protocols are well established as are the subject monitoring protocols. The major risk is form bronchoscopy and the protocol has an acceptable level of risk for the potential societal benefits from the study.

Let me know if you need additional document of my review.

Frank

Buccal cell collections are not invasive and do not pose any serious safety concerns. Although we believe this addition would be of great scientific value, we do not currently have the resources to include this procedure in this clinical study.

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version June 25, 2009

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

Date: April 19, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Name and degrees of Principal Investigator: Melanie J. Jardim, Ph.D.; David Diaz-Sanchez, Ph.D.
Department: US EPA Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514
UNC-CH PID: 715306970 Pager:
Phone #: 919-966-7320 Fax #: 919-966-6271 Email Address: jardim.melanie@epa.gov

For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: David Diaz-Sanchez, Ph.D.
Department: US Epa Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514
Phone #: 919-966-0676 Fax #: 919-966-6271Email Address: diaz-sanchez.david@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any): Michael Schmitt, MSPH
Department: US EPA Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514
Phone #: 919-966-0647 Fax #: 919-966-6271 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 name, location (UNC or specific outside location), role and email address for each person who should receive electronic copies of IRB correspondence to PI.**

Robert Devlin, PhD.; Heidi Hiers, RN; Maryann Bassett, RN; Martin Case, BS; Andrew Ghio MD; Martha Sue Carraway, MD; Robert Truckner, MD;Howard Kehrl, MD; Tracey Montilla, RN; Michael Schmitt, MS.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify):

For external funding, RAMSeS proposal number (from Office of Sponsored Research): The PI is a USEPA scientist and this project is being funded by intramural EPA funds

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

Applications must "stand alone" and should provide all information requested, i.e., complete answers must be contained in the application. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

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 (include DHHS-approved sample, when one exists), fact or information sheets, phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
□	4. All recruitment materials including final copies of printed advertisements, audio/video taped advertisements, scripts, flyers, letters, and 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., Clinical and Translational Research Center (CTRC), Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. For drug studies, Investigator Brochure if one exists. If none, include package insert for previously approved uses..	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*

	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	_X_
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_X_
A.2.4. Do you have specific plans 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_
c. Is this research classified (e.g., requires security clearance)?	__	_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 Clinical and Translational Research Center (CTRC) or is the CTRC involved in any other way with this study? If yes, obtain the ***CTRC Addendum*** *and submit completed application (IRB application and Addendum) directly to the CTRC. The CTRC includes facilities located on the 3rd floor of the Main Hospital (formerly GCRC) and Ground floor Burnett-Womack (formerly CCCT).*	__	_X_
A.2.18. Will gadolinium be administered as a contrast agent?	__	__X
A.2.19. Will subjects' Social Security Number (SSN) be collected for:		
a. processing payments greater than $200 per year, to support IRS reporting (see also B.6)?	__	_X_
b. processing payments of any amount through UNC-CH Accounts Payable?	__	_X_
c. use as a unique identifier for study tracking purposes for national registry or database?	__	_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 personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity engaged in the performance of this project as a subcontractor, sub-recipient or vendor?	__ yes	_X_ no
(d) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_X_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit the form, which is accessible online at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

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

_____ _____

Signature of Faculty Advisor Date

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

Complete answers must be provided. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

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 protocol is to compare the genetic and epigenetic effects between diesel exhaust and ozone exposure in healthy individuals and in mild/moderate asthmatics.

Participants: We will recruit up to 30 mild to moderate asthmatics and up to 30 healthy adults to participate in this study.

Procedures (methods): Subjects will be exposed to clean air, to 300 $\mu g/m^3$ of diesel exhaust for 2 hours and to 0.3 ppm of ozone for 2 hours with intermittent exercise in a controlled environment chamber. Primary endpoints will include spirometry and lung cell changes post-exposure. Secondary endpoints will include analysis of blood clotting/coagulation factors, Holter monitoring of cardiac parameters, analysis of soluble factors present in plasma and bronchial lavage and analysis of intracellular factors present in lung tissue obtained from a brush biopsy.

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.

Several epidemiological studies have shown a strong link between exposure to air pollution and adverse cardiopulmonary effects, such as respiratory tract infections, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke(Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005; Lewtas 2007). Ozone and diesel exhaust are ubiquitous in the urban atmosphere making it difficult to identify the exact source of these effects. Although experimental chamber studies have been previously performed with diesel exhaust or ozone, no one has yet looked at the epigenetic changes resultant from acute exposures to these air pollutants.

Numerous studies have shown that healthy subjects exposed to ozone have decreased pulmonary function and increased neutrophils and soluble pro-inflammatory mediators in the lower airways(Peden, Setzer et al. 1995; Bascom 1996). These include markers of increased permeability, decreased macrophage phagocytic ability, and increases in arachidonic acid metabolites, cytokines, fibronectin, lactase dehydrogenase, coagulation factor VII, tissue factor, elastase and plasminogen activator occurred in response to ozone-induced exposures(Devlin, McDonnell et al. 1991; Koren, Devlin et al. 1991; McDonnell, Kehrl et al. 1991; Devlin 1993; McKinnon, Madden et al. 1993). Diesel exhaust fumes contain primarily fine and ultrafine carbonaceous particles generated by the incomplete combustion of fuel(Diaz-Sanchez, Penichet-Garcia et al. 2000; Lewtas 2007). Diesel exhaust particles (DEP) are the largest single source of vehicular emitted airborne PM and can persist in the air, where they are readily inhaled and deposited throughout the respiratory tract. DEP induced toxicity is mainly attributed to its chemical composition, consisting of a carbonaceous core to which organic and inorganic species can become adsorbed (Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005). Healthy human volunteers exposed to 300 $\mu g/m^3$ diesel exhaust for one hour with intermittent exercise resulted in markers of systemic and redox-sensitive pulmonary inflammatory responses, vascular dysfunction and impaired endogenous fibrinolysis. Although there were no adverse health effects associated with the exposure, acute changes in the aforementioned endpoints can be used to extrapolate information in order to begin to understand the underlying biological mechanisms that link long-

term exposure of diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (Salvi, Blomberg et al. 1999; Behndig, Mudway et al. 2006).

Previous studies reported a wide variation in response to air pollution exposure among individuals in each subject group with no clear understanding in reasons (McDonnell 1991). Asthmatics have been identified as a susceptible subpopulation as seen by their heightened sensitivity to air pollutants, although the severity of health responses of asthmatics exposed to diesel exhaust and ozone are not yet fully understood. It is hypothesized that increased susceptibility could be due to increased baseline airway inflammation and airway responsiveness which characterizes asthma. Indeed, respirable particulate matter and ozone exposure are associated with episodes of increased asthma exacerbation (Devlin 1993; Bascom 1996; Diaz-Sanchez and Riedl 2005). Elevated levels of air pollution can affect airway reactivity with decrements in several indices of pulmonary function especially in those patients with preexisting asthma.

Despite a decade of intensive studies, adverse health effects attributed to air pollutant exposure are still not well understood. It is becoming increasingly clear that epigenetics plays a pivotal role in disease development and outcome, which has been extensively studied in cancer. The term epigenetics refers to the study of heritable gene expression changes, which occur without changing the DNA sequence itself. DNA methylation, histone modifications and microRNA expression all regulate gene expression, and changes in these mechanisms can result in an overall change in gene expression patterns. Unlike the static DNA sequence, these mechanisms are regulated by endogenous and exogenous cues, rendering them more likely to change in response to environmental stimuli. It is not currently known how these changes become permanent, or how epigenetic changes control gene expression in response to acute environmental perturbations. Few studies have examined how the environment can modulate epigenetic marks in individuals. In a pioneering study published in 2005, Fraga et. al. showed that as identical twins age, DNA methylation patters change indicating that differential gene expression observed in older identical twins may be attributed to epigenetic changes, further suggesting that these changes may have been molded by the environment (Fraga, Ballestar et al. 2005). Traffic particle exposure has been associated with methylation changes in retrotransposable elements, randomly dispersed throughout the DNA sequence, however the biological consequence of this is not understood as these are not coding sequences (Baccarelli, Wright et al. 2009). In vitro studies in primary human bronchial epithelial cells grown at air liquid interface showed that microRNA expression profiles change rapidly in response to exposure to diesel exhaust particles(Jardim 2009). Animal studies attempting to address these questions have been difficult as animals have very different epigenetic mechanisms making it difficult to draw conclusions from these studies. Long term exposure to cigarette smoke, a source of particulates, lead to changes in microRNA expression in rat lungs, however the contribution of these changes to biological consequences is not currently known (Izzotti, Calin et al. 2009). Another study, showed that an in utero diet rich in methyl donors enhanced allergic airway disease in mice via changes in methylation of several genes, and that these changes were transgenerational (Hollingsworth, Maruoka et al. 2008). While it is difficult to draw biological meaning from epigenetic animal studies, what these studies show is that the environment can play an important role in regulating gene expression. Although the biological and physiological role epigenetics plays in response to environmental pollutants is not yet understood, however we will begin to address this question in this study by performing in depth gene expression analysis, including microarrays, microRNA arrays and DNA methylation assays on samples collected from patients.

In addition to possible epigenetic mechanisms, genetic polymorphisms, particularly polymorphisms in anti-oxidant genes, have been proposed as a factor to explain variability in these responses. The role of genetic background in mediating airway and vascular inflammation following acute exposure to diesel exhaust and ozone in human subjects will be studied in this protocol as a secondary question. As such, bronchial epithelial cells collected from this study will be further evaluated by in vitro exposure to both gaseous and particulate air pollution.

The purpose of this study is to first, make a direct comparison of epigenetic responses (primarily changes in DNA methylation, microRNA expression and chromatin modification) in individuals exposed to ozone and diesel exhaust; second, to assess whether mild to moderate asthmatics exhibit the similar effects and to the same degree as healthy individuals; and third, to understand the contribution of genetic background and epigenetic mechanisms to cardiopulmonary effects observed in pollutant exposed cells. Although many the observed adverse health effects are generally attributed to oxidative stress upon exposure, we still do not know how these result in physiological changes to the respiratory and cardiovascular organ systems. We hypothesize that exposure to diesel exhaust or ozone will result in changes in epigenetic mechanisms, which may be linked to adverse health effects of exposure. If adverse health effects are induced solely due to oxidative stress, then patients should exhibit similar cardiopulmonary and epigenetic responses when exposed to either, diesel exhaust or ozone. However, if differential

responses to these agents are observed, then alternative mechanisms of cellular injury may exist. Therefore, we will employ several methods to assess potentially important physiological changes to these systems, including cutting edge techniques such as micro-array profiling, DNA methylation assays, microRNA expression profiling and assays for indications of chromatin remodeling.

Baccarelli, A., R. O. Wright, et al. (2009). "Rapid DNA methylation changes after exposure to traffic particles." Am J Respir Crit Care Med **179**(7): 572-8.

Bascom, R. (1996). "Environmental factors and respiratory hypersensitivity: the Americas." Toxicol Lett **86**(2-3): 115-30.

Behndig, A. F., I. S. Mudway, et al. (2006). "Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans." Eur Respir J **27**(2): 359-65.

Brunekreef, B. and S. T. Holgate (2002). "Air pollution and health." Lancet **360**(9341): 1233-42.

Devlin, R. B. (1993). "Identification of subpopulations that are sensitive to ozone exposure: use of end points currently available and potential use of laboratory-based end points under development." Environ Health Perspect **101 Suppl 4**: 225-30.

Devlin, R. B., W. F. McDonnell, et al. (1991). "Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung." Am J Respir Cell Mol Biol **4**(1): 72-81.

Diaz-Sanchez, D., M. Penichet-Garcia, et al. (2000). "Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity." J Allergy Clin Immunol **106**(6): 1140-6.

Diaz-Sanchez, D. and M. Riedl (2005). "Diesel effects on human health: a question of stress?" Am J Physiol Lung Cell Mol Physiol **289**(5): L722-3.

Folinsbee, L. J., J. F. Bedi, et al. (1985). "Pulmonary response to threshold levels of sulfur dioxide (1.0 ppm) and ozone (0.3 ppm)." J Appl Physiol **58**(6): 1783-7.

Fraga, M. F., E. Ballestar, et al. (2005). "Epigenetic differences arise during the lifetime of monozygotic twins." Proc Natl Acad Sci U S A **102**(30): 10604-9.

Hollingsworth, J. W., S. Maruoka, et al. (2008). "In utero supplementation with methyl donors enhances allergic airway disease in mice." J Clin Invest **118**(10): 3462-9.

Izzotti, A., G. A. Calin, et al. (2009). "Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke." FASEB J **23**(3): 806-12.

Jardim, M., Fry, RC, Jaspers, I, Dailey, L and Diaz-Sanchez, D (2009). "Disruption of MicroRNA Expression in Human Airway Cells by Diesel Exhaust Particles is Linked to Tumorgigenesis-Associated Pathways." Environmental Health Perspectives **117**(11).

Koren, H. S., R. B. Devlin, et al. (1991). "Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone." Toxicol Pathol **19**(4 Pt 1): 406-11.

Lewtas, J. (2007). "Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects." Mutat Res **636**(1-3): 95-133.

McDonnell, W. F. (1991). "Intersubject variability in human acute ozone responsiveness." Pharmacogenetics **1**(2): 110-3.

McDonnell, W. F., H. R. Kehrl, et al. (1991). "Respiratory response of humans exposed to low levels of ozone for 6.6 hours." Arch Environ Health **46**(3): 145-50.

McKinnon, K. P., M. C. Madden, et al. (1993). "In vitro ozone exposure increases release of arachidonic acid products from a human bronchial epithelial cell line." Toxicol Appl Pharmacol **118**(2): 215-23.

Peden, D. B., R. W. Setzer, Jr., et al. (1995). "Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics." Am J Respir Crit Care Med **151**(5): 1336-45.

Riedl, M. and D. Diaz-Sanchez (2005). "Biology of diesel exhaust effects on respiratory function." J Allergy Clin Immunol **115**(2): 221-8; quiz 229.

Salvi, S., A. Blomberg, et al. (1999). "Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers." Am J Respir Crit Care Med **159**(3): 702-9.

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).

Subjects for this study will be healthy adults, 18-55 years old, (male and female) and, otherwise healthy, 18-55 year old mild to moderate asthmatics (as defined by NHLBI guidelines), who are non-smoking. Subjects will be recruited through the Westat Corporation (see section B1 below). There are no gender or race based restrictions. Pregnant and lactating women will be excluded because of the unknown effects of air pollutants on inflammatory markers during pregnancy and lactation.

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.

Healthy Individuals

Specific Inclusion Criteria

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66).
2. Normal lung function (pre or post albuterol), defined by Knudson 1976/1984 as:
   a. FVC of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   b. FEV_1 of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   c. FEV_1/FVC ratio of $\geq$ 75 % of predicted values.
3. Oxygen saturation of $\geq$ 96 %.
4. Ability to tolerate intervals of moderate exercise

Exclusion Criteria:

1. A history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease, and lung cancer.
2. If the subject is pregnant, attempting to become pregnant or breastfeeding.
3. Allergy to any medications which may be used or prescribed in the course of this study.
4. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM and/or ozone challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
5. Smoking history within 2 years of the study.
6. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
7. Untreated hypertension (> 150 systolic, > 90 diastolic)
8. Dementia.
9. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
10. History of skin allergy to tape or electrodes.
11. Subjects who do not understand or speak English
12. Subjects who are unable to perform moderate exercise

Temporary exclusions:

1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.

4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:

1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin ≥ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

Mild to Moderate Asthmatics

Inclusion Criteria:

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66) where diagnosis will be judged by EPA physicians.
2. Mild/Moderate asthmatics as defined by NHLBI guidelines.
3. Positive history of asthma (wheezing, chest tightness, and reversible airway obstruction);
4. Baseline $FEV_1/FVC \geq 60\%$;
5. Oxygen saturation of ≥ 94%
6. Ability to tolerate moderate exercise

Exclusion Criteria

1. Use of oral steroid therapy within the past month
2. Physician directed emergency treatment for asthma exacerbation within the preceding 6 months.
3. Abnormal EKG that precludes evaluating heart rate variability.
4. Aside from mild/moderate asthma, a history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma, and cancer (possible exception for history of nonmelanoma skin cancer).
5. If the subject is pregnant, attempting to become pregnant or breastfeeding.
6. Allergy to any medications which may be used or prescribed in the course of this study
7. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
8. Dosing level of an inhaled steroid must be consistent with mild asthma as outlined by the NHLBI NAEPP guidelines. Regular use of oral corticosteroids, or use of inhaled steroid at doses typically used for severe asthma, will result in exclusion of that individual from the protocol.
9. Severe asthmatics as defined by: nighttime symptoms of cough or wheeze greater than 1 time per week at baseline, daily exacerbation of asthma or requirement for albuterol due to asthma symptoms (cough, wheeze, chest tightness, but not to include prophylactic use of albuterol prior to exercise), more than mild interference with normal activity, any episode of physician directed emergency treatment for asthma requiring oral corticosteroid therapy within the past twelve months.
10. Smoking history within 2 years of study.
11. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
12. History of skin allergy to tape or electrodes.
13. History of respiratory diseases other than allergic rhinitis and asthma
14. Untreated hypertension (> 150 systolic, > 90 diastolic)
15. Dementia.
16. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
17. Subjects who do not understand or speak English

18. Subjects who are unable to perform moderate exercise

Allowances: subjects may
1. Use daily theophylline therapy.
2. Use daily inhaled steroids.
3. Use inhaled cromolyn therapy.

Temporary exclusions:
1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.
4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:
1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin ≥ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This is a randomized cross-over study with three arms. This study will compare the epigenetic responses of healthy adults and adults with mild asthma to diesel exhaust and ozone exposures. Up to thirty subjects will be recruited into each group. Subjects will be randomly exposed to clean air, diesel exhaust and ozone with exposures separated by a minimum of two weeks. Responses of primary interest will include: 1) genetic and epigenetic changes 2) FEV1 as measured by spirometry, and 3) lung inflammation and cell changes as evaluated by bronchoalveolar lavage. The performance of these tests and procedures are considered essential to conducting the study. Exploratory endpoints will include: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, telemetry, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18-24 hours post-exposure. All medical procedures will be performed by study personnel.

Physical Examination Day:

Prior to recruitment into the study, subjects will undergo a physical exam. During this visit, a short review of the subject's history will be performed and vital signs, height and weight will be assessed (temperature, pulse, respiratory rate, and blood pressure), to determine whether the individual meets any major exclusion criteria for the study. Up to 50 ml of blood may be collected for a CBC/ differential, chemistry and lipid panels, assessment of

coagulation function, phagocytic function and cell surface markers. At this time the subject may undergo a physical examination for bronchoscopy or may be scheduled for one at another time.

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.
- On the Exposure Day 2, eat a normal breakfast.
- No caffeine for 12 hours prior to all study visits.
- At the end of Exposure Day 2, do not eat or drink anything after midnight in preparation for bronchoscopy on Exposure Day 3.

Training Day

1. Eligible subjects will report to the research lab for an approximate 2-hour training session.
2. Informed consent will be obtained.
3. Assessment of **vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation
4. **Pregnancy tests** will be administered to any women who may have child-bearing potential.
5. Subjects will be trained on the cycle ergometer and the work load to elicit a minute ventilation normalized for body surface of 25L/m^2/BSA will be determined. In most subjects this will be about 50 L/minute (IE. VO^2 of approximately 2.0 L/m). A cycle ergometer work setting of 75 to 100 watts will usually achieve such a physiological response
6. Subjects will perform **spirometry.**
7. Subjects will be shown chamber where exposures will take place

Exposure Day 1

The subjects will be exposed ozone, diesel exhaust and clean air on three separate occasions, with the exposures separated by a minimum of two weeks. .

1. Subjects will be asked to arrive at the study site no sooner than 8 AM and will undergo **assessment of vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation, and **symptom score** assessment.
2. **Pregnancy tests** will be administered to any women who may have child-bearing potential.
3. A **telemetry unit** will be attached for cardiac monitoring.
4. Subject will be fitted with a **Holter monitor** and the subject will be asked to recline in a quiet dark place for 30 minutes. After approximately 20 minutes a time marker will be activated so that heart rate variability frequency readings can be taken over the next 10 minutes. The subject will wear the Holter monitor for the next 24 hrs.
5. **Venipuncture:** Up to 80 ml of blood will be collected for a CBC/ differential, and may be used for assessment of coagulation function and markers, phagocytic function, inflammation, and cell surface markers. A portion of the sample may be used for RNA isolation for microarray analysis and for DNA isolation for genotyping purposes.
6. **Baseline Spirometry**
7. **Exposure sessions**: Exposure to ozone, diesel exhaust or clean air will be conducted in an exposure chamber at the EPA Human Studies Facility on the UNC campus. Each subject will be exposed up to 0.3ppm ozone, diesel exhaust (up to 300 ug/m^3) or clean air for 2 hours. Subjects will begin exercising on an exercise bike. Each exercise session will consist of a 15 minute exercise interval at a level of up to 25 L/m2/BSA followed by a 15 minute rest period. Minute ventilation will be measured for three minutes during each exercise period. Continuous heart rate and rhythm, and oxygen saturation, will be monitored using telemetry and pulse oximetry respectively. Blood pressure will be monitored. Subjects will be asked to refrain but not prohibited from using inhaled bronchodilator during exposures; if medication use is necessary subjects will be instructed to maintain consistent medication use across the two exposures. The subjects will be able to end their exposure and exit the chamber at any time. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, ataxia, or a greater than 5 point drop in saturated oxygen (or a drop to lower than 89%). They will be monitored continuously by trained personnel and a physician is on call whenever a subject is undergoing a procedure at the facility. A detailed description of the physical facilities in the Human Studies Division of the U.S. EPA Health Effects Research Laboratory located on the UNC-CH campus have been published as U.S. Government Publication EPA-600/1-78-064, and is on

file in the office of the UNC Committee on the Protection of the Rights of Human Subjects. The medical station of the Human Studies Division includes a fully-equipped medical examination room, a cardiac/respiratory emergency cart, a subject recovery room, a nurse's station, a waiting room and conference rooms. The facility is staffed by qualified RNs, and an on-site physician is available to respond to a medical emergency in a timely manner. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. The DE will be generated from a diesel generator used to power a load bank 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 up to 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 1080 µg, which assumes that the subject will inhale 2.7 m^3 during the 120 min exposure. 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 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) 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. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured in real time. Particle size distribution may not be determined during each subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. Ozone exposures will be conducted in a (6 ft x 6 ft x 8 ft) stainless steel chamber with a continuous supply of exposure medium. Ozone will be monitored continuously. **Immediately post exposure**, subjects will again undergo **spirometry, Holter monitor frequency reading, symptom scoring and assessment of vital signs.**

8. **Then,** up to 80 ml of blood will be collected for a CBC/ differential, assessment of coagulation function, blood leukocyte phagocytic function, cytokines, cell surface markers and RNA isolation for microarray analysis.
9. Subjects will then be assessed and discharged by the nursing staff.

Exposure Day 2 (approximately 24 hours after exposure)

The subject will arrive at the U.S. EPA HSF for:

1. **Vital sign monitoring.**
2. **Symptom scoring.**
3. **Venipuncture**: up to 80 ml of blood will be collected for a CBC/ differential, coagulation function, assessment of blood monocyte phagocytic function, inflammation, and cell surface markers, and RNA isolation for microarray analysis.
4. **Holter monitor** frequency reading and removal
5. **Spirometry**
6. **Bronchoscopy.** Subjects will have undergone a physical examination including assessment for suitability for transnasal fiberoptic bronchoscopy at the Human Studies Facility by a board certified or board eligible pulmonologist.

   Prior to the procedure, subjects will perform spirometry (as above); bronchoscopy will not be performed if lung function does not meet the recommended performance level (NIH guidelines; FEV1$\geq$ 60 % predicted). Prior to bronchoscopy, a small tube (IV) will be placed in a vein for potential use in administering fluids. Subjects will receive inhaled albuterol prior to the bronchoscopy at the discretion of the pulmonary physician performing the procedure. Bronchoscopy with bronchoalveolar lavage and brush biopsy will be performed by a licensed physician who is Board certified or board eligible in pulmonary medicine and is experienced in fiberoptic bronchoscopy. The physician will be assisted by at least one R.N experienced in bronchoscopy. The subjects will be monitored closely throughout the procedure in the following manner: 1) Chest electrodes for continuous electrocardiogram, 2) Pulse oximetry of arterial blood, and 3) blood pressure monitoring using an electronic sphygmomanometer. Subjects will be premedicated with IV atropine (0.6 mg) to prevent bradycardia and hypotension that could result from vagal stimulation provoked by passing the bronchoscope through the subject's larynx and to minimize the amount of airways secretions. Nasal oxygen will be administered during the procedure. No sedatives and/or narcotics are administered at any time during bronchoscopy. We have found that premedication with sedatives (midazolam) or with opiates (demerol) is not required for research bronchoscopy subjects. Avoidance of these drugs reduces procedure risk and markedly shortens post-procedure recovery time. Topical lidocaine anesthesia will be used for subject comfort and to prevent cough, with a maximum dose of 15 ml of 2% lidocaine solution (360 mg lidocaine). Electrocardiographic and pulse oximeter will be

monitored throughout the procedure. After the airways are visualized, the bronchoscope tip is wedged in a 4th-6th order bronchus (right middle lobe) for bronchial lavage. A total volume of 270 cc of sterile saline, one aliquot of 20 cc and 5 additional aliquots of 50 cc each, will be injected and immediately aspirated through the channel in the bronchoscope using a syringe. The lavage fluid aliquots will be put in polypropylene tubes and kept on ice until processed. A total of up to 4 brush biopsies will then be obtained from the lower trachea, main, and lobar bronchi after topical application of 1% lidocaine. A cytology brush (Bronchoscope Cytology Brush; Bard, Tewksbury, MA) is applied to the region under direct visualization. For a single brushing, six passes, each with a linear excursion of 2-5 cm, are made with the brush along the endobronchial surface.

Subjects are observed and monitored for a minimum of 1 to 2 hours after the procedure in the on-site medical station by a registered nurse with physician supervision. At discharge subjects will be symptom-free. At least 2 physicians, including the responsible bronchoscopist, will be available (24 hours daily) to subjects who develop any symptoms after discharge. The subject is discharged by a physician and is provided with the names and telephone numbers of the bronchoscopist and one other physician. The subject is contacted 24 hours post-bronchoscopy to ask about any untoward effects.

Subjects will return to the U.S. EPA HSF for an additional exposure sessions and post exposure bronchoscopies (air control, ozone or diesel exhaust) after a minimum time interval of two weeks.

We anticipate performing several clinical procedures during the course of this study which include primary, secondary and exploratory endpoints. However, it is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure, because of a investigator problem (such as but not limited too building/equipment failures, weather delays, or staffing issues), then the patient will be compensated for said procedure(s) and time on that day and may be rescheduled.

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.

There will be no direct benefit to the subject for participating, other than information that may be learned during their physical examination about their overall physical status. The primary benefit from the study will be to add to the scientific knowledge about whether exposure to air pollutants induces epigenetic changes.

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.

Risk and Procedures for Minimizing Risks of Diesel Exhaust Exposure

- Diesel Exhaust Exposure: The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban area. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic

polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City. We have seen in IRB-07-0190 that exposure to 300 $\mu g/m^3$of diesel exhaust does not cause adverse health effects.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. In the pilot phase of study #07-0190 (Haiyan Tong MD, Ph.D, PI), subjects reported "moderate" eye irritation following diesel exhaust exposure and the symptom was resolved within a few hours after the exposure. Subjects will be provided with the choice to wear chemical safety goggles during exposures to prevent or minimize the eye irritation. 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 health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

Risk and Procedures for Minimizing Risks of Ozone Exposure

Potential risks may likely 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. It is likely that most subjects participating in this study will experience some degree of tracheobronchial irritation and cough.

Risks and Procedures for Minimizing Risks of Venipuncture:

Insertion of the needle may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.

Risks and Procedures for Minimizing Risks of Bronchoscopy:

The most significant risk to a volunteer in this protocol involves bronchoscopy. Over 1000 procedures have been performed within the EPA Human Studies Facility over the past 15 years. From January 1, 1986 through December 30, 1995, 736 procedures were performed in normal nonasthmatic subjects, and these have been reported in the

Journal of Bronchology 1998; 5:185-194. The overall complication rate was less than 1% (actually less than 0.1%). The most frequent complication was a low grade fever. Bronchoscopy with BAL can be associated with more significant complications, including hypoxemia, cough, wheezing, and post-bronchoscopy fever. These may be increased in asthmatic subjects, and some investigators have found hypoxemia to be more severe in asthmatics compared to normal subjects (Djukanović, R., J. W. Wilson, C. K. Lai, S. T. Holgate, and P. H. Howarth. 1991. The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am. Rev. Respir. Dis.* 143: 772-777), while others have not (Van Vyve, T., P. Chanez, J. Bousquet, J. Y. Lacoste, F. B. Michel, and P. Godard. 1992. Safety of bronchoalveolar lavage and bronchial biopsies in patients with asthma of variable severity. *Am. Rev. Respir. Dis.* 146: 116-121); Anonymous. Workshop summary and guidelines: investigative use of bronchoscopy, lavage, and bronchial biopsies in asthma and other airway disease. *J Allergy Clin Immunol* 88:808-14, 1991.;
Jarjour NN, Peters SP, Djukanovic R, Calhoun WJ. Investigative use of bronchoscopy in asthma. Am J Respir Crit Care Med 157:692-97, 1998.) Furthermore, hypoxemia is rarely severe enough to require premature termination of the procedure and can usually be treated or prevented by supplemental oxygen (Van Wyre).

Bronchoscopies are performed by experienced physicians who are either Board-certified or Board-eligible in pulmonary medicine. The physician performing the bronchoscopy is always assisted by at least one nurse familiar with the procedure. The subject's vital signs and ECG are continuously monitored during the bronchoscopic procedures and at regular intervals during the recovery phase. The procedure is immediately terminated if the subject experiences any alterations such as tachypnea, depressed respiration, tachycardia, bradycardia, abnormal rhythms or significant changes in blood pressure. As indicated earlier, a fully equipped emergency cart including a defibrillator, endotracheal intubation equipment and emergency medications (atropine, epinephrine) is available at all times. An on-site physician is always available to respond and the UNC Memorial Hospital Emergency Room is within 1/4 mile of Human Studies Facility. Before the subject is sent home, he/she receives a brief physical examination by a physician and is given phone numbers and a pager number with which to contact a physician should he/she experience any problems or have any questions.

The complication rate during medical bronchoscopy is approximately 0.08 to 0.12% for all events, including low oxygen saturation, respiratory embarrassment, myocardial infarction, or death. The medical screening of the subjects is specifically designed to exclude subjects with a history of medical problems which might put them at risk from the particle exposure and bronchoscopic procedures. Medical problems which could arise as a result of bronchoalveolar lavage and brush biopsy include the following:

Discomfort of the nose and throat is a primary risk of bronchoscopy. As outlined, the lidocaine gargle, spray and liquid is used to anesthetize these areas prior to passing the bronchoscope to reach the lower airways. The procedure will be discontinued if adequate anesthesia cannot be achieved by the means described.

Low-grade fever (38-38.5 °C) can occur in approximately 25 % of hospitalized subjects undergoing bronchoscopy. The fever invariably resolves within 18 hours without treatment or with acetaminophen. Our experience at HSD, EPA has shown that this fever and the associated malaise can almost always be prevented by administering ibuprofen following the procedure. The subject will be asked to contact the physician who performed the procedure or one of the nurses at the Medical Station if fever persists or is higher than 38.5 °C. As part of the standard protocol, a nurse or physician will contact the subject by telephone between 24 and 48 hours after the procedure to inquire on the general health of the subject and specifically about the presence of fever.

Cough is a common, albeit minor, problem encountered during bronchoscopy. Cough results from mechanical stimulation of cough receptors in the airway by the bronchoscope or the cytologic brush used for the brush biopsies. In addition to the discomfort, prolonged coughing with the bronchoscope in place can result in mild mechanical trauma to the vocal cords. Lidocaine solution is passed through the bronchoscope channel at the main carina and at the level of the right mainstream bronchus to suppress the cough reflex during the procedure, more can be used as needed. The procedure will be terminated if adequate cough suppression cannot be attained.

Epistaxis is caused by trauma to the nose by the bronchoscope. This condition is expected to be minor and resolve on its own. Small streaks of blood in nasal secretions may be present for up to 12 hours following the procedure. If the bleeding becomes moderate or severe during bronchoscopy, the procedure will be terminated and the bronchoscope removed. The subject's anterior nasal passage will be packed with sterile gauze to stop the bleeding. If the bleeding does not resolve with packing, the subject will be transferred to the UNC Hospital Emergency Room.

Lidocaine use presents a small risk to the subjects, as a variable fraction of the medication is absorbed through the airway mucosa. If a significant amount of lidocaine is absorbed, adverse reactions such as bradycardia, hypotension, urticarial reactions, confusion, lightheadedness, euphoria, tremors and seizures can result. We take particular precautions to avoid the possibility of overdose of topical lidocaine and limit the total amount of lidocaine instilled into the airways to a maximum of 360 mg. Also, subjects will be screened by history prior to the procedure for known allergies to lidocaine or other similar topical anesthetics.

Bronchospasm, manifested as wheezing, chest tightness or dyspnea, is a risk of bronchoscopy that is caused by stimulation of irritant receptors in the airways by the bronchoscope. This risk is minimized by 1) only allowing persons with stable mild to moderate stable asthma to participate in the study, 2) pretreatment with inhaled

bronchodilator before the procedure per request of the bronchoscopy physician and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physicians responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. In the unlikely event that the subject requires additional treatment because the asthma attack continues, they will be transported by ambulance to the emergency room of the UNC Hospital Emergency Room.

Pneumothorax is a risk of bronchoscopic procedures such as transbronchial biopsy, peripheral lung protected brushings or peripheral lung cytology brushings. Because these procedures will not be performed, the risk of pneumothorax in this study is extremely small. There is a very small risk of a pneumothorax resulting from a endobronchial brush biopsy. The symptoms include dyspnea and chest pain. The subjects will be interviewed and examined by the bronchoscopy physician 2 hours after the procedure with these symptoms in mind. If a pneumothorax is suspected, the subject will be transferred to the UNC Hospital Emergency Room by ambulance for further evaluation and treatment.

Bleeding in the lower airway may occur from trauma caused by the bronchoscope or by brush biopsy. Bleeding is typically very minor, in the order of 1/10 cc, is not clinically significant in an otherwise healthy subject and should spontaneously resolve in a matter of minutes. Epinephrine, diluted 1/20,000 fold will be administered through the bronchoscope to the affected site if bleeding is minor to moderate. The maximum dose of epinephrine used will be 2-3 cc to prevent local tissue necrosis. The site will be monitored through the bronchoscope until the bleeding stops. If bleeding fails to resolve with epinephrine or if it is sufficiently severe to cause hemoptysis or hemoglobin desaturation, oxygen supplementation will be provided to keep arterial oxygen saturation above 90% and the subject will be transported to the NC Memorial Hospital Emergency Room by ambulance. If it becomes necessary, the subject can be intubated before transport.

The use of epinephrine to control bleeding can produce systemic effects resulting from mucosal absorption of the drug. These symptoms are transient and include headache, palpitations and tachycardia when the dose is greater than 1 mg and is given intravenously. Two cc of a 1/20, 000 dilution of epinephrine contains 0.10 mg of the drug, a dose that is very unlikely to induce any untoward effects. This is especially true when the drug is applied to the mucosa, since only a fraction is expected to be absorbed.

Bradycardia and/or hypotension may result from increased vagal nerve output provoked by coughing or Valsalva during the passage of the bronchoscope through the vocal cords and trachea. Atropine (0.6 mg iv) is prophylactically administered to the subjects before the start of the procedure to minimize this risk. The primary side effects associated with atropine administration is mild sinus tachycardia which typically resolves within 30 minutes as the drug is cleared from the subject's system. In rare cases (less than 1 percent) the sinus tachycardia is accompanied by hypotension.

Hypoxemia: Arterial blood oxygen saturation is continuously monitored during the procedure with a finger pulse oximeter. Subjects are supplemented with oxygen at a flow rate of 2 l/min via nasal cannula during the procedure. The oxygen flow can be increased to a maximum of 6 l/min if the arterial oxygen saturation falls below 93 %. If oxygen supplementation is not effective, the procedure will be discontinued immediately. Removal of the bronchoscope and treatment with inhaled bronchodilator should be sufficient to allow the subject's arterial oxygen saturation to return to normal. Under normal conditions, oxygen supplementation is ceased once the procedure is completed. However, supplementation will be continued if arterial oxygen saturation is below 93 % on room air. In the event that low oxygen saturation persists, if the subject cannot be weaned from the oxygen supplementation, or if it falls precipitously at any time, an emergency situation will be declared and the subject will be transported immediately by ambulance to the UNC Hospital Emergency Room. These risks are minimized during the research procedure at the Human Studies Facility, in part because premedication with sedatives (midazolam) or with opiates (demerol) (as is used in medical bronchosopy) is not required for healthy research subjects. Avoiding these drugs reduces procedure risk and allows the participants to enjoy a normal day after leaving the EPA Human Studies Facility.

The risk of pneumonia as a result of bronchoalveolar lavage in the lobe involved in the procedure is less than 1%. Symptoms of pneumonia could include fever, dyspnea, persistent cough, productive cough and chest pain. The subject will be instructed to call the physician who performed the procedure or the Medical Station if these symptoms occur. The medical staff will evaluate the subject over the phone between 24 and 48 hours after the procedure for signs of pneumonia.

The subject will be urged to contact the Medical Station or the bronchoscopy physician should he/she experience any of the following symptoms: 1) Epistaxis, 2) Persistent cough, 3) Hemoptysis, 4) Chest pain, 5) Dyspnea, 6) Wheezing, 7) Sputum production, 8) Hoarseness or sore throat.

Confidentiality Risk

Risk of breach of confidentiality is minimal. For the mild asthmatic subject, it is conceivable that an employer or insurance company could learn of the diagnosis if the subject requires medical intervention as a result of

participating in the study. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files which may contain the subject's name or screening number are secure in the EPA building which has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, spirometry) will be discussed with the subject and the subject will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data 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 changes in epigenetic mechanisms in bronchial epithelial cells can be linked to physiological changes in healthy and mild/moderate asthmatic volunteers safely exposed to low levels of O3 and diesel exhaust. The study will follow a randomized, repeated measures design with each subject exposed to clean air, diesel exhaust and ozone. The data analysis will be focused on changes in lung function measurements, primarily FVC and FEV_1, and secondarily, HRV measurements between pre and post 2hr exposure for each exposure and lung cell composition changes. Also blood endpoints include inflammatory factors (eg IL-6), blood clotting factors (eg fibrinogen), and susceptibility factors (eg specific genotypes associated with susceptibility to air pollution).

In evaluating exposure effects of O3 and DE 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 (Folinsbee, Bedi et al. 1985) for a constant acute exposure to 0.3 ppm O_3 versus filtered air. In 22 subjects, the pre exposure FEV_1 was 4.608 ± 0.514 (mean $\pm$ SEM) in FA exposure and 4.656 ± 0.592 in O_3. The post exposure FEV_1 was 4.64 ± 0.476 in FA 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 the required sample size for each group is 30 subjects. We will initially recruit 30 subjects for each group (healthy and mild/moderate asthmatics) for this study to ensure the statistical power that can analyze small differences expected from low concentration exposures.

Other Exploratory Observations:
We hypothesize that subjects harboring polymorphisms in antioxidant genes will have increased responses to diesel exhaust and ozone compared to subjects who do not. In order to address this we will use collected cell samples for in vitro exposure experiments

Exploratory endpoints will include the effect of diesel exhaust and ozone on changes in: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values.

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, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data.** Are the identifiers in A.4.9 above linked or maintained with the research data?

_X_ yes __ no

A.4.11. **Confidentiality of the data.** Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in a locked file cabinet in the Medical Station at the U.S. EPA 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 a day for seven days a week

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

_X_ No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data stored on a desk top computer:
_X_ Secure network _X_ Password access __ Data encryption__ Password protected file(s)
__ Other comparable safeguard (describe):

For portable computing devices/external storage devices (e.g. laptop computer, PDA, CDs, memory sticks):
__ Power-on password __ Automatic log-off __ Data encryption __ Password protected file(s)
__ Other comparable safeguard (describe):

For hardcopy data (including human biological specimens, CDs, tapes, etc.):
__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office _X_ Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples. Subjects at any time may request that their samples be no longer stored in the repository. Any analysis in progress at the time of the request or already performed prior to the request being received by the researcher will continue to be used as part of the research study. Once the researcher is notified, the subject's specimens will be destroyed.

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.*

The subject will be given an opportunity to read the consent form. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent.** *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *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 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 __ yes __ no

Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).*
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Subjects for U.S. EPA Studies (95-EPA-66). Typical methods for recruiting may include mass mailings, fliers and print ads. 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. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of this study will be approximately eighteen months. Subject recruitment and screening is expected to be continuous throughout the study until the intended number of subjects is reached.

Scheduling constraints imposed by concurrent studies in the U.S. EPA Human Studies Division are expected to limit the rate at which subjects can be exposed to 1-2 per week.

The physical examination evaluation may take up to one hour. If the subject is eligible for the study, the individual may have up to nine more visits to the research facility over approximately 6 months. A physical examination for bronchoscopy will be performed. The training day (Day 1) visit will require approximately 2 hours. Exposure day (Day 2) will last approximately 6 hours. Subjects will report to the facility at no sooner than 8 AM. The morning following the exposure, the subject will return for Day 3 which a follow-up visit which may last approximately 4 hours. Subjects will report to the facility at no sooner than 8 AM. Subjects will then return and repeat the Day 2-3 visits with the second and third exposure session. Exposure sessions will be separated by a minimum of two weeks.

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

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators for involuntary reasons after enrollment in the study but prior to completion will be paid for their participation up to that point

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and for any procedures cancelled up to a maximum of $100. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study:
Subjects will be paid approximately $12 per hour for participation in this study. If the subjects complete all three exposures with bronchoscopy, the total compensation including prestudy qualifications will be approximately $2516.

Pre-study qualifications		
recruitment screening	$15	Previously paid
physical exam	$15	Previously paid

bronchoscopy physical exam	$20
	Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24
	Training Day Total = $ 44

Subjects will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure
Endobronchial Brush biopsies	$ 25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus	**$100**

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures	**$1548**
TOTAL for completion of THREE exposures (including completion bonus)	**$2422**

If a subject begins but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, they will receive compensation for participation up to that point.

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

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***This section applies even if records are only used to identify potential subjects.***

→ ***If your study does not use existing data, records or specimens for any purpose, 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
If applicant was involved in the original collection, please explain role:

__ 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, do you have permission from the custodians of the data, records or human biological specimens (e.g., pathology dept, tissue bank, original researcher)? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 24 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE (not present)
1 = TRACE/NOTICED (barely detectable)
2 = MILD/LIGHT (present, but not annoying)
3 = MODERATE (present, but somewhat annoying)
4 = SEVERE/HEAVY (present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4

4/22/10 9/27/10

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study and Consent for Genotype Screening
With Identifying Information
Subjects 18 years of age or older
Biomedical Form

IRB Study:# 09-1625
Consent Form Version Date: January 4, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D. and David Diaz-Sanchez, Ph.D.
Department: United States Environmental Protection Agency
Phone number: 919-966-7320

Co-Investigators: Michael T. Schmitt, MSPH

Sponsor: U.S. Environmental Protection Agency

You are being asked to take part in a research study. The investigators listed above are in charge of the study; other professional persons may help them or act for them.

What are some general things you should know about research studies?

Research studies are designed to gain scientific knowledge that may help other people in the future. You may not receive any direct benefit from participating. There may also be risks associated with participating in research studies.

Your participation is voluntary. You may refuse to participate, or may withdraw your consent to participate in any study at any time, and for any reason, without jeopardizing your ability to participate with EPA-sponsored research.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want to participate. You will be given a copy of this consent form. You are urged to ask the investigators 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 research study is to understand how ozone exposure compares to that of diesel exhaust exposure in order to investigate mechanisms of injury by environmental pollutants. This may be especially important for asthmatics, because studies have shown an association between exposure to air pollution and increased medication usage and emergency room visits in this population. To accomplish this we will:

- Document changes in lung function (spirometry) and the presence of mild inflammation in the respiratory tract (measured by bronchoscopy to collect cells from the lower respiratory tract) after each exposure.
- Determine if air pollution exposure can affect components of the blood involved in inflammation and clotting.
- See if air pollution particles can cause changes in the ability of the nervous system to correctly regulate one's heart rate.
- Cells from your airways will be collected and stored. All specimen collections will be stored throughout the course of this study and then any remaining samples will be stored indefinitely as described in an accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information.***
- The cell samples will be used to identify the expression of a panel of inflammation- and oxidative stress-related genes. As new genes are identified that may be associated with adverse health effects of air pollutant exposure, expression of these genes may also be investigated.
- The cell samples will be used to identify epigenetic changes (mechanisms which control the expression of genes) associated with air pollutant exposure.
- Research reports have suggested that people with particular genes are more susceptible to air pollutants. The cells will be studied for those specific genes related to adverse health effects of air pollutant exposure.

Are there any reasons you should not participate?

You should not participate in this study if:

- You are pregnant, attempting to become pregnant or nursing an infant
- You have a history of significant chronic illness other than asthma, or your asthma is not well-controlled
- You are not 18 to 55 years of age
- You cannot perform intervals of moderate exercise
- You are not fluent with the English language
- You are taking more than 81 mg aspirin regularly
- You are unwilling or unable to refrain from the following medications for the week prior to the study: anti-inflammatory agents such as ibuprofen (Advil), naproxen (Aleve, Naprosyn) or aspirin
- You have skin allergy to tape or electrodes
- You are currently taking or have taken anti-coagulant medication in the week prior to the study
- You are currently smoking or have a smoking history within 2 years of the study
- You have been diagnosed as a severe asthmatic as defined by physician-directed emergency treatment for an asthma exacerbation within the preceding 12 months, use of systemic steroid therapy within the preceding 12 months, nighttime symptoms of cough or wheeze greater than 2 times per month, asthma symptoms (cough, wheeze, chest tightness) more than 2 times per week, requirement for albuterol due to asthma symptoms more than 2 times per week, (not including prophylactic use of albuterol prior to exercise), have chronic and continuous allergic rhinitis.

- Your dosing level of an inhaled steroid is not consistent with the severity of your asthma as outlined by the NHLBI NAEPP guidelines
- You have a history of fainting in response to pain
- You currently have or have had a viral respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of the study. If this is the case, your visit will be rescheduled
- You currently have or have had seasonal exacerbation of allergic rhinitis symptoms within 1 weeks of the study. If this is the case, your visit will be rescheduled

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

- No over the counter pain medications such as aspirin (aspirin $\leq$ 81 mg/day is allowed), Advil, Aleve or other non-steroidal anti-inflammatory medications for 7 days prior to all visits. Tylenol (acetaminophen) is permitted.
- No vitamin C or E (or multivitamins which contain Vitamins C or E) for 7 days prior to all 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.
- Avoid eating or drinking anything after midnight on the evening of the Exposure Day 1, before the bronchoscopy to take place on Exposure Day 2.
- No caffeine for 12 hours prior to all study visits.
- Avoid use of antihistamines for one week prior to exposures.

How many subjects will participate in this study?

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

How long will your participation last?

Your participation in this study may require up to 9 visits to the research facility over approximately 6 months if you are eligible for the study.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone a general screening visit and physical examination, and a physical exam and pulmonary function test for bronchoscopy to certify that you are a suitable candidate. For the exposure study, we will perform the following procedures:

Training Day

- Report to the EPA medical station at the requested time.

- 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 this consent form. We will give one copy to you. We will check your vital signs (heart rate, respiratory rate, blood pressure, oxygen saturation level and a symptom questionnaire) and do a pregnancy test if applicable(WOMEN ONLY) For the pregnancy test, you will be asked to provide a urine sample. The purpose of this test is to provide evidence that you are not pregnant at the time of the study. The nurse will also ask you when your most recent menstrual cycle began. The results of the pregnancy test will be held in strict confidence. A positive pregnancy test will preclude further participation.
- You will undergo a breathing test. This is known as spirometry, and 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. The information gained from the breathing tests will be used by the investigators to determine whether you qualify for the study.
- You will be trained on the exercise bike and the work load to elicit a minute ventilation normalized for body surface of $25L/m^2/BSA$ will be determined.

Exposure Day 1: You will be asked to eat a normal breakfast and arrive at the EPA medical station no sooner than 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level) and fill out a symptom questionnaire. Have a pregnancy test if applicable.
- Have monitors placed on you so that we can watch your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on for 24 hours. Before exposure, after exposure and the following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate. This will consist of a 20 minute rest period and a 10 minute data collection. The monitor will be removed after the last data collection. These monitors will allow us to determine if air pollutants cause small changes in the ability of your nervous system to regulate how your heart beats.
- Have up to 80 mL blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust or ozone exposure affects the ability of your blood to clot correctly, and to look at changes in proteins and/or blood cells.
- 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.
- Given a Symptom Questionnaire for you to fill out.
- Testing will be postponed if it is determined that your medication history is not within previously established limits, respiratory tract illness has occurred within the previous 6 weeks or if respiratory symptoms are present.

During the exposure, you will:

Undergo a chamber exposure of 2 hours duration, once to clean air, once to diesel exhaust and once to ozone; the amount of pollution you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. You will be blinded as to the exposure condition. The exposure chamber is 6 ft wide by 6. ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. You will exercise for 15 minutes at the previously determined pace and rest for 15 minutes. While exercising, minute ventilation will be measured for three minutes. Your blood pressure will be measured intermittently. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid for your participation up to that point, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Following the exposure, you will:

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have your vital signs checked.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Fill out a symptom score sheet.
- Have blood drawn (up to 80 mL or about 5 tablespoons).
- Be assessed and discharged by the nursing staff.

Exposure Day 2 (24 hour follow up visit): You will come back the next morning and asked to arrive at the EPA medical station at no sooner than 8 am. (approximately 24 hours after exposure) You will:

- Have your vital signs taken.
- Fill out a symptom questionnaire.
- Have blood draw (up to 80 mL or about 5 tablespoons).
- Have spirometry testing.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Have the Holter monitor removed.
- We are asking all subjects to have bronchoscopy on the day following each exposure. Prior to the bronchoscopy you may be pretreated with inhaled bronchodilator (albuterol). The bronchoscopy will not be performed if the baseline lung function shows more than a minimal amount of airway obstruction and this does not resolve with inhaled bronchodilator. During the bronchoscopy, a fiberoptic scope will be inserted through the nose into one of your airways where a portion of one of your

lungs will be washed with sterile saline and a sample of airway lining cells will be obtained with a special brush device.

Bronchoalveolar Lavage (BAL) and Brush Biopsy is frequently used in the diagnosis of lung disease in both adult and pediatric patients. The procedure has also been used in numerous research studies of persons with respiratory disease and in healthy volunteers. The procedure has been used safely many times in studies conducted at our laboratory. The purpose of the BAL procedure is to obtain fluids and cells from the lower regions of the respiratory tract, i.e., the trachea, bronchi and smaller airways. These materials will be analyzed to obtain information about the role of various chemical substances and cells in the pulmonary response to particle exposure of persons with asthma. The bronchoscope is a highly flexible fiberoptic tube that is about two feel long and 1 centimeter in diameter (about the diameter of a pencil). It is an optical device with a light at the end which can be used to transmit images to a camera connected at the other end. Using the bronchoscope, the physician can see into your airways and direct the placement of the bronchoscope. A small channel allows fluid to pass through the bronchoscope.

The BAL procedure will be performed at the laboratory medical station by specially trained experienced pulmonary physicians. You will be expected at the medical station at 8:00 on the morning after the exposure. If you have had anything to drink or eat since midnight the night before the bronchoscopy you will not be allowed to proceed. Before you undergo bronchoscopy, your lung function will be evaluated and you may be treated with inhaled bronchodilator (albuterol). The procedure will not be performed if your lung function does not exceed a predetermined level of function recommended by the National Institute of Health for performing research bronchoscopies in persons with asthma. You may terminate the bronchoscopy procedure at any time. If the physician deems that your are too uncomfortable or anxious, the procedure will be terminated.

A saline lock intravenous catheter will be placed in a vein in your arm to give you medication before the procedure starts (a saline lock is a flexible catheter that stays in your arm for a short time). The saline lock will remain in place so that it can be used to administer medications in case there are any problems during the procedure. You will also be connected to a telemetry monitor that will display heart rate and rhythm, a blood pressure cuff will be placed on your arm, and an oximeter sensor (small band like device will be placed on a finger to allow the medical staff to monitor you during the procedure. No sedatives and/or narcotics will be administered at any time during bronchoscopy.

Before proceeding, the medical staff will again make certain that you have had nothing to eat or drink since midnight the previous night. You will be asked to decongest your nose by administering 2 puffs of neosynephrine nasal spray to each nostril. They will then give you a lidocaine solution and ask you to gargle with it for a few seconds to anesthetize your throat. You will then be asked to inhale (snort) a small amount of lidocaine jelly through one nostril to anesthetize your nose and the back of your throat. A Q-tip with lidocaine jelly will be gently inserted into your nose to ensure that your nose is completely numb before the bronchoscope is inserted. The procedure will not begin until your nose and throat are well anesthetized. If this cannot be

accomplished, the bronchoscopy will not be performed. A tube delivering oxygen will be placed inside your other nostril. Delivery of supplemental oxygen is done as a precaution during all bronchoscopies conducted at our facility.

To start the procedure, the physician will pass the bronchoscope through your anesthetized nostril to the back of your throat and then to above your vocal cords. He will then inject a lidocaine solution to numb your vocal cords before passing the bronchoscope into your trachea (windpipe). More lidocaine, up to a safe maximum dose is injected at various points in your trachea and airways to minimize coughing during the procedure. You will experience some cough during the procedure. This is a normal reflex caused by the presence of the bronchoscope in your airway.

The bronchoscope will be gently wedged in an airway in the right lung and sterile saline will be injected in your lung through a channel in the bronchoscope. The saline will then be gently suctioned from your lung through the channel in the bronchoscope. This sequence will constitute a wash. Six (6) washes will be performed. A total of 270 cc (about one-half pint) of sterile saline will be used during the BAL procedure. Approximately 75% of the saline injected into your lungs will be recovered by aspiration (suction) through the bronchoscope. The remaining 25% (75 cc) is expected to remain in your lungs. The saline left in your lungs will not generally cause you any difficulty breathing or harm you and should be completely absorbed by your lungs within 48 hours.

After the BAL is performed, brush biopsies of your airway epithelium will be taken. A very small brush (3 millimeters in diameter) will be inserted through the channel in the bronchoscope. The brush is visible to the physician performing the procedure through the bronchoscope. Small amounts of surface cells are scraped from the airway by gently brushing the airway several times. Two (2) brush biopsies are taken at one site in the right lung. After the brush biopsies are obtained, the procedure will be complete and the bronchoscope will be removed from your airway. The total time the bronchoscope will reside in your airways will be 15-30 minutes.

After the procedure the oxygen cannula will be removed if your oxygen saturation is acceptable. The nurses will check your vital sign immediately after the procedure and every half hour thereafter until discharge. During this time you will sit in a recliner at the medical station for an observation period of approximately two hours. You will not have any food or drink during this time. After the recovery period, the nurses will check your gag reflex. Since the gag reflex will be absent due to anesthesia during the procedure, you will not be allowed to ear or drink until the anesthesia wears off. This normally takes about one to one-and-one half hours. Once your gag reflex returns, you will then be given some juice to sip and then some crackers.

The physician who performed the procedure will check you after the recovery period and you will repeat the spirometry lung function test. You will be discharged if your vital signs are stable, chest examination is normal, and lung function is greater than 80% of your pre-procedure value. Prior to discharge you will be requested to take 600 mgm of ibuprofen by mouth; administration of ibuprofen/motrin almost always prevents the post-bronchoscopy malaise and low grade fever that would otherwise occur in about 25% of persons undergoing the procedure (acetaminophen/tylenol is a less effective alternative medication).

Before going home, you will be give the phone number of the medical station (966-6232) and pager number to the physician who performed the bronchoscopy with instructions to call if you experience any adverse symptoms such as: 1) persistent fever or fever above 101 degrees Fahrenheit, 2) persistent cough, 3) sputum (phlegm) production, 4) chest pain, 5) coughing up any amount of blood, 6) nose bleeds, or 7) shortness of breath

Are there any reasons you should not participate in bronchoscopy?
You should not participate in this study if you have problems with excessive bleeding after minor cuts or abrasions. You should not participate if you have had a recent exacerbation in your asthma that was not controlled with your usual medications. The physician and medical staff will explain other exclusionary condition in detail to you. In addition, during your participation in this study, you will be asked to refrain from, limit, or control taking the following medications:

*No more than 81 mg aspirin or similar medication on a regular basis or during the week of testing.
*Withhold antihistamines for 7 days prior to testing.
*Withhold inhaled bronchodilators 12 hours prior to testing.
You should not participate in this study if you are unwilling or unable to remain in the local area for 24 hours following each bronchosocpy

With your permission, DNA from the collected cells will be genotyped for specific genes related to adverse health effects associated with air pollution exposure. Unwillingness to have samples genotyped will NOT exclude you from participating in the study. If you do not wish for your cells to be used for genotyping but do wish to participate in the study do not sign the section ***Subject's Agreement for Genotype Screening With Identifying Information*** located at the end of this document.

What if we learn about new risks 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.

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. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely. With your permission, we will continue to store any samples that we collect to possibly use for other studies. This allows us to make the best use of the samples we collect from our volunteers. Again, you do not have to allow us to keep these samples if you do not want to.

What are the possible benefits from being in this study?
Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study. We will share with you any information that we learn about your lung function or other medical findings. We can also send you the results of some of the blood tests we perform.

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

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

2. 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. Also, the forceful breathing during this test can cause wheezing or shortness of breath to occur.

3. The adhesive patches used as part of the heart monitoring system may cause some mild skin discomfort or discoloration. If this occurs you should notify the nursing staff.

4. During air pollution exposure, you may experience some minor degree of airway irritation, cough, shortness of breath or wheezing. Some studies suggest that persons with asthma may be at risk for developing an asthma attack as a result of exposure to fine air pollution particles. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. Your symptoms will be closely monitored during the exposure and the study terminated if more than modest respiratory symptoms occur during the exposure. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. If you experience a significant drop in your lung function, albuterol will be administered.

Air pollution may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. There is a small possibility that you could have more asthma symptoms for several days, and even less likely, for several weeks, after the particle exposure. This flare in your asthma would be similar to what happens to you and other asthmatics during the allergy season or with a viral infection. Such a flare is considered highly unlikely and the exposure risk is comparable to walking around a major metropolitan area such as Los Angeles or Houston on a smoggy day. If an asthma flare occurs, you will be asked to seek medical treatment from your regular physician who treats you for your asthma.

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 6 ft in width, 6 ft in height, and 8

ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator at all times.

5. Bronchoscopy: As with other medical procedures, there are risks associated with bronchoscopy. However, experience has shown these risks are extremely small in research subjects. The risks associated with bronchoscopy are described in detail below.

A primary risk is bronchoscopic procedures is **discomfort to the nose and throat** brought about by the insertion of the bronchoscope through the nasopharynx. This discomfort should be alleviated or eliminated by the use of local anesthetics such as lidocaine. For this reason, you will be asked to gargle with a lidocaine solution and have lidocaine jelly placed in your nose prior to beginning the procedure. The effectiveness of the anesthesia will be tested by using a cotton swab gently inserted into your nose before inserting the bronchoscope. If you experience pain or discomfort during insertion of the bronchoscope, more lidocaine will be used up to a safe maximum amount. If adequate anesthesia cannot be achieved using this amount of lidocaine, the procedure will not take place.

A second risk of bronchoscopy is **coughing** that results from the irritation of the airway caused by the presence of the bronchoscope in the airway. Coughing is a normal reflex to expel foreign substances from the airways. Because coughing itself can cause mild trauma and discomfort to the airways and vocal cords, lidocaine liquid is sprayed on the airways at various sites to suppress the cough reflex. This technique is usually not completely successful and it is expected that you will have some cough during the procedure, especially during the initial insertion of the bronchoscope into the airway. If coughing is not controllable with the lidocaine or causes you discomfort, the procedure will be terminated.

There is a slight chance that the bronchoscopic procedure will precipitate an **asthma attack**. This risk is minimized by 1) only allowing persons with mild stable asthma to participate in the study, 2) pretreatment with inhaled bronchodilator (albuterol) before the procedure if your lung function is impaired and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physician responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. You will not be discharged from the EPA Medical Station until your lung function has returned to 80% of your pre-procedure performance. . In the unlikely event that you require additional treatment because the asthma attach continues, you will be transported by ambulance to the emergency room of the UNC Hospital, located 1/4 mile away.

Nose bleeds are another risk of bronchoscopy. This is caused by trauma to the nose during the bronchoscopy procedure. Usually, the bleeding is minor and is noticed as a few drops of blood in mucous secretions in the nostril. The bleeding usually stops on its own within an hour after the bronchoscopy. On very rare occasions, if the bleeding becomes moderate to severe, your nostril will be packed

with sterile gauze to absorb the blood and put pressure on the site of bleeding. Should you require additional treatment, you will be transported to the UNC Hospital Emergency Room. Major nose bleeds during bronchoscopy are very rare and generally limited to persons with kidney disease or a blood clotting disorder. It could also be promoted by chronic use of aspirin (>81 mg), ibuprofen or similar medications. You will not be allowed to participate in this study if you are taking such medications. Therefore the probability of getting a major nose bleed is very small.

Major **bleeding of the airways** is another rare (less than one percent) complication of bronchoscopic procedures. Most episodes of major bleeding due to bronchoscopy occur in persons with underlying predisposing conditions and are due to forceps biopsies of the airways mucosa which will **not** be performed in this study. People who suffer from kidney disease, blood clotting disorders or are chronic users of aspirin-like drugs are at increased risk of bleeding during bronchoscopy. In addition, people with lung cancer or with serious lung infection are more likely to suffer from airway bleeding during bronchoscopy. Your screening medical history, physical examination, and blood analysis will reveal most conditions that would increase your risk of bleeding during the bronchoscopy. You will not be allowed to participate if you have any of these conditions. Therefore your chances of incurring a serious airway bleed during bronchoscopy are extremely small. Nevertheless, if you do experience bleeding during bronchoscopy, you will be under the care of an experienced physician who will take steps to stop the bleeding immediately. A special cart with emergency supplies is available to handle medical emergencies. In the event of a major bleeding episode, you will be immediately transported to the Emergency Room of the UNC Hospital.

Pneumothorax or collapsed lung is another possible complication of bronchoscopic procedures. This complication is more likely to occur during tissue biopsy in the lung periphery, not the major central airways which will be the focus of this study. However you will be advised of the symptoms in the event that you experience a pneumothorax. The symptoms include chest pain and shortness of breath. If you experience these symptoms, you should contact the medical station (966-6232), or page the physician who performed the bronchoscopy. The vast majority of pneumothoraces occur within 24-48 hour of the procedure being completed. While a serious condition, a pneumothorax can be readily treated in an emergency room.

The **lidocaine** used to anesthetize your nasopharynx and reduce your cough can pose certain risks as well. A small fraction of the lidocaine applied to your nose and airways will be absorbed into your bloodstream, where it could cause adverse effects. Your risk of suffering side effects are very small, but would be much greater and potentially life-threatening if you are allergic to lidocaine or other topical anesthetics. You will not be allowed to participate in this study if you have drug allergy to topical anesthetics. When used at high doses, lidocaine can also cause symptoms in your central nervous system (confusion, tremor, euphoria, or, rarely, seizures) or heart rate disturbances (very fast or very slow heart rate) if an excessive dose of medication is used. Finally, a death in a volunteer receiving an overdose (over 1000 milligrams) of lidocaine during bronchoscopy has been

reported from Rochester, New York. However, no serious side effects of this medication have been noted at lower doses such as those described in this protocol which uses up to 360 milligrams of lidocaine during the entire procedure. If any problems develop secondary to the use of lidocaine, the physician bronchoscopist and the doctor on duty that day at the Human Studies Division of the EPA will be available to handle these problems.

Prior to the start of the bronchoscopy **atropine** may be given though a vein in your arm in order to counteract any changes in blood pressure that would result from the placement of the bronchoscope in your airway and to limit the quantity of secretions in your nose and throat. Atropine can cause dry mouth and nose for 30 to 60 minutes after the procedure is completed. This effect should wear off about an hour after the procedure is completed.

Placing an **intravenous catheter** in your arm carries a small risk as well. The needle stick can be momentarily painful and more rarely can leave a bruise in your arm. The medical staff who will be placing the catheter are very experienced and therefore the risk is minimal. Infection is also a very small risk that can result from placing the catheter. Again, the medical staff is very experienced in the use of sterile technique to prevent infection. If you notice redness or swelling in the area surrounding the catheter site, you should contact the medical station at 966-6232 or the physician on call for treatment.

A low grade fever (less than 101EF) during the 24 hours following the procedure occurs in about 25% of patients with respiratory disease undergoing bronchoscopy and BAL. The fever is benign and is usually effectively prevented by administering ibuprofen following the procedure. However, if you experience fever greater than 101 EF or lasting longer than 24 hours you could have pneumonia and should contact the Medical Station immediately so that you can be examined and treated.

Pneumonia occurs in less than 1 percent of patients undergoing bronchoscopy and BAL. Signs of pneumonia include fever greater than 101 EF, or persisting for more than 24 hours, persistent cough with or without sputum production, coughing up blood, chest pain, and shortness of breath or wheeze. Should you experience these symptoms, it is imperative that you contact the Medical Station to be seen and evaluated by a physician. As a routine precaution the Medical Station Staff will contact you between 24 and 48 hours following the procedure to inquire about any symptoms you may be experiencing.

In addition, there may be uncommon or previously unrecognized risks that might occur.

There are no risks 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 may cause some irritation, itching, or burning in some people. If this occurs you should notify the nursing staff.

The following are policies and procedures to minimize risk during the experiment:

1. During this study, skilled personnel, in an adequately equipped facility, will be observing you for any adverse effects. You may, without penalty, stop the test at any time by telling the monitor.
2. A physician will be on call at all times during the study to deal with any medical problems that may arise.
3. Every test has some degree of risk and there may be some risks associated with this experiment which are unforeseeable. You should not participate in the study if you have an acute illness.
4. You may terminate your participation in the experiment at any time and for any reason that you wish without penalty; you will be paid an amount for participation prorated for the time and procedures in which you took part. You understand that you are not under any obligation to complete any test procedures which you find objectionable or uncomfortable.
5. The investigator may terminate your participation in the experiment at any time. If this happens, you will be entitled to full payment for your time commitment and any experimental activities up to that point.
6. Your participation in the study may be postponed if your pre-exposure lung function shows airflow obstruction or if you develop a cold or asthma exacerbation.

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. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

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, the U.S. EPA and/or UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

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

You may withdraw from this study at any time. If you choose to withdraw or are unable to complete the study because of not following the instructions in this consent form, you will receive compensation only for your participation up to that point.

The investigators have the right to stop your participation at any time. This could be because you have had an unexpected reaction, have failed to follow instructions, or because the study has been stopped. After you enroll, if you are dismissed by the investigators before completing the study for reasons that are not your fault, you will be paid for your participation up to that point.

We made need to cancel your study activity due to adverse weather conditions, equipment failure, or other unforeseen events. If a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid for the time scheduled and cancelled at the standard hourly rate of $12 per hour plus any cancelled activities up to a maximum of $100. When possible, you will be rescheduled at a later time.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study. Total compensation will be based on the number of exposure sessions that will be required.

1. If you are complete all three exposures sessions of this study, your total compensation including screening will be approximately $2516.

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

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is

normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. 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. Compensation for the visits will be as follows:

You will be exposed to diesel exhaust ,ozone and have an air control exposure in three separate sessions. Exposure Days 1-2 will be repeated for the second and third chamber exposure session, but a second training Day is not needed. If you complete three exposures, your compensation including the pre-study qualifications and training day will be approximately $2516.

Pre-study qualifications

recruitment screening	$15	Previously paid
physical exam	$15	Previously paid
bronchoscopy physical exam	$20	Previously paid

Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24

Training Day Total = $ 44

You will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure

Endobronchial Brush biopsies	$25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus	**$100**

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures (excluding Prestudy qualifications and training day)	**$1548**
TOTAL for completion of THREE exposures (excluding Prestudy qualifications/training day but including completion bonus)	**$2422**

If you begin but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, you will receive compensation for your participation up to that point however in you arrive in the lab for the procedure having not followed instructions (eating food, taking NSAIDs or aspirin, or failing to report cold or flu symptoms the day prior to the bronchoscopy) you will be compensated $15 for your time, but will not receive compensation for the bronchoscopy.

You will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available.

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 or follow directions of the investigators, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and cancelled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during a current visit if cancelation occurs while you are in the facility. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled. The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be reimbursed for time and procedures completed up to that 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/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

Who is sponsoring this study?
This research is funded by the United States Environmental Protection Agency. This means that the research team is being compensated by the sponsor for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the product being studied.

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 US Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, 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 the Chairman of the IRB Committee at 919-966-1344 and /or the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What specimens will be collected and stored?

Bronchial epithelial cells collected via bronchoscopy and all blood specimens will be stored with your permission. If you agree to have your specimens stored for future research you will need to sign the accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information***.

IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D. and David Diaz-Sanchez, Ph.D.

Subject's Agreement to Participate in the Research Study with Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I voluntarily **AGREE** to have my provided bronchial epithelial cells genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

Signing here and agreeing to participate in this study DOES NOT allow us to store your samples.

Signature of Research Subject ____________________ Date ____________

Printed Name of Research Subject ____________________

Signature of Person Obtaining Consent ____________________ Date ____________

Printed Name of Person Obtaining Consent ____________________

IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D. and David Diaz-Sanchez, Ph.D.

Subject's Agreement to Participate in the Research Study without Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I **REFUSE** to have my provided bronchial epithelial cells genotyped for any

genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

______________________________ ______________

Signature of Research Subject Date

____

Printed Name of Research Subject

______________________________ ______________

Signature of Person Obtaining Consent Date

____

Printed Name of Person Obtaining Consent

11/20/09 9/27/10

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

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IRB Study # Consent Form Version Date: June 2009

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D and David Diaz-Sanchez, Ph.D.
UNC-Chapel Hill Department: US EPA
UNC-Chapel Hill Phone number: 919-966-7320
Email Address: jardim.melanie@epa.gov
Co-Investigators:
Funding Source and/or Sponsor: EPA funds

Study Contact telephone number: 919-966-7320
Study Contact email:
jardim.melanie@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. If you are a patient with an illness, you do not have to be in research in order to receive treatment.

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

What is the purpose of this specimen repository or "biobank?"
Research with blood, tissue 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."

The purpose of this particular repository or biobank is to:

- Document changes in lung function (spirometry) and the presence of mild inflammation in the respiratory tract (measured by bronchoscopy to collect cells from the lower respiratory tract) after each exposure.

- Determine if air pollution exposure can affect components of the blood involved in inflammation and clotting.
- See if air pollution particles can cause changes in the ability of the nervous system to correctly regulate one's heart rate.
- Store samples of respiratory cells and blood samples. These collections are part of the protocol described in the accompanying consent form **Consent to Participate in a Research Study** and the components stored represent excess tissues that are not immediately required for the study but may have value for future research.
- Cells from the lung will be used to identify the expression of a panel of inflammation- and oxidative stress-related genes. As new genes are identified that may be associated with adverse health effects of air pollutant exposure, expression of these genes may also be investigated.
- Research reports have suggested that people with particular genes are more susceptible to air pollutants. The epithelial specimens will be genotyped for those specific genes related to adverse health effects of air pollutant exposure.
- Studies of gene expression represent one type of genetic research, thus future investigations involving these specimens are intrinsically of a genetic nature.

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

How will the specimens be collected?
Samples will be collected in the following manner.

- Lung cell collection. Cells will be collected from the airways and airspaces of the lung by bronchoscopy with bronchoalveolar lavage and brush biopsy. (This procedure is explained in separate consent.) Fluid will be collected into specimen tubes and placed on ice. The cells will be separated from the fluid by centrifugation (very fast spinning whereby the cells will separate from the liquid and fall to the bottom of the tube), and both samples may be processed immediately, or frozen for later analysis.
- Blood collection. You will have up to 80 cc (about 5 tablespoons) of blood per sample collected from an arm vein by our trained nursing staff. The blood will be placed into specially designed blood collection tubes which allow separation of the fluid and cell portions of blood. Those samples may be processed immediately, or be frozen for later analysis.

What will happen to the specimens?
Samples will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill, North Carolina. Only project members of the study will have access to the samples. Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the air pollution exposure. Specimens may be released to other investigators if there is a need to analyze samples with equipment that is not located at our facility.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding asthma and how environmental factors affect the disease

What are the possible risks or discomforts involved with the use of your specimens?
This study might involve the following risks and/or discomforts to you:

Risk of breach of confidentiality is minimal. For the mild asthmatic subject, it is conceivable that an employer or insurance company could learn of the diagnosis if you required medical intervention as a result of participating in this study. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

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

Will you receive anything for the use of your specimens?
You will not receive anything for taking part in this research.

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

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

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires

the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, U.S. EPA and/or 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.

You will not be identified in any report or publication about research using your specimens. 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 could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

A Federal law called the Genetic Information Nondiscrimination Act (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. GINA does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. GINA also does not protect you against discrimination based on an already-diagnosed genetic condition or disease.

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants. In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive results from research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you personally. There are no plans to re-contact you or other subjects with information about research results.

Can you withdraw the specimens from the research repository?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. It is best to make your request in writing.

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

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this research?
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 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.

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

Epigenetic Effects of Diesel Exhaust and Ozone Exposure, IRB Study #_____
Web Site Announcement
www.epastudies.org

NOV 2 0 2009

[ACRONYM TO BE DECIDED]

What is the purpose of the research study?

The purpose of this research study is to compare changes in lung function, lung cells, blood and heart rate between people with and without asthma when they are exposed to diesel exhaust and ozone. This study is for healthy people who have mild to moderate asthma and those who do not have asthma.

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

- Are between 18 and 55 and
- Are healthy or
- Are healthy but have mild to moderate asthma and
- Do not smoke and
- Can perform moderate exercise and
- Can stop taking certain drugs and food supplements for a week before each exposure

What will I be asked to do?

- Have a free physical exam
- Have blood drawn
- Take part in breathing tests, including spirometry (explanation link provided)
- Have your heart rate monitored (explanation link provided)
- Have your blood pressure monitored
- Use an exercise bike during testing
- Come in 3 different times for 2 hour exposure sessions when you will either
  - 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
- Return the next day for bronchoscopy (explanation link provided) and more testing

How long will it take?

- After 2 screening visits there will be 7 study visits, including 1 training visit and 3 sets of 2-day exposure visits, each set separated by at least 2 weeks
- In total, the study will take about 32 hours over 6 to 8 weeks.

What will I get for volunteering?

- You will be paid for screening, for the study, and for out of town travel.

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

NOV 2 1 2005

[STUDY ACRONYM] Study E-mail Announcement

SUBJECT: INFORMATIONAL: Research Study about Ozone, Diesel Exhaust and Exercise for People With and Without Asthma

This research study is for healthy non-smoking people between 18 and 55 who have mild to moderate asthma, and others who do not have asthma. The purpose of the study is to compare changes in lung function, lung cells, blood and heart rate between people with and without asthma when they are exposed to diesel exhaust and ozone. The study involves 7 visits after screening over about 6 to 8 weeks. It requires a commitment of about 32 hours, including 3 2-day exposure sessions. You will be paid for screening and the study. 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 # _____: Epigenetic effects of diesel exhaust and ozone exposure.

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

Advertising: Epigenetic effects of diesel exhaust and ozone exposure.

The US Environmental Protection Agency is seeking

Adults With Asthma

For Research Study

Do you have asthma or think you have asthma?
Are you between 18 and 55?
This is an air pollution study about diesel exhaust & ozone. It requires screening plus 7 visits over about 6 to 8 weeks and includes three 2-day exposure sessions with exercise.

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

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

NOV 2 0 2009

Advertising: Epigenetic effects of diesel exhaust and ozone exposure.

The US Environmental Protection Agency is seeking

Adult Volunteers

For Research Study

Now recruiting healthy adults with and without asthma ages 18 to 55 for an air pollution study about diesel exhaust & ozone. Study requires screening plus 7 visits over about 6 to 8 weeks. Includes three 2-day exposure sessions with exercise.

919-966-0694 or 1-888-279-9353
www.epastudies.org

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

NOV 4 2009

APPROVED - IRB, UNC-CH
APR 22 2010

TO:

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

STUDY: **Lamarck, IRB #09-1625**

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 6 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 for 7 days prior to all visits. Tylenol and daily 81 mg aspirin are permitted.
- No antihistamines for 7 days before exposures.
- No inhaled bronchodilators for 12 hours prior to bronchoscopy

Diet and Exercise
- No vitamins or multivitamins with C or E and no fish oil supplements for 7 days prior to each visit.
- No drinking alcohol 24 hours before all visits
- No strenuous exercise for 24 hours prior to and after all visits
- No caffeine for 12 hours before to all study visits.
- Eat a normal breakfast on Exposure days.
- Nothing to eat or drink after midnight the evening before the bronchoscopy (Exposure Day 2)

Other
- No smoke and fumes for 24 hours before all visits
- Wear comfortable clothes and shoes suitable for exercise.

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

Thank You! Westat EPA Support Services

Recruitment Script

APPROVED - IRB, UNC-CH

IRB Study # 09-1625

APR 22 2010

Title: **Epigenetic effects of diesel exhaust and ozone exposure (Lamarck)**

Purpose:
The purpose of this research study is to compare changes in lung function, lung cells, blood and heart rate between people with and without asthma when they are exposed to diesel exhaust and ozone. This study is for healthy people ages 18 to 55 who have mild to moderate asthma and those who do not have asthma.

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 training session and three 2-day exposure sessions, each separated by at least two weeks. On the second day of each exposure session volunteers will have a bronchoscopy, a procedure used to get a lung cell sample. During the procedure, a doctor passes a thin tube through your nose and throat into the airways. Cells are removed either by a squirt of salt water or a gentle brushing. Before inserting the tube, the doctor will give you anesthetic to numb your nose and throat. You will be given an intravenous (IV) line. After the procedure, nurses will monitor your heart rate and blood pressure. The bronchoscopy itself usually lasts about 30 minutes, but the entire procedure, including preparation and recovery time, takes about 4 hours, and we ask that you stay in the Chapel Hill area for 48 hours afterward.

Training takes about 2 hours. Each exposure day takes about 6 hours and the follow up day takes 4. During exposure sessions you will be asked to give blood samples, ride an exercise bike, and perform several different breathing tests. You will also wear a heart monitor during the study sessions and over night. The total amount of time is about 32 hours over about 6 to 8 weeks. Each set of exposures is separated by 2 weeks.

If you complete the screening visits, the training visit, and all three 2-day study sessions, you will be paid $2,516.

Study Schedule

Screening, 2 visits, as needed: 3 hours

Training visit, *days & times to be decided*: 2 hours

Session 1, Day 1, 8 AM, *days to be decided*: 6 hours

Session 1, Day 2, 8 AM, day after Day 1, 4 hours

Sessions 2 and 3 are separated by at least 2 weeks. Does this seem like something that interests you?

Study Restrictions:
In addition to meeting all study eligibility criteria (*see 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 for 7 days prior to all visits. Tylenol is permitted.
- No vitamins or multivitamins with C or E and no fish oil supplements for 7 days prior to each visit.
- No antihistamines for 7 days before all visits.
- No smoke and fumes for 24 hours before all visits
- No drinking alcohol 24 hours before all visits
- No strenuous exercise for 24 hours prior to and after all visits
- No caffeine for 12 hours before to all study visits.
- No inhaled bronchodilators for 12 hours prior to bronchoscopy
- Nothing to eat or drink after midnight the evening before the bronchoscopy (Exposure Day 2)

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

104 Airport Drive Suite 2200, CB 1350
Chapel Hill, NC 27599-1350
Phone: (919) 966-3411
Fax: (919) 962-5011 or (919) 962-3352

December 16, 2009

To Whom It May Concern:

In accordance with the National Institutes of Health (NIH) notices OD-00-039 of August 25, 2000 and OD-01-061 of September 5, 2001, The University of North Carolina at Chapel Hill submits the following information concerning the education in the protection of human research participants undertaken by the key personnel involved with this project. The following persons have been trained in the ethical and regulatory requirements for protection of human research participants in compliance with NIH requirements and The University of North Carolina at Chapel Hill "Policy on Education and Certification of Investigators Involved in Human Subjects Research." http://ohre.unc.edu/irbtraining/

Name	Dept	Training
Bob Truckner	Environment Sciences & Engi	CITI Biomedical & Social/Behavioral
Bob Truckner	Environment Sciences & Engi	CITI Group 2: Social and Behavioral
Bob Truckner	Environment Sciences & Engi	CITI Group 2: Social and Behavioral
Melanie Jardim		CITI Group 1: Biomedical
Melanie Jardim		CITI Group 3: Data and Specimens ONLY
Michael Schmitt	Inactive	CITI Group 1: Biomedical
Michael Schmitt	Inactive	CITI Group 3: Data and Specimens ONLY
David Diaz-Sanchez		CITI Group 1: Biomedical
Robert Devlin	Pediatrics	CITI Group 1: Biomedical
Tracey Montilla		CITI Group 1: Biomedical
Maryann Bassett		CITI Group 1: Biomedical
Heidi Hiers	Epa	CITI Group 1: Biomedical
Martha Carraway		CITI Group 1: Biomedical
Andrew Ghio	Medicine	CITI Group 1: Biomedical
Howard Kehrl	Medicine	CITI Group 1: Biomedical
Martin Case		CITI Group 1: Biomedical

Should any additional persons come to work on this project and meet the definition of key personnel, they will be trained similarly; their names and certification of their training in the protection of human research participants will be forwarded.

Sincerely,

Margaret Vigiolto
Director
Office of Sponsored Research

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
Federalwide Assurance (FWA) #4801

TO: Melanie Jardim
Epa
CB: 7315

FROM: Biomedical IRB

DATE: 1/20/2010

RE: Notice of Receipt of Modification Submission on 1/20/2010
STUDY #: 09-1625

STUDY TITLE: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

Your submission for the above-referenced study has been received by the Office of Human Research Ethics and will be reviewed by the appropriate IRB.

Please refer to the above study number when corresponding with our office about this study.

If you have disclosed any potential conflict of interest in "Part A.3" it must be resolved with the COI review committee before your study can be reviewed by the IRB. Further information can be obtained at http://coi.unc.edu or 919.843.5328.

CC:
David Diaz Sanchez, Environmental Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
□	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
□	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. If adding personnel, include name, location (UNC or specific outside location), role, and email address for each person who should receive electronic copies of IRB correspondence to PI. For those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/) include documentation of required training in human research ethics.	1

1 List and describe each proposed change: .

1. On page 14 of the IRB application it states "This safety limit will prevent an inhalational exposure greater than 600 μg, which assumes that the resting subject will inhale 2.0 m^3 during the 120 min exposure." These numbers were calculated for a resting individual. The new values have been calculated for an individual who is exercising during the exposure as is being done in this study. The sentence now reads: ". This safety limit will prevent an inhalational exposure greater than 1080 μg, which assumes that the subject will inhale 2.7 m^3 during the 120 min exposure."

2. On page 16 of the IRB application there is a reference toanother study conducted by Haiyan Tond MD, Ph.D, PI. There was a typo in her name so this part now reads "Haiyan Tong MD, Ph.D, PI"

3. On page 12 of the consent form a sentence reads "Prior to the start of the bronchoscopy **atropine** will be given through a vein in your arm in order to counteract any changes in blood pressure that would result from the placement of the bronchoscope in your airway and to limit the quantity of secretions in your nose and throat." Atrpoine will only be administered if necessary so the sentence now reads "Prior to the start of the bronchoscopy **atropine** may be given through a vein in your arm in order to counteract any changes in blood pressure that would result from the placement of the bronchoscope in your airway and to limit the quantity of secretions in your nose and throat."

4. The last sentence on page 16 of the consent ford read "If you are dismissed by the investigators prior to completion, you will be paid for the entire study excluding the completion bonus." This has been edited to be

consistent with the IRB protocol and consent form to read: "If you are dismissed by the investigators prior to completion, you will be reimbursed for time and procedures up to that point."

6. On page 4 of the consent form a line has been added to indicate that subjects will fill out a symptom score sheet prior to exposure.

7. Two new pieces of recruitment material have been added (recruitment script and appointment reminder).

2. Is this modification being submitted in response to an unanticipated problem/adverse event or new findings? ___yes X_no
If yes, explain, including whether these events or findings are relevant to participants' willingness to continue.

3. Do any of the proposed changes increase risk? ___yes __X_no If yes, explain.

IRB study #: 09-1625 **Date:** January 20, 2010

Title of Study: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

Principal Investigator: Melanie j. Jardim, Ph.D. **Faculty advisor:** David Diaz-Sanchez, Ph.D. (if applicable)

For industry sponsored research (if applicable):
Sponsor's master protocol version #: Version date:
Investigator Brochure version #: Version date:
Any other details you need documented on IRB approval:

_____ _____
Signature of Principal Investigator or designee Date

_____ _____
Signature of Faculty Advisor (if applicable) Date

This application is to seek approval for a modification to a currently approved study. Any proposed changes to previously approved human subjects research must be reviewed and approved by the IRB prior to implementation. This includes modifications to the study, inclusion or exclusion criteria, recruitment methods, research personnel, or *any* new or revised study materials. Approval is required for all modifications whether initiated by the investigator or external sponsor. **This form should not be used to report violations and deviations.**

Instructions for Submitting

Include with your submission the items indicated in the list on the next page, where applicable.

Unless otherwise instructed, submit to the same IRB that previously reviewed this study. Complete submission instructions can be found at http://ohre.unc.edu/submission_instructions.php. ***All application and consent materials should be copied or printed on one side only***.

Special submission instructions apply for studies that require additional review. Examples include the General Clinical Research Center (GCRC; http://gcrc.med.unc.edu/investigators/admin/gcrcapp.htm) or the Oncology Protocol Review Committee (PRC; http://cancer.med.unc.edu/research/prc/default.asp). See their web sites for details.

Address for all Applications and Other Correspondence

IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

---- Instructions – Do not submit this page with your application ----

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
Federalwide Assurance (FWA) #4801

To: Melanie Jardim
EPA, CB: 7315

From: Biomedical IRB

Date: 2/05/2010
RE: Contingencies to be addressed following IRB Review
Submission Type: Modification
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

This submission has been reviewed by the above IRB. This is not an IRB approval. You may not implement the research activities described in your submission until you have received a memo indicating final IRB approval. The IRB determined that this submission MAY BE APPROVED, pending stipulated change(s) and/or clarification(s) as detailed below.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the item(s) listed. **When you respond, please be sure to cite the IRB study number noted above, and indicate that you are responding to this memo as dated above; failure to link your response to this study, in general, and this memo, in particular, may result in delays.** Any additional changes (including new materials) must also be listed and discussed in the memo. If the application or any study materials must be revised, please submit 2 copies of all revised materials, one in which all changes are underlined and the other a "clean" copy with no underlining.

Study Specific Details:
From preliminary review of the submitted modification request, the following information is needed before review can proceed:

1. From a reviewer with expertise in this area: " Resting adults would be expected to inhale 5-10 Liters of air per minute. Assuming an average value of 8.3 L/min, the "average" subject would inhale 500 L/hour (60 x 8.3) and 1000 L (i.e., 1.0 cubic meter) over 2 hours. Thus, a 2-hour resting exposure to an atmosphere containing 300 micrograms of particulate matter per cubic meter of air would result in the inhalation of 300 (not 600) micrograms. The intermittent exercise protocol proposed by Dr. Jardim would double the total volume of air inhaled over a two hour period as compared to the resting exposure protocol. By my calculation this would be 2.0 cubic meters of air for the "average" adult." In light of this information, please explain where the number 2.7 cubic meters comes from.
2. If there are data supporting the absence of adverse health effects of spontaneous or controlled exposure of healthy adults to total inhaled doses of diesel exhaust particles higher than 300 micrograms, please supply the data to support the estimated risk in the proposed exposure.

CC:David Diaz Sanchez, Environmental Protection Agency; Robert Truckner, (EPA), Non-IRB Review Contact

Date: 2/08/2010
RE: Contingencies to be addressed following IRB Review
Submission Type: Modification
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

We are writing in regards to a request made by the IRB Committee dated February 5, 2010 about our study 09-1625, "Epigenetic Effects of Diesel Exhaust and Ozone Exposure". The answers to the questions put forth by the committee follow the text of the question which appears in red font.

1. From a reviewer with expertise in this area: " Resting adults would be expected to inhale 5-10 Liters of air per minute. Assuming an average value of 8.3 L/min, the "average" subject would inhale 500 L/hour (60 x 8.3) and 1000 L (i.e., 1.0 cubic meter) over 2 hours. Thus, a 2-hour resting exposure to an atmosphere containing 300 micrograms of particulate matter per cubic meter of air would result in the inhalation of 300 (not 600) micrograms. The intermittent exercise protocol proposed by Dr. Jardim would double the total volume of air inhaled over a two hour period as compared to the resting exposure protocol. By my calculation this would be 2.0 cubic meters of air for the "average" adult." In light of this information, please explain where the number 2.7 cubic meters comes from.

The outside reviewer has used an average ventilation rate (8.3 L/min) to make his/her calculation and has assumed that exercise should double this rate. Although it is not wrong to make these assumptions, we chose to make these calculations using values on the higher end of the scale, which leads to an over-estimated value in the amount of air inhaled. Safety for the subjects has always been a priority for us and for this reason we believe that our over-estimations have been more useful in guiding the appropriate dosage of particles. Our calculations are based on a resting ventilation rate of 10 L/min and 35 L/min for an exercising individual, which lead to a total volume of 2.7 cubic meters during the 2-hour session, with intermittent exercise. As pointed out by the reviewer, the "average" resting adult will likely inhale approximately 8.3 Liters of air in a minute. We agree with these assessments and admit to utilizing higher rates of ventilation in our calculations in order to ensure subject safety.

2. If there are data supporting the absence of adverse health effects of spontaneous or controlled exposure of healthy adults to total inhaled doses of diesel exhaust particles higher than 300 micrograms, please supply the data to support the estimated risk in the proposed exposure.

Dr. Andy Ghio from our group conducted a chamber study on the health effects of woodsmoke (). In this study, subjects were exposed to an average dose of 500 micrograms which did not elicit any adverse health effects. Although these particles are not diesel exhaust particles, there is no data to suggest that diesel exhaust is more toxic than woodsmoke. In addition, a study conducted at UCLA nasally instilled 1000

micrograms of diesel exhaust particles in subjects. No adverse health effects were observed. Finally, much of what we know about the toxicity of diesel exhaust has been studied in occupational settings. Firefighters, for example, are continually exposed to diesel exhaust for several hours a day. These exposures have been reported to reach up to 480 micrograms/cubic meter. Although data does support an increased risk for workers chronically exposed to diesel exhaust, no acute effects, including cardiac events, have been reported.

We hope that these clarifications have answered all of the questions the IRB Committee may have regarding the study at this time.

Sincerely,

Melanie J. Jardim, Ph.D.

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
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CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
Federalwide Assurance (FWA) #4801

To: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

Date: 3/25/2010
RE: Notice of IRB Deferral following Full Board Review on 3/08/2010
Submission Type: Modification
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

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. Please clarify on the actual amount of micrograms to be inhaled since the number proposed by the reviewer and your estimate vary greatly.
2. The risk of exposure to diesel exhaust to excerising is not clear. What evidence supports this level of exposure?Please clarify.
3. A review of recent literature on this subject suggests that animal and culture models are sufficient for documentation in the United States. Looking about at the initial review, this question was asked at that point. Please comment on the possible use of animals in place of humans for some of the work and please include this information in A4.2 background section of the application.

   In addition to your written responses, the committee invites you and your faculty advisor to attend the full board meeting when this modification is scheduled to re-reviewed.

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:
David Diaz Sanchez, Environmental Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

Date: 3/25/2010
RE: Notice of IRB Deferral following Full Board Review on 3/08/2010
Submission Type: Modification
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

We are writing in response to a letter we received from the IRB committee on 3/25/2010 regarding study # 09-1625, "Epigenetic Effects of Diesel Exhaust and Ozone Exposure." Our responses to the questions asked appear in red font and any changes to the protocol have been underlined in the body of the application form

Study Specific Details:

1. Please clarify on the actual amount of micrograms to be inhaled since the number proposed by the reviewer and your estimate vary greatly.

The outside reviewer is correct in the above calculations. S/He used an average ventilation rate (8.3 L/min) to make his/her calculation and has assumed that exercise should double this rate. These assumptions are completely appropriate. However, we chose to err on the side of caution and make these calculations using values on the higher end of the scale. Our calculations are based on a resting ventilation rate of 10 L/min and 35 L/min for an exercising individual, which lead to a total volume of 2.7 cubic meters during the 2-hour session, with intermittent exercise. The expert consultant stated that "inhalation of 300 (not 600) micrograms" will occur. This is indeed the theoretical highest total concentration during the two-hour exposure period if we accept 2.0 cubic meters of air for the "average" adult. However, the reality is that not all particles are deposited in the lung. Although this likely result in an over-estimation of the value of air and thus particles inhaled, safety for the subjects has always been paramount. Our conservative approach leads us to be able to estimate the "worst-case scenario" and as such has been useful in guiding the appropriate dosage of particles. Approximately 25% of the total particle load is estimated to be deposited, for a more realistic estimate of 150 micrograms for the entire two-hour exposure.

2. The risk of exposure to diesel exhaust to excerising is not clear. What evidence supports this level of exposure?Please clarify.

Evidence supporting the safety of inhaled total dose (rather than concentrations) of particles higher than 300 micrograms comes from experimental and real-world settings. Dr. Andy Ghio from our group conducted a chamber study on the health effects of woodsmoke (IRB# 08-0334). In this study, subjects were exposed to an average dose of 500 micrograms which did not elicit any adverse health effects. Although these particles are not diesel exhaust particles, there is no reason to suppose that diesel exhaust is more toxic than woodsmoke. Diaz-Sanchez et al (. J. Allergy Clin. Immunol.., 98:114-123, 1996) demonstrated that 1000 micrograms of diesel exhaust particles instilled in the upper airways cause no systemic or health effects. Finally, exposure to levels of diesel exhaust at greater than 600 ug/m3 is observed in occupational settings. For examples in

coalmines and in Fire stations, diesel engines were run permanently so as to avoid "warming up" periods. Analysis of the health effects in these situations (eg by the EPA Health Effects Assessment Document) show that chronic lifetime exposure can cause an increased incidence of lung cancer. However it is important to note that no short-term exposure effects have been observed. We include a risk calculation for a 2 hour exposure at 100 ug/m3 (with exercise) from our original IRB application for a diesel exposure in 1999. The risk for 300 ug/m3 is 3 times the 1 in a billion calculated in that document.

3. A review of recent literature on this subject suggests that animal and culture models are sufficient for documentation in the United States. Looking about at the initial review, this question was asked at that point. Please comment on the possible use of animals in place of humans for some of the work and please include this information in A4.2 background section of the application.

The contribution of using in vitro cell culture systems and in vivo animals is valuable and what they have taught us regarding regulation of epigenetic mechanisms has broadened our overall understanding of the field. As already stated on page 8 of the application, there have been a few groups which have linked exposure to environmental toxins with changes in epigenetic mechanisms. Since the original submission of this protocol, a few more papers using animal models to show that epigenetic mechanisms can change as a result of environmental exposure have been published. Although these results are promising we are still faced with the same problem as before, in that we still do not know if such changes can be expected or are even observed in controlled human exposures. As stated on page 8 of our IRB application, regulation of epigenetic mechanisms is different between animals and humans, making it difficult to draw conclusions regarding human biology using animals as the sole source of information. Although several biological mechanisms may be similar between humans and animals, there are dramatic differences in the complexity of these mechanisms, which can lead to biologically different outcomes.

We would be more than happy to attend the full board meeting when this study is to be reviewed. Please forward us the details regarding time and location of this meeting at your earliest convenience.

Sincerely,

Melanie J. Jardim, Ph.D.

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
Federalwide Assurance (FWA) #4801

To: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

Date: 4/14/2010
RE: Minor Contingencies to be addressed following Full Board IRB Review on 4/12/2010
Submission Type: Modification
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

This submission has been reviewed by the above IRB at a convened meeting. This is not an IRB approval. You may not implement the research activities described in your submission until you have received a memo indicating final IRB approval. The IRB determined that this submission MAY BE APPROVED, pending stipulated change(s) and/or clarification(s) as detailed below.

Please address these contingencies in a revised submission, and provide a memo that includes a point-by-point response to the item(s) listed. **When you respond, please be sure to cite the IRB study number noted above, and indicate that you are responding to this memo as dated above; failure to link your response to this study, in general, and this memo, in particular, may result in delays.** Any additional changes (including new materials) must also be listed and discussed in the memo. If the application or any study materials must be revised, please submit 2 copies of all revised materials, one in which all changes are underlined and the other a "clean" copy with no underlining.

Study Specific Details:
The PI is requested to meet with the IRB Chair and Coordinator to address the final issue of level of subject exposure to pollutants in this study and risk. Of specific concern, in the IRB Application, page 7, Section A.4.2, the sentence at the bottom of the page that begins, "Healthy human volunteers exposed to 300 ug/m3 with intermittent exercise….myocardial infarction (Salvi, Blomberg et al. ….2006)" suggests a high degree of risk for that level of exposure which is the same exposure with exercise to be used in this study (Application p. 13, A.4.5.). This is in contrast to the exposure described on page 16 of 300ug/m3 where the implication is that that level of exposure is "fairly safe" for 18-35 healthy adults. While acknowledging the many safety measures built into the study, the obvious concern is for the asthmatic group. The meeting is to get clarification on a few remaining points. The committee thanks the investigators for their due diligence in responding to earlier stipulations. Please contact Joy Bryde 966-3113 to set up an appointment.

CC:
David Diaz Sanchez, Environmental Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
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Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 4/22/2010
Expiration Date of Approval: 9/27/2010
RE: Notice of IRB Approval by Full Board Review
Submission Type: Modification
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure
Sponsors: US Environmental Protection Agency - GRANTS

This submission has been approved by the above IRB for the period indicated. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment, dated 01/20/2010, addresses:

1. new calculated exposure values for exercising subjects, correction of name, clarification that atropine may be given (not will be given), clarification in consent to be consistent with application that payment to subjects will be pro-rated and that subjects will complete a symptom score sheet.
2. submission of recruitment materials.
3. updated IRB application
4. updated consent

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

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
David Diaz Sanchez, Environmental Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

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Web site: ohre.unc.edu
Federalwide Assurance (FWA) #4801

To: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

_____
Authorized signature on behalf of IRB

Approval Date: 4/22/2010
Expiration Date of Approval: 9/27/2010
RE: Notice of IRB Approval by Full Board Review
Submission Type: Modification
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure
Sponsors: US Environmental Protection Agency - GRANTS

This submission has been approved by the above IRB for the period indicated. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

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4. updated consent

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

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CC:
David Diaz Sanchez, Environmental Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version June 25, 2009

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

Date: April 19, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Name and degrees of Principal Investigator: Melanie J. Jardim, Ph.D.; David Diaz-Sanchez, Ph.D.
Department: US EPA Mailing address/CB #: 104 Mason Farm Road
Chapel Hill, NC 27514

UNC-CH PID: 715306970 Pager:
Phone #: 919-966-7320 Fax #: 919-966-6271 Email Address: jardim.melanie@epa.gov

For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: David Diaz-Sanchez, Ph.D.
Department: US Epa Mailing address/CB #: 104 Mason Farm Road
Chapel Hill, NC 27514
Phone #: 919-966-0676 Fax #: 919-966-6271Email Address: diaz-sanchez.david@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any): Michael Schmitt, MSPH
Department: US EPA Mailing address/CB #: 104 Mason Farm Road
Chapel Hill, NC 27514
Phone #: 919-966-0647 Fax #: 919-966-6271 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 name, location (UNC or specific outside location), role and email address for each person who should receive electronic copies of IRB correspondence to PI.**

Robert Devlin, PhD.; Heidi Hiers, RN; Maryann Bassett, RN; Martin Case, BS; Andrew Ghio MD; Martha Sue Carraway, MD; Robert Truckner, MD;Howard Kehrl, MD; Tracey Montilla, RN; Michael Schmitt, MS.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify):

For external funding, RAMSeS proposal number (from Office of Sponsored Research): The PI is a USEPA scientist and this project is being funded by intramural EPA funds

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

Applications must "stand alone" and should provide all information requested, i.e., complete answers must be contained in the application. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

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 (include DHHS-approved sample, when one exists), fact or information sheets, phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
□	4. All recruitment materials including final copies of printed advertisements, audio/video taped advertisements, scripts, flyers, letters, and 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., Clinical and Translational Research Center (CTRC), Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. For drug studies, Investigator Brochure if one exists. If none, include package insert for previously approved uses..	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	_X_
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_X_
A.2.4. Do you have specific plans 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_
c. Is this research classified (e.g., requires security clearance)?	__	_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 Clinical and Translational Research Center (CTRC) or is the CTRC involved in any other way with this study? If yes, obtain the ***CTRC Addendum*** *and submit completed application (IRB application and Addendum) directly to the CTRC. The CTRC includes facilities located on the 3rd floor of the Main Hospital (formerly GCRC) and Ground floor Burnett-Womack (formerly CCCT).*	__	_X_
A.2.18. Will gadolinium be administered as a contrast agent?	__	__X
A.2.19. Will subjects' Social Security Number (SSN) be collected for:		
a. processing payments greater than $200 per year, to support IRS reporting (see also B.6)?	__	_X_
b. processing payments of any amount through UNC-CH Accounts Payable?	__	_X_
c. use as a unique identifier for study tracking purposes for national registry or database?	__	_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 personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity engaged in the performance of this project as a subcontractor, sub-recipient or vendor?	__ yes	_X_ no
(d) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_X_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit the form, which is accessible online at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

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

_____ _____

Signature of Faculty Advisor Date

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

Complete answers must be provided. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

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 protocol is to compare the genetic and epigenetic effects between diesel exhaust and ozone exposure in healthy individuals and in mild/moderate asthmatics.

Participants: We will recruit up to 30 mild to moderate asthmatics and up to 30 healthy adults to participate in this study.

Procedures (methods): Subjects will be exposed to clean air, to 300 $\mu g/m^3$ of diesel exhaust for 2 hours and to 0.3 ppm of ozone for 2 hours with intermittent exercise in a controlled environment chamber. Primary endpoints will include spirometry and lung cell changes post-exposure. Secondary endpoints will include analysis of blood clotting/coagulation factors, Holter monitoring of cardiac parameters, analysis of soluble factors present in plasma and bronchial lavage and analysis of intracellular factors present in lung tissue obtained from a brush biopsy.

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.

Several epidemiological studies have shown a strong link between exposure to air pollution and adverse cardiopulmonary effects, such as respiratory tract infections, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke(Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005; Lewtas 2007). Ozone and diesel exhaust are ubiquitous in the urban atmosphere making it difficult to identify the exact source of these effects. Although experimental chamber studies have been previously performed with diesel exhaust or ozone, no one has yet looked at the epigenetic changes resultant from acute exposures to these air pollutants.

Numerous studies have shown that healthy subjects exposed to ozone have decreased pulmonary function and increased neutrophils and soluble pro-inflammatory mediators in the lower airways(Peden, Setzer et al. 1995; Bascom 1996). These include markers of increased permeability, decreased macrophage phagocytic ability, and increases in arachidonic acid metabolites, cytokines, fibronectin, lactase dehydrogenase, coagulation factor VII, tissue factor, elastase and plasminogen activator occurred in response to ozone-induced exposures(Devlin, McDonnell et al. 1991; Koren, Devlin et al. 1991; McDonnell, Kehrl et al. 1991; Devlin 1993; McKinnon, Madden et al. 1993). Diesel exhaust fumes contain primarily fine and ultrafine carbonaceous particles generated by the incomplete combustion of fuel(Diaz-Sanchez, Penichet-Garcia et al. 2000; Lewtas 2007). Diesel exhaust particles (DEP) are the largest single source of vehicular emitted airborne PM and can persist in the air, where they are readily inhaled and deposited throughout the respiratory tract. DEP induced toxicity is mainly attributed to its chemical composition, consisting of a carbonaceous core to which organic and inorganic species can become adsorbed (Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005). Healthy human volunteers exposed to 300 $\mu g/m^3$ diesel exhaust for one hour with intermittent exercise resulted in markers of systemic and redox-sensitive pulmonary inflammatory responses, vascular dysfunction and impaired endogenous fibrinolysis. Although there were no adverse health effects associated with the exposure, acute changes in the aforementioned endpoints can be used to extrapolate information in order to begin to understand the underlying biological mechanisms that link long-

term exposure of diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (Salvi, Blomberg et al. 1999; Behndig, Mudway et al. 2006).

Previous studies reported a wide variation in response to air pollution exposure among individuals in each subject group with no clear understanding in reasons (McDonnell 1991). Asthmatics have been identified as a susceptible subpopulation as seen by their heightened sensitivity to air pollutants, although the severity of health responses of asthmatics exposed to diesel exhaust and ozone are not yet fully understood. It is hypothesized that increased susceptibility could be due to increased baseline airway inflammation and airway responsiveness which characterizes asthma. Indeed, respirable particulate matter and ozone exposure are associated with episodes of increased asthma exacerbation (Devlin 1993; Bascom 1996; Diaz-Sanchez and Riedl 2005). Elevated levels of air pollution can affect airway reactivity with decrements in several indices of pulmonary function especially in those patients with preexisting asthma.

Despite a decade of intensive studies, adverse health effects attributed to air pollutant exposure are still not well understood. It is becoming increasingly clear that epigenetics plays a pivotal role in disease development and outcome, which has been extensively studied in cancer. The term epigenetics refers to the study of heritable gene expression changes, which occur without changing the DNA sequence itself. DNA methylation, histone modifications and microRNA expression all regulate gene expression, and changes in these mechanisms can result in an overall change in gene expression patterns. Unlike the static DNA sequence, these mechanisms are regulated by endogenous and exogenous cues, rendering them more likely to change in response to environmental stimuli. It is not currently known how these changes become permanent, or how epigenetic changes control gene expression in response to acute environmental perturbations. Few studies have examined how the environment can modulate epigenetic marks in individuals. In a pioneering study published in 2005, Fraga et. al. showed that as identical twins age, DNA methylation patters change indicating that differential gene expression observed in older identical twins may be attributed to epigenetic changes, further suggesting that these changes may have been molded by the environment (Fraga, Ballestar et al. 2005). Traffic particle exposure has been associated with methylation changes in retrotransposable elements, randomly dispersed throughout the DNA sequence, however the biological consequence of this is not understood as these are not coding sequences (Baccarelli, Wright et al. 2009). In vitro studies in primary human bronchial epithelial cells grown at air liquid interface showed that microRNA expression profiles change rapidly in response to exposure to diesel exhaust particles(Jardim 2009). Animal studies attempting to address these questions have been difficult as animals have very different epigenetic mechanisms making it difficult to draw conclusions from these studies. Long term exposure to cigarette smoke, a source of particulates, lead to changes in microRNA expression in rat lungs, however the contribution of these changes to biological consequences is not currently known (Izzotti, Calin et al. 2009). Another study, showed that an in utero diet rich in methyl donors enhanced allergic airway disease in mice via changes in methylation of several genes, and that these changes were transgenerational (Hollingsworth, Maruoka et al. 2008). While it is difficult to draw biological meaning from epigenetic animal studies, what these studies show is that the environment can play an important role in regulating gene expression. Although the biological and physiological role epigenetics plays in response to environmental pollutants is not yet understood, however we will begin to address this question in this study by performing in depth gene expression analysis, including microarrays, microRNA arrays and DNA methylation assays on samples collected from patients.

In addition to possible epigenetic mechanisms, genetic polymorphisms, particularly polymorphisms in anti-oxidant genes, have been proposed as a factor to explain variability in these responses. The role of genetic background in mediating airway and vascular inflammation following acute exposure to diesel exhaust and ozone in human subjects will be studied in this protocol as a secondary question. As such, bronchial epithelial cells collected from this study will be further evaluated by in vitro exposure to both gaseous and particulate air pollution.

The purpose of this study is to first, make a direct comparison of epigenetic responses (primarily changes in DNA methylation, microRNA expression and chromatin modification) in individuals exposed to ozone and diesel exhaust; second, to assess whether mild to moderate asthmatics exhibit the similar effects and to the same degree as healthy individuals; and third, to understand the contribution of genetic background and epigenetic mechanisms to cardiopulmonary effects observed in pollutant exposed cells. Although many the observed adverse health effects are generally attributed to oxidative stress upon exposure, we still do not know how these result in physiological changes to the respiratory and cardiovascular organ systems. We hypothesize that exposure to diesel exhaust or ozone will result in changes in epigenetic mechanisms, which may be linked to adverse health effects of exposure. If adverse health effects are induced solely due to oxidative stress, then patients should exhibit similar cardiopulmonary and epigenetic responses when exposed to either, diesel exhaust or ozone. However, if differential

responses to these agents are observed, then alternative mechanisms of cellular injury may exist. Therefore, we will employ several methods to assess potentially important physiological changes to these systems, including cutting edge techniques such as micro-array profiling, DNA methylation assays, microRNA expression profiling and assays for indications of chromatin remodeling.

Baccarelli, A., R. O. Wright, et al. (2009). "Rapid DNA methylation changes after exposure to traffic particles." Am J Respir Crit Care Med **179**(7): 572-8.

Bascom, R. (1996). "Environmental factors and respiratory hypersensitivity: the Americas." Toxicol Lett **86**(2-3): 115-30.

Behndig, A. F., I. S. Mudway, et al. (2006). "Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans." Eur Respir J **27**(2): 359-65.

Brunekreef, B. and S. T. Holgate (2002). "Air pollution and health." Lancet **360**(9341): 1233-42.

Devlin, R. B. (1993). "Identification of subpopulations that are sensitive to ozone exposure: use of end points currently available and potential use of laboratory-based end points under development." Environ Health Perspect **101 Suppl 4**: 225-30.

Devlin, R. B., W. F. McDonnell, et al. (1991). "Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung." Am J Respir Cell Mol Biol **4**(1): 72-81.

Diaz-Sanchez, D., M. Penichet-Garcia, et al. (2000). "Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity." J Allergy Clin Immunol **106**(6): 1140-6.

Diaz-Sanchez, D. and M. Riedl (2005). "Diesel effects on human health: a question of stress?" Am J Physiol Lung Cell Mol Physiol **289**(5): L722-3.

Folinsbee, L. J., J. F. Bedi, et al. (1985). "Pulmonary response to threshold levels of sulfur dioxide (1.0 ppm) and ozone (0.3 ppm)." J Appl Physiol **58**(6): 1783-7.

Fraga, M. F., E. Ballestar, et al. (2005). "Epigenetic differences arise during the lifetime of monozygotic twins." Proc Natl Acad Sci U S A **102**(30): 10604-9.

Hollingsworth, J. W., S. Maruoka, et al. (2008). "In utero supplementation with methyl donors enhances allergic airway disease in mice." J Clin Invest **118**(10): 3462-9.

Izzotti, A., G. A. Calin, et al. (2009). "Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke." FASEB J **23**(3): 806-12.

Jardim, M., Fry, RC, Jaspers, I, Dailey, L and Diaz-Sanchez, D (2009). "Disruption of MicroRNA Expression in Human Airway Cells by Diesel Exhaust Particles is Linked to Tumorgigenesis-Associated Pathways." Environmental Health Perspectives **117**(11).

Koren, H. S., R. B. Devlin, et al. (1991). "Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone." Toxicol Pathol **19**(4 Pt 1): 406-11.

Lewtas, J. (2007). "Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects." Mutat Res **636**(1-3): 95-133.

McDonnell, W. F. (1991). "Intersubject variability in human acute ozone responsiveness." Pharmacogenetics **1**(2): 110-3.

McDonnell, W. F., H. R. Kehrl, et al. (1991). "Respiratory response of humans exposed to low levels of ozone for 6.6 hours." Arch Environ Health **46**(3): 145-50.

McKinnon, K. P., M. C. Madden, et al. (1993). "In vitro ozone exposure increases release of arachidonic acid products from a human bronchial epithelial cell line." Toxicol Appl Pharmacol **118**(2): 215-23.

Peden, D. B., R. W. Setzer, Jr., et al. (1995). "Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics." Am J Respir Crit Care Med **151**(5): 1336-45.

Riedl, M. and D. Diaz-Sanchez (2005). "Biology of diesel exhaust effects on respiratory function." J Allergy Clin Immunol **115**(2): 221-8; quiz 229.

Salvi, S., A. Blomberg, et al. (1999). "Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers." Am J Respir Crit Care Med **159**(3): 702-9.

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).

Subjects for this study will be healthy adults, 18-55 years old, (male and female) and, otherwise healthy, 18-55 year old mild to moderate asthmatics (as defined by NHLBI guidelines), who are non-smoking. Subjects will be recruited through the Westat Corporation (see section B1 below). There are no gender or race based restrictions. Pregnant and lactating women will be excluded because of the unknown effects of air pollutants on inflammatory markers during pregnancy and lactation.

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.

Healthy Individuals

Specific Inclusion Criteria

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66).
2. Normal lung function (pre or post albuterol), defined by Knudson 1976/1984 as:
   a. FVC of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   b. FEV_1 of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   c. FEV_1/FVC ratio of $\geq$ 75 % of predicted values.
3. Oxygen saturation of $\geq$ 96 %.
4. Ability to tolerate intervals of moderate exercise

Exclusion Criteria:

1. A history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease, and lung cancer.
2. If the subject is pregnant, attempting to become pregnant or breastfeeding.
3. Allergy to any medications which may be used or prescribed in the course of this study.
4. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM and/or ozone challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
5. Smoking history within 2 years of the study.
6. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
7. Untreated hypertension (> 150 systolic, > 90 diastolic)
8. Dementia.
9. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
10. History of skin allergy to tape or electrodes.
11. Subjects who do not understand or speak English
12. Subjects who are unable to perform moderate exercise

Temporary exclusions:

1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.

4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:

1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin $\geq$ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

Mild to Moderate Asthmatics

Inclusion Criteria:

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66) where diagnosis will be judged by EPA physicians.
2. Mild/Moderate asthmatics as defined by NHLBI guidelines.
3. Positive history of asthma (wheezing, chest tightness, and reversible airway obstruction);
4. Baseline $FEV_1/FVC \geq 60\%$;
5. Oxygen saturation of $\geq 94\%$
6. Ability to tolerate moderate exercise

Exclusion Criteria

1. Use of oral steroid therapy within the past month
2. Physician directed emergency treatment for asthma exacerbation within the preceding 6 months.
3. Abnormal EKG that precludes evaluating heart rate variability.
4. Aside from mild/moderate asthma, a history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma, and cancer (possible exception for history of nonmelanoma skin cancer).
5. If the subject is pregnant, attempting to become pregnant or breastfeeding.
6. Allergy to any medications which may be used or prescribed in the course of this study
7. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
8. Dosing level of an inhaled steroid must be consistent with mild asthma as outlined by the NHLBI NAEPP guidelines. Regular use of oral corticosteroids, or use of inhaled steroid at doses typically used for severe asthma, will result in exclusion of that individual from the protocol.
9. Severe asthmatics as defined by: nighttime symptoms of cough or wheeze greater than 1 time per week at baseline, daily exacerbation of asthma or requirement for albuterol due to asthma symptoms (cough, wheeze, chest tightness, but not to include prophylactic use of albuterol prior to exercise), more than mild interference with normal activity, any episode of physician directed emergency treatment for asthma requiring oral corticosteroid therapy within the past twelve months.
10. Smoking history within 2 years of study.
11. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
12. History of skin allergy to tape or electrodes.
13. History of respiratory diseases other than allergic rhinitis and asthma
14. Untreated hypertension (> 150 systolic, > 90 diastolic)
15. Dementia.
16. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
17. Subjects who do not understand or speak English

18. Subjects who are unable to perform moderate exercise

Allowances: subjects may
1. Use daily theophylline therapy.
2. Use daily inhaled steroids.
3. Use inhaled cromolyn therapy.

Temporary exclusions:
1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.
4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:
1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin $\geq$ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This is a randomized cross-over study with three arms. This study will compare the epigenetic responses of healthy adults and adults with mild asthma to diesel exhaust and ozone exposures. Up to thirty subjects will be recruited into each group. Subjects will be randomly exposed to clean air, diesel exhaust and ozone with exposures separated by a minimum of two weeks. Responses of primary interest will include: 1) genetic and epigenetic changes 2) FEV1 as measured by spirometry, and 3) lung inflammation and cell changes as evaluated by bronchoalveolar lavage. The performance of these tests and procedures are considered essential to conducting the study. Exploratory endpoints will include: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, telemetry, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18-24 hours post-exposure. All medical procedures will be performed by study personnel.

Physical Examination Day:

Prior to recruitment into the study, subjects will undergo a physical exam. During this visit, a short review of the subject's history will be performed and vital signs, height and weight will be assessed (temperature, pulse, respiratory rate, and blood pressure), to determine whether the individual meets any major exclusion criteria for the study. Up to 50 ml of blood may be collected for a CBC/ differential, chemistry and lipid panels, assessment of

coagulation function, phagocytic function and cell surface markers. At this time the subject may undergo a physical examination for bronchoscopy or may be scheduled for one at another time.

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.
- On the Exposure Day 2, eat a normal breakfast.
- No caffeine for 12 hours prior to all study visits.
- At the end of Exposure Day 2, do not eat or drink anything after midnight in preparation for bronchoscopy on Exposure Day 3.

Training Day

1. Eligible subjects will report to the research lab for an approximate 2-hour training session.
2. Informed consent will be obtained.
3. Assessment of **vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation
4. **Pregnancy tests** will be administered to any women who may have child-bearing potential.
5. Subjects will be trained on the cycle ergometer and the work load to elicit a minute ventilation normalized for body surface of $25L/m^2/BSA$ will be determined. In most subjects this will be about 50 L/minute (IE. VO^2 of approximately 2.0 L/m). A cycle ergometer work setting of 75 to 100 watts will usually achieve such a physiological response
6. Subjects will perform **spirometry.**
7. Subjects will be shown chamber where exposures will take place

Exposure Day 1

The subjects will be exposed ozone, diesel exhaust and clean air on three separate occasions, with the exposures separated by a minimum of two weeks. .

1. Subjects will be asked to arrive at the study site no sooner than 8 AM and will undergo **assessment of vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation, and **symptom score** assessment.
2. **Pregnancy tests** will be administered to any women who may have child-bearing potential.
3. A **telemetry unit** will be attached for cardiac monitoring.
4. Subject will be fitted with a **Holter monitor** and the subject will be asked to recline in a quiet dark place for 30 minutes. After approximately 20 minutes a time marker will be activated so that heart rate variability frequency readings can be taken over the next 10 minutes. The subject will wear the Holter monitor for the next 24 hrs.
5. **Venipuncture:** Up to 80 ml of blood will be collected for a CBC/ differential, and may be used for assessment of coagulation function and markers, phagocytic function, inflammation, and cell surface markers. A portion of the sample may be used for RNA isolation for microarray analysis and for DNA isolation for genotyping purposes.
6. **Baseline Spirometry**
7. **Exposure sessions**: Exposure to ozone, diesel exhaust or clean air will be conducted in an exposure chamber at the EPA Human Studies Facility on the UNC campus. Each subject will be exposed up to 0.3ppm ozone, diesel exhaust (up to 300 ug/m^3) or clean air for 2 hours. Subjects will begin exercising on an exercise bike. Each exercise session will consist of a 15 minute exercise interval at a level of up to 25 L/m2/BSA followed by a 15 minute rest period. Minute ventilation will be measured for three minutes during each exercise period. Continuous heart rate and rhythm, and oxygen saturation, will be monitored using telemetry and pulse oximetry respectively. Blood pressure will be monitored. Subjects will be asked to refrain but not prohibited from using inhaled bronchodilator during exposures; if medication use is necessary subjects will be instructed to maintain consistent medication use across the two exposures. The subjects will be able to end their exposure and exit the chamber at any time. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, ataxia, or a greater than 5 point drop in saturated oxygen (or a drop to lower than 89%). They will be monitored continuously by trained personnel and a physician is on call whenever a subject is undergoing a procedure at the facility. A detailed description of the physical facilities in the Human Studies Division of the U.S. EPA Health Effects Research Laboratory located on the UNC-CH campus have been published as U.S. Government Publication EPA-600/1-78-064, and is on

file in the office of the UNC Committee on the Protection of the Rights of Human Subjects. The medical station of the Human Studies Division includes a fully-equipped medical examination room, a cardiac/respiratory emergency cart, a subject recovery room, a nurse's station, a waiting room and conference rooms. The facility is staffed by qualified RNs, and an on-site physician is available to respond to a medical emergency in a timely manner. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. The DE will be generated from a diesel generator used to power a load bank 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 up to 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 1080 µg, which assumes that the subject will inhale 2.7 m^3 during the 120 min exposure. 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 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) 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. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured in real time. Particle size distribution may not be determined during each subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. Ozone exposures will be conducted in a (6 ft x 6 ft x 8 ft) stainless steel chamber with a continuous supply of exposure medium. Ozone will be monitored continuously. **Immediately post exposure**, subjects will again undergo **spirometry, Holter monitor frequency reading, symptom scoring and assessment of vital signs.**

8. **Then**, up to 80 ml of blood will be collected for a CBC/ differential, assessment of coagulation function, blood leukocyte phagocytic function, cytokines, cell surface markers and RNA isolation for microarray analysis.
9. Subjects will then be assessed and discharged by the nursing staff.

Exposure Day 2 (approximately 24 hours after exposure)

The subject will arrive at the U.S. EPA HSF for:

1. **Vital sign monitoring**.
2. **Symptom scoring**.
3. **Venipuncture**: up to 80 ml of blood will be collected for a CBC/ differential, coagulation function, assessment of blood monocyte phagocytic function, inflammation, and cell surface markers, and RNA isolation for microarray analysis.
4. **Holter monitor** frequency reading and removal
5. **Spirometry**
6. **Bronchoscopy.** Subjects will have undergone a physical examination including assessment for suitability for transnasal fiberoptic bronchoscopy at the Human Studies Facility by a board certified or board eligible pulmonologist.

   Prior to the procedure, subjects will perform spirometry (as above); bronchoscopy will not be performed if lung function does not meet the recommended performance level (NIH guidelines; FEV1$\geq$ 60 % predicted). Prior to bronchoscopy, a small tube (IV) will be placed in a vein for potential use in administering fluids. Subjects will receive inhaled albuterol prior to the bronchoscopy at the discretion of the pulmonary physician performing the procedure. Bronchoscopy with bronchoalveolar lavage and brush biopsy will be performed by a licensed physician who is Board certified or board eligible in pulmonary medicine and is experienced in fiberoptic bronchoscopy. The physician will be assisted by at least one R.N experienced in bronchoscopy. The subjects will be monitored closely throughout the procedure in the following manner: 1) Chest electrodes for continuous electrocardiogram, 2) Pulse oximetry of arterial blood, and 3) blood pressure monitoring using an electronic sphygmomanometer. Subjects will be premedicated with IV atropine (0.6 mg) to prevent bradycardia and hypotension that could result from vagal stimulation provoked by passing the bronchoscope through the subject's larynx and to minimize the amount of airways secretions. Nasal oxygen will be administered during the procedure. No sedatives and/or narcotics are administered at any time during bronchoscopy. We have found that premedication with sedatives (midazolam) or with opiates (demerol) is not required for research bronchoscopy subjects. Avoidance of these drugs reduces procedure risk and markedly shortens post-procedure recovery time. Topical lidocaine anesthesia will be used for subject comfort and to prevent cough, with a maximum dose of 15 ml of 2% lidocaine solution (360 mg lidocaine). Electrocardiographic and pulse oximeter will be

monitored throughout the procedure. After the airways are visualized, the bronchoscope tip is wedged in a 4th-6th order bronchus (right middle lobe) for bronchial lavage. A total volume of 270 cc of sterile saline, one aliquot of 20 cc and 5 additional aliquots of 50 cc each, will be injected and immediately aspirated through the channel in the bronchoscope using a syringe. The lavage fluid aliquots will be put in polypropylene tubes and kept on ice until processed. A total of up to 4 brush biopsies will then be obtained from the lower trachea, main, and lobar bronchi after topical application of 1% lidocaine. A cytology brush (Bronchoscope Cytology Brush; Bard, Tewksbury, MA) is applied to the region under direct visualization. For a single brushing, six passes, each with a linear excursion of 2-5 cm, are made with the brush along the endobronchial surface.

Subjects are observed and monitored for a minimum of 1 to 2 hours after the procedure in the on-site medical station by a registered nurse with physician supervision. At discharge subjects will be symptom-free. At least 2 physicians, including the responsible bronchoscopist, will be available (24 hours daily) to subjects who develop any symptoms after discharge. The subject is discharged by a physician and is provided with the names and telephone numbers of the bronchoscopist and one other physician. The subject is contacted 24 hours post-bronchoscopy to ask about any untoward effects.

Subjects will return to the U.S. EPA HSF for an additional exposure sessions and post exposure bronchoscopies (air control, ozone or diesel exhaust) after a minimum time interval of two weeks.

We anticipate performing several clinical procedures during the course of this study which include primary, secondary and exploratory endpoints. However, it is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure, because of a investigator problem (such as but not limited too building/equipment failures, weather delays, or staffing issues), then the patient will be compensated for said procedure(s) and time on that day and may be rescheduled.

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.

There will be no direct benefit to the subject for participating, other than information that may be learned during their physical examination about their overall physical status. The primary benefit from the study will be to add to the scientific knowledge about whether exposure to air pollutants induces epigenetic changes.

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.

Risk and Procedures for Minimizing Risks of Diesel Exhaust Exposure

- Diesel Exhaust Exposure: The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban area. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic

polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City. We have seen in IRB-07-0190 that exposure to 300 $\mu g/m^3$ of diesel exhaust does not cause adverse health effects.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. In the pilot phase of study #07-0190 (Haiyan Tong MD, Ph.D, PI), subjects reported "moderate" eye irritation following diesel exhaust exposure and the symptom was resolved within a few hours after the exposure. Subjects will be provided with the choice to wear chemical safety goggles during exposures to prevent or minimize the eye irritation. 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 health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

Risk and Procedures for Minimizing Risks of Ozone Exposure

Potential risks may likely 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. It is likely that most subjects participating in this study will experience some degree of tracheobronchial irritation and cough.

Risks and Procedures for Minimizing Risks of Venipuncture:

Insertion of the needle may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.

Risks and Procedures for Minimizing Risks of Bronchoscopy:

The most significant risk to a volunteer in this protocol involves bronchoscopy. Over 1000 procedures have been performed within the EPA Human Studies Facility over the past 15 years. From January 1, 1986 through December 30, 1995, 736 procedures were performed in normal nonasthmatic subjects, and these have been reported in the

Journal of Bronchology 1998; 5:185-194. The overall complication rate was less than 1% (actually less than 0.1%). The most frequent complication was a low grade fever. Bronchoscopy with BAL can be associated with more significant complications, including hypoxemia, cough, wheezing, and post-bronchoscopy fever. These may be increased in asthmatic subjects, and some investigators have found hypoxemia to be more severe in asthmatics compared to normal subjects (Djukanović, R., J. W. Wilson, C. K. Lai, S. T. Holgate, and P. H. Howarth. 1991. The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am. Rev. Respir. Dis.* 143: 772-777), while others have not (Van Vyve, T., P. Chanez, J. Bousquet, J. Y. Lacoste, F. B. Michel, and P. Godard. 1992. Safety of bronchoalveolar lavage and bronchial biopsies in patients with asthma of variable severity. *Am. Rev. Respir. Dis.* 146: 116-121); Anonymous. Workshop summary and guidelines: investigative use of bronchoscopy, lavage, and bronchial biopsies in asthma and other airway disease. *J Allergy Clin Immunol* 88:808-14, 1991.;
Jarjour NN, Peters SP, Djukanovic R, Calhoun WJ. Investigative use of bronchoscopy in asthma. Am J Respir Crit Care Med 157:692-97, 1998.) Furthermore, hypoxemia is rarely severe enough to require premature termination of the procedure and can usually be treated or prevented by supplemental oxygen (Van Wyre).

Bronchoscopies are performed by experienced physicians who are either Board-certified or Board-eligible in pulmonary medicine. The physician performing the bronchoscopy is always assisted by at least one nurse familiar with the procedure. The subject's vital signs and ECG are continuously monitored during the bronchoscopic procedures and at regular intervals during the recovery phase. The procedure is immediately terminated if the subject experiences any alterations such as tachypnea, depressed respiration, tachycardia, bradycardia, abnormal rhythms or significant changes in blood pressure. As indicated earlier, a fully equipped emergency cart including a defibrillator, endotracheal intubation equipment and emergency medications (atropine, epinephrine) is available at all times. An on-site physician is always available to respond and the UNC Memorial Hospital Emergency Room is within 1/4 mile of Human Studies Facility. Before the subject is sent home, he/she receives a brief physical examination by a physician and is given phone numbers and a pager number with which to contact a physician should he/she experience any problems or have any questions.

The complication rate during medical bronchoscopy is approximately 0.08 to 0.12% for all events, including low oxygen saturation, respiratory embarrassment, myocardial infarction, or death. The medical screening of the subjects is specifically designed to exclude subjects with a history of medical problems which might put them at risk from the particle exposure and bronchoscopic procedures. Medical problems which could arise as a result of bronchoalveolar lavage and brush biopsy include the following:

Discomfort of the nose and throat is a primary risk of bronchoscopy. As outlined, the lidocaine gargle, spray and liquid is used to anesthetize these areas prior to passing the bronchoscope to reach the lower airways. The procedure will be discontinued if adequate anesthesia cannot be achieved by the means described.

Low-grade fever (38-38.5 °C) can occur in approximately 25 % of hospitalized subjects undergoing bronchoscopy. The fever invariably resolves within 18 hours without treatment or with acetaminophen. Our experience at HSD, EPA has shown that this fever and the associated malaise can almost always be prevented by administering ibuprofen following the procedure. The subject will be asked to contact the physician who performed the procedure or one of the nurses at the Medical Station if fever persists or is higher than 38.5 °C. As part of the standard protocol, a nurse or physician will contact the subject by telephone between 24 and 48 hours after the procedure to inquire on the general health of the subject and specifically about the presence of fever.

Cough is a common, albeit minor, problem encountered during bronchoscopy. Cough results from mechanical stimulation of cough receptors in the airway by the bronchoscope or the cytologic brush used for the brush biopsies. In addition to the discomfort, prolonged coughing with the bronchoscope in place can result in mild mechanical trauma to the vocal cords. Lidocaine solution is passed through the bronchoscope channel at the main carina and at the level of the right mainstream bronchus to suppress the cough reflex during the procedure, more can be used as needed. The procedure will be terminated if adequate cough suppression cannot be attained.

Epistaxis is caused by trauma to the nose by the bronchoscope. This condition is expected to be minor and resolve on its own. Small streaks of blood in nasal secretions may be present for up to 12 hours following the procedure. If the bleeding becomes moderate or severe during bronchoscopy, the procedure will be terminated and the bronchoscope removed. The subject's anterior nasal passage will be packed with sterile gauze to stop the bleeding. If the bleeding does not resolve with packing, the subject will be transferred to the UNC Hospital Emergency Room.

Lidocaine use presents a small risk to the subjects, as a variable fraction of the medication is absorbed through the airway mucosa. If a significant amount of lidocaine is absorbed, adverse reactions such as bradycardia, hypotension, urticarial reactions, confusion, lightheadedness, euphoria, tremors and seizures can result. We take particular precautions to avoid the possibility of overdose of topical lidocaine and limit the total amount of lidocaine instilled into the airways to a maximum of 360 mg. Also, subjects will be screened by history prior to the procedure for known allergies to lidocaine or other similar topical anesthetics.

Bronchospasm, manifested as wheezing, chest tightness or dyspnea, is a risk of bronchoscopy that is caused by stimulation of irritant receptors in the airways by the bronchoscope. This risk is minimized by 1) only allowing persons with stable mild to moderate stable asthma to participate in the study, 2) pretreatment with inhaled

bronchodilator before the procedure per request of the bronchoscopy physician and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physicians responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. In the unlikely event that the subject requires additional treatment because the asthma attack continues, they will be transported by ambulance to the emergency room of the UNC Hospital Emergency Room.

Pneumothorax is a risk of bronchoscopic procedures such as transbronchial biopsy, peripheral lung protected brushings or peripheral lung cytology brushings. Because these procedures will not be performed, the risk of pneumothorax in this study is extremely small. There is a very small risk of a pneumothorax resulting from a endobronchial brush biopsy. The symptoms include dyspnea and chest pain. The subjects will be interviewed and examined by the bronchoscopy physician 2 hours after the procedure with these symptoms in mind. If a pneumothorax is suspected, the subject will be transferred to the UNC Hospital Emergency Room by ambulance for further evaluation and treatment.

Bleeding in the lower airway may occur from trauma caused by the bronchoscope or by brush biopsy. Bleeding is typically very minor, in the order of 1/10 cc, is not clinically significant in an otherwise healthy subject and should spontaneously resolve in a matter of minutes. Epinephrine, diluted 1/20,000 fold will be administered through the bronchoscope to the affected site if bleeding is minor to moderate. The maximum dose of epinephrine used will be 2-3 cc to prevent local tissue necrosis. The site will be monitored through the bronchoscope until the bleeding stops. If bleeding fails to resolve with epinephrine or if it is sufficiently severe to cause hemoptysis or hemoglobin desaturation, oxygen supplementation will be provided to keep arterial oxygen saturation above 90% and the subject will be transported to the NC Memorial Hospital Emergency Room by ambulance. If it becomes necessary, the subject can be intubated before transport.

The use of epinephrine to control bleeding can produce systemic effects resulting from mucosal absorption of the drug. These symptoms are transient and include headache, palpitations and tachycardia when the dose is greater than 1 mg and is given intravenously. Two cc of a 1/20, 000 dilution of epinephrine contains 0.10 mg of the drug, a dose that is very unlikely to induce any untoward effects. This is especially true when the drug is applied to the mucosa, since only a fraction is expected to be absorbed.

Bradycardia and/or hypotension may result from increased vagal nerve output provoked by coughing or Valsalva during the passage of the bronchoscope through the vocal cords and trachea. Atropine (0.6 mg iv) is prophylactically administered to the subjects before the start of the procedure to minimize this risk. The primary side effects associated with atropine administration is mild sinus tachycardia which typically resolves within 30 minutes as the drug is cleared from the subject's system. In rare cases (less than 1 percent) the sinus tachycardia is accompanied by hypotension.

Hypoxemia: Arterial blood oxygen saturation is continuously monitored during the procedure with a finger pulse oximeter. Subjects are supplemented with oxygen at a flow rate of 2 l/min via nasal cannula during the procedure. The oxygen flow can be increased to a maximum of 6 l/min if the arterial oxygen saturation falls below 93 %. If oxygen supplementation is not effective, the procedure will be discontinued immediately. Removal of the bronchoscope and treatment with inhaled bronchodilator should be sufficient to allow the subject's arterial oxygen saturation to return to normal. Under normal conditions, oxygen supplementation is ceased once the procedure is completed. However, supplementation will be continued if arterial oxygen saturation is below 93 % on room air. In the event that low oxygen saturation persists, if the subject cannot be weaned from the oxygen supplementation, or if it falls precipitously at any time, an emergency situation will be declared and the subject will be transported immediately by ambulance to the UNC Hospital Emergency Room. These risks are minimized during the research procedure at the Human Studies Facility, in part because premedication with sedatives (midazolam) or with opiates (demerol) (as is used in medical bronchosopy) is not required for healthy research subjects. Avoiding these drugs reduces procedure risk and allows the participants to enjoy a normal day after leaving the EPA Human Studies Facility.

The risk of pneumonia as a result of bronchoalveolar lavage in the lobe involved in the procedure is less than 1%. Symptoms of pneumonia could include fever, dyspnea, persistent cough, productive cough and chest pain. The subject will be instructed to call the physician who performed the procedure or the Medical Station if these symptoms occur. The medical staff will evaluate the subject over the phone between 24 and 48 hours after the procedure for signs of pneumonia.

The subject will be urged to contact the Medical Station or the bronchoscopy physician should he/she experience any of the following symptoms: 1) Epistaxis, 2) Persistent cough, 3) Hemoptysis, 4) Chest pain, 5) Dyspnea, 6) Wheezing, 7) Sputum production, 8) Hoarseness or sore throat.

Confidentiality Risk

Risk of breach of confidentiality is minimal. For the mild asthmatic subject, it is conceivable that an employer or insurance company could learn of the diagnosis if the subject requires medical intervention as a result of

participating in the study. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files which may contain the subject's name or screening number are secure in the EPA building which has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, spirometry) will be discussed with the subject and the subject will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data 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 changes in epigenetic mechanisms in bronchial epithelial cells can be linked to physiological changes in healthy and mild/moderate asthmatic volunteers safely exposed to low levels of O3 and diesel exhaust. The study will follow a randomized, repeated measures design with each subject exposed to clean air, diesel exhaust and ozone. The data analysis will be focused on changes in lung function measurements, primarily FVC and FEV_1, and secondarily, HRV measurements between pre and post 2hr exposure for each exposure and lung cell composition changes. Also blood endpoints include inflammatory factors (eg IL-6), blood clotting factors (eg fibrinogen), and susceptibility factors (eg specific genotypes associated with susceptibility to air pollution).

In evaluating exposure effects of O3 and DE 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 (Folinsbee, Bedi et al. 1985) for a constant acute exposure to 0.3 ppm O_3 versus filtered air. In 22 subjects, the pre exposure FEV_1 was 4.608 ± 0.514 (mean $\pm$ SEM) in FA exposure and 4.656 ± 0.592 in O_3. The post exposure FEV_1 was 4.64 ± 0.476 in FA 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 the required sample size for each group is 30 subjects. We will initially recruit 30 subjects for each group (healthy and mild/moderate asthmatics) for this study to ensure the statistical power that can analyze small differences expected from low concentration exposures.

Other Exploratory Observations:
We hypothesize that subjects harboring polymorphisms in antioxidant genes will have increased responses to diesel exhaust and ozone compared to subjects who do not. In order to address this we will use collected cell samples for in vitro exposure experiments

Exploratory endpoints will include the effect of diesel exhaust and ozone on changes in: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values.

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, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data**. Are the identifiers in A.4.9 above linked or maintained with the research data?

_X_ yes __ no

A.4.11. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in a locked file cabinet in the Medical Station at the U.S. EPA 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 a day for seven days a week

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

_X_ No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data stored on a desk top computer:
_X_ Secure network _X_ Password access __ Data encryption__ Password protected file(s)
__ Other comparable safeguard (describe):

For portable computing devices/external storage devices (e.g. laptop computer, PDA, CDs, memory sticks):
__ Power-on password __ Automatic log-off __ Data encryption __ Password protected file(s)
__ Other comparable safeguard (describe):

For hardcopy data (including human biological specimens, CDs, tapes, etc.):
__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office _X_ Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples. Subjects at any time may request that their samples be no longer stored in the repository. Any analysis in progress at the time of the request or already performed prior to the request being received by the researcher will continue to be used as part of the research study. Once the researcher is notified, the subject's specimens will be destroyed.

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.*

The subject will be given an opportunity to read the consent form. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *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 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 __ yes __ no

Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).*
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Subjects for U.S. EPA Studies (95-EPA-66). Typical methods for recruiting may include mass mailings, fliers and print ads. 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. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of this study will be approximately eighteen months. Subject recruitment and screening is expected to be continuous throughout the study until the intended number of subjects is reached.

Scheduling constraints imposed by concurrent studies in the U.S. EPA Human Studies Division are expected to limit the rate at which subjects can be exposed to 1-2 per week.

The physical examination evaluation may take up to one hour. If the subject is eligible for the study, the individual may have up to nine more visits to the research facility over approximately 6 months. A physical examination for bronchoscopy will be performed. The training day (Day 1) visit will require approximately 2 hours. Exposure day (Day 2) will last approximately 6 hours. Subjects will report to the facility at no sooner than 8 AM. The morning following the exposure, the subject will return for Day 3 which a follow-up visit which may last approximately 4 hours. Subjects will report to the facility at no sooner than 8 AM. Subjects will then return and repeat the Day 2-3 visits with the second and third exposure session. Exposure sessions will be separated by a minimum of two weeks.

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

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators for involuntary reasons after enrollment in the study but prior to completion will be paid for their participation up to that point

.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and for any procedures cancelled up to a maximum of $100. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study:
Subjects will be paid approximately $12 per hour for participation in this study. If the subjects complete all three exposures with bronchoscopy, the total compensation including prestudy qualifications will be approximately $2516.

Pre-study qualifications		
recruitment screening	$15	Previously paid
physical exam	$15	Previously paid

bronchoscopy physical exam	$20
	Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24
	Training Day Total = $ 44

Subjects will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure
Endobronchial Brush biopsies	$ 25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus	**$100**

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures	**$1548**
TOTAL for completion of THREE exposures (including completion bonus)	**$2422**

If a subject begins but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, they will receive compensation for participation up to that point.

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

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***This section applies even if records are only used to identify potential subjects.***

→ ***If your study does not use existing data, records or specimens for any purpose, 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
If applicant was involved in the original collection, please explain role:

__ 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, do you have permission from the custodians of the data, records or human biological specimens (e.g., pathology dept, tissue bank, original researcher)? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 24 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE (not present)
1 = TRACE/NOTICED (barely detectable)
2 = MILD/LIGHT (present, but not annoying)
3 = MODERATE (present, but somewhat annoying)
4 = SEVERE/HEAVY (present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4

THIS CONSENT DOCUMENT HAS BEEN APPROVED
FOR USE IN THIS STUDY BY THE UNIVERSITY OF NORTH CAROLINA
INSTITUTIONAL REVIEW BOARD FOR PROTECTION OF HUMAN SUBJECTS
REVIEW DATE: 4/22/10 9/27/10

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study and Consent for Genotype Screening
With Identifying Information
Subjects 18 years of age or older
Biomedical Form

IRB Study:# 09-1625
Consent Form Version Date: January 4, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D. and David Diaz-Sanchez, Ph.D.
Department: United States Environmental Protection Agency
Phone number: 919-966-7320

Co-Investigators: Michael T. Schmitt, MSPH

Sponsor: U.S. Environmental Protection Agency

You are being asked to take part in a research study. The investigators listed above are in charge of the study; other professional persons may help them or act for them.

What are some general things you should know about research studies?

Research studies are designed to gain scientific knowledge that may help other people in the future. You may not receive any direct benefit from participating. There may also be risks associated with participating in research studies.

Your participation is voluntary. You may refuse to participate, or may withdraw your consent to participate in any study at any time, and for any reason, without jeopardizing your ability to participate with EPA-sponsored research.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want to participate. You will be given a copy of this consent form. You are urged to ask the investigators 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 research study is to understand how ozone exposure compares to that of diesel exhaust exposure in order to investigate mechanisms of injury by environmental pollutants. This may be especially important for asthmatics, because studies have shown an association between exposure to air pollution and increased medication usage and emergency room visits in this population. To accomplish this we will:

- Document changes in lung function (spirometry) and the presence of mild inflammation in the respiratory tract (measured by bronchoscopy to collect cells from the lower respiratory tract) after each exposure.
- Determine if air pollution exposure can affect components of the blood involved in inflammation and clotting.
- See if air pollution particles can cause changes in the ability of the nervous system to correctly regulate one's heart rate.
- Cells from your airways will be collected and stored. All specimen collections will be stored throughout the course of this study and then any remaining samples will be stored indefinitely as described in an accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information.***
- The cell samples will be used to identify the expression of a panel of inflammation- and oxidative stress-related genes. As new genes are identified that may be associated with adverse health effects of air pollutant exposure, expression of these genes may also be investigated.
- The cell samples will be used to identify epigenetic changes (mechanisms which control the expression of genes) associated with air pollutant exposure.
- Research reports have suggested that people with particular genes are more susceptible to air pollutants. The cells will be studied for those specific genes related to adverse health effects of air pollutant exposure.

Are there any reasons you should not participate?

You should not participate in this study if:

- You are pregnant, attempting to become pregnant or nursing an infant
- You have a history of significant chronic illness other than asthma, or your asthma is not well-controlled
- You are not 18 to 55 years of age
- You cannot perform intervals of moderate exercise
- You are not fluent with the English language
- You are taking more than 81 mg aspirin regularly
- You are unwilling or unable to refrain from the following medications for the week prior to the study: anti-inflammatory agents such as ibuprofen (Advil), naproxen (Aleve, Naprosyn) or aspirin
- You have skin allergy to tape or electrodes
- You are currently taking or have taken anti-coagulant medication in the week prior to the study
- You are currently smoking or have a smoking history within 2 years of the study
- You have been diagnosed as a severe asthmatic as defined by physician-directed emergency treatment for an asthma exacerbation within the preceding 12 months, use of systemic steroid therapy within the preceding 12 months, nighttime symptoms of cough or wheeze greater than 2 times per month, asthma symptoms (cough, wheeze, chest tightness) more than 2 times per week, requirement for albuterol due to asthma symptoms more than 2 times per week, (not including prophylactic use of albuterol prior to exercise), have chronic and continuous allergic rhinitis.

- Your dosing level of an inhaled steroid is not consistent with the severity of your asthma as outlined by the NHLBI NAEPP guidelines
- You have a history of fainting in response to pain
- You currently have or have had a viral respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of the study. If this is the case, your visit will be rescheduled
- You currently have or have had seasonal exacerbation of allergic rhinitis symptoms within 1 weeks of the study. If this is the case, your visit will be rescheduled

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

- No over the counter pain medications such as aspirin (aspirin $\leq$ 81 mg/day is allowed), Advil, Aleve or other non-steroidal anti-inflammatory medications for 7 days prior to all visits. Tylenol (acetaminophen) is permitted.
- No vitamin C or E (or multivitamins which contain Vitamins C or E) for 7 days prior to all 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.
- Avoid eating or drinking anything after midnight on the evening of the Exposure Day 1, before the bronchoscopy to take place on Exposure Day 2.
- No caffeine for 12 hours prior to all study visits.
- Avoid use of antihistamines for one week prior to exposures.

How many subjects will participate in this study?

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

How long will your participation last?

Your participation in this study may require up to 9 visits to the research facility over approximately 6 months if you are eligible for the study.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone a general screening visit and physical examination, and a physical exam and pulmonary function test for bronchoscopy to certify that you are a suitable candidate. For the exposure study, we will perform the following procedures:

Training Day

- Report to the EPA medical station at the requested time.

- 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 this consent form. We will give one copy to you. We will check your vital signs (heart rate, respiratory rate, blood pressure, oxygen saturation level and a symptom questionnaire) and do a pregnancy test if applicable(WOMEN ONLY) For the pregnancy test, you will be asked to provide a urine sample. The purpose of this test is to provide evidence that you are not pregnant at the time of the study. The nurse will also ask you when your most recent menstrual cycle began. The results of the pregnancy test will be held in strict confidence. A positive pregnancy test will preclude further participation.
- You will undergo a breathing test. This is known as spirometry, and 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. The information gained from the breathing tests will be used by the investigators to determine whether you qualify for the study.
- You will be trained on the exercise bike and the work load to elicit a minute ventilation normalized for body surface of $25L/m^2/BSA$ will be determined.

Exposure Day 1: You will be asked to eat a normal breakfast and arrive at the EPA medical station no sooner than 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level) and fill out a symptom questionnaire. Have a pregnancy test if applicable.
- Have monitors placed on you so that we can watch your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on for 24 hours. Before exposure, after exposure and the following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate. This will consist of a 20 minute rest period and a 10 minute data collection. The monitor will be removed after the last data collection. These monitors will allow us to determine if air pollutants cause small changes in the ability of your nervous system to regulate how your heart beats.
- Have up to 80 mL blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust or ozone exposure affects the ability of your blood to clot correctly, and to look at changes in proteins and/or blood cells.
- 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.
- Given a Symptom Questionnaire for you to fill out.
- Testing will be postponed if it is determined that your medication history is not within previously established limits, respiratory tract illness has occurred within the previous 6 weeks or if respiratory symptoms are present.

During the exposure, you will:

Undergo a chamber exposure of 2 hours duration, once to clean air, once to diesel exhaust and once to ozone; the amount of pollution you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. You will be blinded as to the exposure condition. The exposure chamber is 6 ft wide by 6. ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. You will exercise for 15 minutes at the previously determined pace and rest for 15 minutes. While exercising, minute ventilation will be measured for three minutes. Your blood pressure will be measured intermittently. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid for your participation up to that point, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Following the exposure, you will:

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have your vital signs checked.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Fill out a symptom score sheet.
- Have blood drawn (up to 80 mL or about 5 tablespoons).
- Be assessed and discharged by the nursing staff.

Exposure Day 2 (24 hour follow up visit): You will come back the next morning and asked to arrive at the EPA medical station at no sooner than 8 am. (approximately 24 hours after exposure) You will:

- Have your vital signs taken.
- Fill out a symptom questionnaire.
- Have blood draw (up to 80 mL or about 5 tablespoons).
- Have spirometry testing.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Have the Holter monitor removed.
- We are asking all subjects to have bronchoscopy on the day following each exposure. Prior to the bronchoscopy you may be pretreated with inhaled bronchodilator (albuterol). The bronchoscopy will not be performed if the baseline lung function shows more than a minimal amount of airway obstruction and this does not resolve with inhaled bronchodilator. During the bronchoscopy, a fiberoptic scope will be inserted through the nose into one of your airways where a portion of one of your

lungs will be washed with sterile saline and a sample of airway lining cells will be obtained with a special brush device.

Bronchoalveolar Lavage (BAL) and Brush Biopsy is frequently used in the diagnosis of lung disease in both adult and pediatric patients. The procedure has also been used in numerous research studies of persons with respiratory disease and in healthy volunteers. The procedure has been used safely many times in studies conducted at our laboratory. The purpose of the BAL procedure is to obtain fluids and cells from the lower regions of the respiratory tract, i.e., the trachea, bronchi and smaller airways. These materials will be analyzed to obtain information about the role of various chemical substances and cells in the pulmonary response to particle exposure of persons with asthma. The bronchoscope is a highly flexible fiberoptic tube that is about two feel long and 1 centimeter in diameter (about the diameter of a pencil). It is an optical device with a light at the end which can be used to transmit images to a camera connected at the other end. Using the bronchoscope, the physician can see into your airways and direct the placement of the bronchoscope. A small channel allows fluid to pass through the bronchoscope.

The BAL procedure will be performed at the laboratory medical station by specially trained experienced pulmonary physicians. You will be expected at the medical station at 8:00 on the morning after the exposure. If you have had anything to drink or eat since midnight the night before the bronchoscopy you will not be allowed to proceed. Before you undergo bronchoscopy, your lung function will be evaluated and you may be treated with inhaled bronchodilator (albuterol). The procedure will not be performed if your lung function does not exceed a predetermined level of function recommended by the National Institute of Health for performing research bronchoscopies in persons with asthma. You may terminate the bronchoscopy procedure at any time. If the physician deems that your are too uncomfortable or anxious, the procedure will be terminated.

A saline lock intravenous catheter will be placed in a vein in your arm to give you medication before the procedure starts (a saline lock is a flexible catheter that stays in your arm for a short time). The saline lock will remain in place so that it can be used to administer medications in case there are any problems during the procedure. You will also be connected to a telemetry monitor that will display heart rate and rhythm, a blood pressure cuff will be placed on your arm, and an oximeter sensor (small band like device will be placed on a finger to allow the medical staff to monitor you during the procedure. No sedatives and/or narcotics will be administered at any time during bronchoscopy.

Before proceeding, the medical staff will again make certain that you have had nothing to eat or drink since midnight the previous night. You will be asked to decongest your nose by administering 2 puffs of neosynephrine nasal spray to each nostril. They will then give you a lidocaine solution and ask you to gargle with it for a few seconds to anesthetize your throat. You will then be asked to inhale (snort) a small amount of lidocaine jelly through one nostril to anesthetize your nose and the back of your throat. A Q-tip with lidocaine jelly will be gently inserted into your nose to ensure that your nose is completely numb before the bronchoscope is inserted. The procedure will not begin until your nose and throat are well anesthetized. If this cannot be

accomplished, the bronchoscopy will not be performed. A tube delivering oxygen will be placed inside your other nostril. Delivery of supplemental oxygen is done as a precaution during all bronchoscopies conducted at our facility.

To start the procedure, the physician will pass the bronchoscope through your anesthetized nostril to the back of your throat and then to above your vocal cords. He will then inject a lidocaine solution to numb your vocal cords before passing the bronchoscope into your trachea (windpipe). More lidocaine, up to a safe maximum dose is injected at various points in your trachea and airways to minimize coughing during the procedure. You will experience some cough during the procedure. This is a normal reflex caused by the presence of the bronchoscope in your airway.

The bronchoscope will be gently wedged in an airway in the right lung and sterile saline will be injected in your lung through a channel in the bronchoscope. The saline will then be gently suctioned from your lung through the channel in the bronchoscope. This sequence will constitute a wash. Six (6) washes will be performed. A total of 270 cc (about one-half pint) of sterile saline will be used during the BAL procedure. Approximately 75% of the saline injected into your lungs will be recovered by aspiration (suction) through the bronchoscope. The remaining 25% (75 cc) is expected to remain in your lungs. The saline left in your lungs will not generally cause you any difficulty breathing or harm you and should be completely absorbed by your lungs within 48 hours.

After the BAL is performed, brush biopsies of your airway epithelium will be taken. A very small brush (3 millimeters in diameter) will be inserted through the channel in the bronchoscope. The brush is visible to the physician performing the procedure through the bronchoscope. Small amounts of surface cells are scraped from the airway by gently brushing the airway several times. Two (2) brush biopsies are taken at one site in the right lung. After the brush biopsies are obtained, the procedure will be complete and the bronchoscope will be removed from your airway. The total time the bronchoscope will reside in your airways will be 15-30 minutes.

After the procedure the oxygen cannula will be removed if your oxygen saturation is acceptable. The nurses will check your vital sign immediately after the procedure and every half hour thereafter until discharge. During this time you will sit in a recliner at the medical station for an observation period of approximately two hours. You will not have any food or drink during this time. After the recovery period, the nurses will check your gag reflex. Since the gag reflex will be absent due to anesthesia during the procedure, you will not be allowed to ear or drink until the anesthesia wears off. This normally takes about one to one-and-one half hours. Once your gag reflex returns, you will then be given some juice to sip and then some crackers.

The physician who performed the procedure will check you after the recovery period and you will repeat the spirometry lung function test. You will be discharged if your vital signs are stable, chest examination is normal, and lung function is greater than 80% of your pre-procedure value. Prior to discharge you will be requested to take 600 mgm of ibuprofen by mouth; administration of ibuprofen/motrin almost always prevents the post-bronchoscopy malaise and low grade fever that would otherwise occur in about 25% of persons undergoing the procedure (acetaminophen/tylenol is a less effective alternative medication).

Before going home, you will be give the phone number of the medical station (966-6232) and pager number to the physician who performed the bronchoscopy with instructions to call if you experience any adverse symptoms such as: 1) persistent fever or fever above 101 degrees Fahrenheit, 2) persistent cough, 3) sputum (phlegm) production, 4) chest pain, 5) coughing up any amount of blood, 6) nose bleeds, or 7) shortness of breath

Are there any reasons you should not participate in bronchoscopy?
You should not participate in this study if you have problems with excessive bleeding after minor cuts or abrasions. You should not participate if you have had a recent exacerbation in your asthma that was not controlled with your usual medications. The physician and medical staff will explain other exclusionary condition in detail to you. In addition, during your participation in this study, you will be asked to refrain from, limit, or control taking the following medications:

*No more than 81 mg aspirin or similar medication on a regular basis or during the week of testing.
*Withhold antihistamines for 7 days prior to testing.
*Withhold inhaled bronchodilators 12 hours prior to testing.
You should not participate in this study if you are unwilling or unable to remain in the local area for 24 hours following each bronchosocpy

With your permission, DNA from the collected cells will be genotyped for specific genes related to adverse health effects associated with air pollution exposure. Unwillingness to have samples genotyped will NOT exclude you from participating in the study. If you do not wish for your cells to be used for genotyping but do wish to participate in the study do not sign the section ***Subject's Agreement for Genotype Screening With Identifying Information*** located at the end of this document.

What if we learn about new risks 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.

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. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely. With your permission, we will continue to store any samples that we collect to possibly use for other studies. This allows us to make the best use of the samples we collect from our volunteers. Again, you do not have to allow us to keep these samples if you do not want to.

What are the possible benefits from being in this study?
Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study. We will share with you any information that we learn about your lung function or other medical findings. We can also send you the results of some of the blood tests we perform.

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

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

2. 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. Also, the forceful breathing during this test can cause wheezing or shortness of breath to occur.

3. The adhesive patches used as part of the heart monitoring system may cause some mild skin discomfort or discoloration. If this occurs you should notify the nursing staff.

4. During air pollution exposure, you may experience some minor degree of airway irritation, cough, shortness of breath or wheezing. Some studies suggest that persons with asthma may be at risk for developing an asthma attack as a result of exposure to fine air pollution particles. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. Your symptoms will be closely monitored during the exposure and the study terminated if more than modest respiratory symptoms occur during the exposure. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. If you experience a significant drop in your lung function, albuterol will be administered.

Air pollution may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. There is a small possibility that you could have more asthma symptoms for several days, and even less likely, for several weeks, after the particle exposure. This flare in your asthma would be similar to what happens to you and other asthmatics during the allergy season or with a viral infection. Such a flare is considered highly unlikely and the exposure risk is comparable to walking around a major metropolitan area such as Los Angeles or Houston on a smoggy day. If an asthma flare occurs, you will be asked to seek medical treatment from your regular physician who treats you for your asthma.

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 6 ft in width, 6 ft in height, and 8

ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator at all times.

5. Bronchoscopy: As with other medical procedures, there are risks associated with bronchoscopy. However, experience has shown these risks are extremely small in research subjects. The risks associated with bronchoscopy are described in detail below.

A primary risk is bronchoscopic procedures is **discomfort to the nose and throat** brought about by the insertion of the bronchoscope through the nasopharynx. This discomfort should be alleviated or eliminated by the use of local anesthetics such as lidocaine. For this reason, you will be asked to gargle with a lidocaine solution and have lidocaine jelly placed in your nose prior to beginning the procedure. The effectiveness of the anesthesia will be tested by using a cotton swab gently inserted into your nose before inserting the bronchoscope. If you experience pain or discomfort during insertion of the bronchoscope, more lidocaine will be used up to a safe maximum amount. If adequate anesthesia cannot be achieved using this amount of lidocaine, the procedure will not take place.

A second risk of bronchoscopy is **coughing** that results from the irritation of the airway caused by the presence of the bronchoscope in the airway. Coughing is a normal reflex to expel foreign substances from the airways. Because coughing itself can cause mild trauma and discomfort to the airways and vocal cords, lidocaine liquid is sprayed on the airways at various sites to suppress the cough reflex. This technique is usually not completely successful and it is expected that you will have some cough during the procedure, especially during the initial insertion of the bronchoscope into the airway. If coughing is not controllable with the lidocaine or causes you discomfort, the procedure will be terminated.

There is a slight chance that the bronchoscopic procedure will precipitate an **asthma attack**. This risk is minimized by 1) only allowing persons with mild stable asthma to participate in the study, 2) pretreatment with inhaled bronchodilator (albuterol) before the procedure if your lung function is impaired and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physician responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. You will not be discharged from the EPA Medical Station until your lung function has returned to 80% of your pre-procedure performance. . In the unlikely event that you require additional treatment because the asthma attach continues, you will be transported by ambulance to the emergency room of the UNC Hospital, located 1/4 mile away.

Nose bleeds are another risk of bronchoscopy. This is caused by trauma to the nose during the bronchoscopy procedure. Usually, the bleeding is minor and is noticed as a few drops of blood in mucous secretions in the nostril. The bleeding usually stops on its own within an hour after the bronchoscopy. On very rare occasions, if the bleeding becomes moderate to severe, your nostril will be packed

with sterile gauze to absorb the blood and put pressure on the site of bleeding. Should you require additional treatment, you will be transported to the UNC Hospital Emergency Room. Major nose bleeds during bronchoscopy are very rare and generally limited to persons with kidney disease or a blood clotting disorder. It could also be promoted by chronic use of aspirin (>81 mg), ibuprofen or similar medications. You will not be allowed to participate in this study if you are taking such medications. Therefore the probability of getting a major nose bleed is very small.

Major **bleeding of the airways** is another rare (less than one percent) complication of bronchoscopic procedures. Most episodes of major bleeding due to bronchoscopy occur in persons with underlying predisposing conditions and are due to forceps biopsies of the airways mucosa which will **not** be performed in this study. People who suffer from kidney disease, blood clotting disorders or are chronic users of aspirin-like drugs are at increased risk of bleeding during bronchoscopy. In addition, people with lung cancer or with serious lung infection are more likely to suffer from airway bleeding during bronchoscopy. Your screening medical history, physical examination, and blood analysis will reveal most conditions that would increase your risk of bleeding during the bronchoscopy. You will not be allowed to participate if you have any of these conditions. Therefore your chances of incurring a serious airway bleed during bronchoscopy are extremely small. Nevertheless, if you do experience bleeding during bronchoscopy, you will be under the care of an experienced physician who will take steps to stop the bleeding immediately. A special cart with emergency supplies is available to handle medical emergencies. In the event of a major bleeding episode, you will be immediately transported to the Emergency Room of the UNC Hospital.

Pneumothorax or collapsed lung is another possible complication of bronchoscopic procedures. This complication is more likely to occur during tissue biopsy in the lung periphery, not the major central airways which will be the focus of this study. However you will be advised of the symptoms in the event that you experience a pneumothorax. The symptoms include chest pain and shortness of breath. If you experience these symptoms, you should contact the medical station (966-6232), or page the physician who performed the bronchoscopy. The vast majority of pneumothoraces occur within 24-48 hour of the procedure being completed. While a serious condition, a pneumothorax can be readily treated in an emergency room.

The **lidocaine** used to anesthetize your nasopharynx and reduce your cough can pose certain risks as well. A small fraction of the lidocaine applied to your nose and airways will be absorbed into your bloodstream, where it could cause adverse effects. Your risk of suffering side effects are very small, but would be much greater and potentially life-threatening if you are allergic to lidocaine or other topical anesthetics. You will not be allowed to participate in this study if you have drug allergy to topical anesthetics. When used at high doses, lidocaine can also cause symptoms in your central nervous system (confusion, tremor, euphoria, or, rarely, seizures) or heart rate disturbances (very fast or very slow heart rate) if an excessive dose of medication is used. Finally, a death in a volunteer receiving an overdose (over 1000 milligrams) of lidocaine during bronchoscopy has been

reported from Rochester, New York. However, no serious side effects of this medication have been noted at lower doses such as those described in this protocol which uses up to 360 milligrams of lidocaine during the entire procedure. If any problems develop secondary to the use of lidocaine, the physician bronchoscopist and the doctor on duty that day at the Human Studies Division of the EPA will be available to handle these problems.

Prior to the start of the bronchoscopy **atropine** may be given though a vein in your arm in order to counteract any changes in blood pressure that would result from the placement of the bronchoscope in your airway and to limit the quantity of secretions in your nose and throat. Atropine can cause dry mouth and nose for 30 to 60 minutes after the procedure is completed. This effect should wear off about an hour after the procedure is completed.

Placing an **intravenous catheter** in your arm carries a small risk as well. The needle stick can be momentarily painful and more rarely can leave a bruise in your arm. The medical staff who will be placing the catheter are very experienced and therefore the risk is minimal. Infection is also a very small risk that can result from placing the catheter. Again, the medical staff is very experienced in the use of sterile technique to prevent infection. If you notice redness or swelling in the area surrounding the catheter site, you should contact the medical station at 966-6232 or the physician on call for treatment.

A low grade fever (less than 101EF) during the 24 hours following the procedure occurs in about 25% of patients with respiratory disease undergoing bronchoscopy and BAL. The fever is benign and is usually effectively prevented by administering ibuprofen following the procedure. However, if you experience fever greater than 101 EF or lasting longer than 24 hours you could have pneumonia and should contact the Medical Station immediately so that you can be examined and treated.

Pneumonia occurs in less than 1 percent of patients undergoing bronchoscopy and BAL. Signs of pneumonia include fever greater than 101 EF, or persisting for more than 24 hours, persistent cough with or without sputum production, coughing up blood, chest pain, and shortness of breath or wheeze. Should you experience these symptoms, it is imperative that you contact the Medical Station to be seen and evaluated by a physician. As a routine precaution the Medical Station Staff will contact you between 24 and 48 hours following the procedure to inquire about any symptoms you may be experiencing.

In addition, there may be uncommon or previously unrecognized risks that might occur.

There are no risks 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 may cause some irritation, itching, or burning in some people. If this occurs you should notify the nursing staff.

The following are policies and procedures to minimize risk during the experiment:

1. During this study, skilled personnel, in an adequately equipped facility, will be observing you for any adverse effects. You may, without penalty, stop the test at any time by telling the monitor.
2. A physician will be on call at all times during the study to deal with any medical problems that may arise.
3. Every test has some degree of risk and there may be some risks associated with this experiment which are unforeseeable. You should not participate in the study if you have an acute illness.
4. You may terminate your participation in the experiment at any time and for any reason that you wish without penalty; you will be paid an amount for participation prorated for the time and procedures in which you took part. You understand that you are not under any obligation to complete any test procedures which you find objectionable or uncomfortable.
5. The investigator may terminate your participation in the experiment at any time. If this happens, you will be entitled to full payment for your time commitment and any experimental activities up to that point.
6. Your participation in the study may be postponed if your pre-exposure lung function shows airflow obstruction or if you develop a cold or asthma exacerbation.

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. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

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, the U.S. EPA and/or UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

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

You may withdraw from this study at any time. If you choose to withdraw or are unable to complete the study because of not following the instructions in this consent form, you will receive compensation only for your participation up to that point.

The investigators have the right to stop your participation at any time. This could be because you have had an unexpected reaction, have failed to follow instructions, or because the study has been stopped. After you enroll, if you are dismissed by the investigators before completing the study for reasons that are not your fault, you will be paid for your participation up to that point.

We made need to cancel your study activity due to adverse weather conditions, equipment failure, or other unforeseen events. If a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid for the time scheduled and cancelled at the standard hourly rate of $12 per hour plus any cancelled activities up to a maximum of $100. When possible, you will be rescheduled at a later time.

Will you receive anything for being in this study?

You will be paid approximately $12 per hour for your participation in this study. Total compensation will be based on the number of exposure sessions that will be required.

1. If you are complete all three exposures sessions of this study, your total compensation including screening will be approximately $2516.

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

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is

normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. 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. Compensation for the visits will be as follows:

You will be exposed to diesel exhaust ,ozone and have an air control exposure in three separate sessions. Exposure Days 1-2 will be repeated for the second and third chamber exposure session, but a second training Day is not needed. If you complete three exposures, your compensation including the pre-study qualifications and training day will be approximately $2516.

Pre-study qualifications

recruitment screening	$15	Previously paid
physical exam	$15	Previously paid
bronchoscopy physical exam	$20	Previously paid

Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24

Training Day Total = $ 44

You will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure

Endobronchial Brush biopsies	$25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus	**$100**

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures (excluding Prestudy qualifications and training day)	**$1548**
TOTAL for completion of THREE exposures (excluding Prestudy qualifications/training day but including completion bonus)	**$2422**

If you begin but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, you will receive compensation for your participation up to that point however in you arrive in the lab for the procedure having not followed instructions (eating food, taking NSAIDs or aspirin, or failing to report cold or flu symptoms the day prior to the bronchoscopy) you will be compensated $15 for your time, but will not receive compensation for the bronchoscopy.

You will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available.

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 or follow directions of the investigators, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and cancelled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during a current visit if cancelation occurs while you are in the facility. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled. The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be reimbursed for time and procedures completed up to that 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/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

Who is sponsoring this study?
This research is funded by the United States Environmental Protection Agency. This means that the research team is being compensated by the sponsor for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the product being studied.

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 US Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, 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 the Chairman of the IRB Committee at 919-966-1344 and /or the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What specimens will be collected and stored?

Bronchial epithelial cells collected via bronchoscopy and all blood specimens will be stored with your permission. If you agree to have your specimens stored for future research you will need to sign the accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information***.

- -

IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D. and David Diaz-Sanchez, Ph.D.

Subject's Agreement to Participate in the Research Study with Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I voluntarily **AGREE** to have my provided bronchial epithelial cells genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

Signing here and agreeing to participate in this study DOES NOT allow us to store your samples.

_______________________________ _______________

Signature of Research Subject Date

____

Printed Name of Research Subject

_______________________________ _______________

Signature of Person Obtaining Consent Date

____

Printed Name of Person Obtaining Consent

IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D. and David Diaz-Sanchez, Ph.D.

Subject's Agreement to Participate in the Research Study without Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I **REFUSE** to have my provided bronchial epithelial cells genotyped for any

genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

Signature of Research Subject | Date

Printed Name of Research Subject

Signature of Person Obtaining Consent | Date

Printed Name of Person Obtaining Consent

- -

APPROVED - IRB, UNC-CH
APR 22 2010

TO:

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

STUDY: **Lamarck, IRB #09-1625**

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 6 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 for 7 days prior to all visits. Tylenol and daily 81 mg aspirin are permitted.
- No antihistamines for 7 days before exposures.
- No inhaled bronchodilators for 12 hours prior to bronchoscopy

Diet and Exercise
- No vitamins or multivitamins with C or E and no fish oil supplements for 7 days prior to each visit.
- No drinking alcohol 24 hours before all visits
- No strenuous exercise for 24 hours prior to and after all visits
- No caffeine for 12 hours before to all study visits.
- Eat a normal breakfast on Exposure days.
- Nothing to eat or drink after midnight the evening before the bronchoscopy (Exposure Day 2)

Other
- No smoke and fumes for 24 hours before all visits
- Wear comfortable clothes and shoes suitable for exercise.

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

Thank You! Westat EPA Support Services

Recruitment Script

APPROVED - IRB, UNC-CH

IRB Study # 09-1625

APR 22 2010

Title: Epigenetic effects of diesel exhaust and ozone exposure (Lamarck)

Purpose:
The purpose of this research study is to compare changes in lung function, lung cells, blood and heart rate between people with and without asthma when they are exposed to diesel exhaust and ozone. This study is for healthy people ages 18 to 55 who have mild to moderate asthma and those who do not have asthma.

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 training session and three 2-day exposure sessions, each separated by at least two weeks. On the second day of each exposure session volunteers will have a bronchoscopy, a procedure used to get a lung cell sample. During the procedure, a doctor passes a thin tube through your nose and throat into the airways. Cells are removed either by a squirt of salt water or a gentle brushing. Before inserting the tube, the doctor will give you anesthetic to numb your nose and throat. You will be given an intravenous (IV) line. After the procedure, nurses will monitor your heart rate and blood pressure. The bronchoscopy itself usually lasts about 30 minutes, but the entire procedure, including preparation and recovery time, takes about 4 hours, and we ask that you stay in the Chapel Hill area for 48 hours afterward.

Training takes about 2 hours. Each exposure day takes about 6 hours and the follow up day takes 4. During exposure sessions you will be asked to give blood samples, ride an exercise bike, and perform several different breathing tests. You will also wear a heart monitor during the study sessions and over night. The total amount of time is about 32 hours over about 6 to 8 weeks. Each set of exposures is separated by 2 weeks.

If you complete the screening visits, the training visit, and all three 2-day study sessions, you will be paid $2,516.

Study Schedule

Screening, 2 visits, as needed: 3 hours

Training visit, *days & times to be decided*: 2 hours

Session 1, Day 1, 8 AM, *days to be decided*: 6 hours

Session 1, Day 2, 8 AM, day after Day 1, 4 hours

Sessions 2 and 3 are separated by at least 2 weeks. Does this seem like something that interests you?

Study Restrictions:
In addition to meeting all study eligibility criteria (*see 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 for 7 days prior to all visits. Tylenol is permitted.
- No vitamins or multivitamins with C or E and no fish oil supplements for 7 days prior to each visit.
- No antihistamines for 7 days before all visits.
- No smoke and fumes for 24 hours before all visits
- No drinking alcohol 24 hours before all visits
- No strenuous exercise for 24 hours prior to and after all visits
- No caffeine for 12 hours before to all study visits.
- No inhaled bronchodilators for 12 hours prior to bronchoscopy
- Nothing to eat or drink after midnight the evening before the bronchoscopy (Exposure Day 2)

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: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 6/03/2010
Expiration Date of Approval: 9/27/2010

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Approved Research
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure
Sponsors: US Environmental Protection Agency - GRANTS

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment received 6/3/10 addresses:

1. addition of Kelly Duncan
2. decrease in amount of wash fluid used for bronchoscopy & increased # of brushes
3. minute ventilation measurement may be used during exercise procedure, but not necessary
4. blood pressure measurements collected intermittently
5. addition of new information/instruction sheets

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

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: David Diaz Sanchez, Environmental Protection Agency; Robert Truckner, (EPA), Non-IRB Review Contact

OFFICE OF HUMAN RESEARCH ETHICS -- Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

This application is to seek approval for a modification to a currently approved study. Any proposed changes to previously approved human subjects research must be reviewed and approved by the IRB prior to implementation. This includes modifications to the study, inclusion or exclusion criteria, recruitment methods, research personnel, or *any* new or revised study materials. Approval is required for all modifications whether initiated by the investigator or external sponsor. **This form should not be used to report violations and deviations.**

Instructions for Submitting

Include with your submission the items indicated in the list on the next page, where applicable.

Unless otherwise instructed, submit to the same IRB that previously reviewed this study. Complete submission instructions can be found at http://ohre.unc.edu/submission_instructions.php. ***All application and consent materials should be copied or printed on one side only.***

Special submission instructions apply for studies that require additional review. Examples include the General Clinical Research Center (GCRC; http://gcrc.med.unc.edu/investigators/admin/gcrcapp.htm) or the Oncology Protocol Review Committee (PRC; http://cancer.med.unc.edu/research/prc/default.asp). See their web sites for details.

Address for all Applications and Other Correspondence

IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

---- Instructions – Do not submit this page with your application ----

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
X	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
X	5. If adding personnel, include name, location (UNC or specific outside location), role, and email address for each person who should receive electronic copies of IRB correspondence to PI. For those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/) include documentation of required training in human research ethics.	1

1 List and describe each proposed change:

All changes have been made where appropriate in the study protocol and all consents

(1). Added Kelly Duncan as a Co-PI

(2). On page 15 of the protocol we decreased the amount of wash fluid used for the bronchoscopy procedure and increased the amount of brushes. We determined that the collections of the Epithelial cells obtained via the brushes are more important than the cells/proteins obtained from the large volume of lavage fluid.

(3). On page 13 of the protocol, we may use the minute ventilation measurement during the exercise procedure but it is not necessary to do the measurement at each interval. Also we determined that the blood pressure measurements may be collected intermittently, mainly because it is not feasible during exercise.

(4). Also included in this modification are two new instruction/information sheets we will give to the volunteers.

2. Is this modification being submitted in response to an unanticipated problem/adverse event or new findings? ___yes __X_no

If yes, explain, including whether these events or findings are relevant to participants' willingness to continue.

3. Do any of the proposed changes increase risk? ___yes _X__no If yes, explain.

IRB study #: 09-1625 **Date:** 6-3-2010

Title of Study: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

Principal Investigator: Melanie Jardim, Ph.D. **Faculty advisor:** (if applicable)

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

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X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
X	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
X	5. If adding personnel, include name, location (UNC or specific outside location), role, and email address for each person who should receive electronic copies of IRB correspondence to PI. For those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/) include documentation of required training in human research ethics.	1

1 List and describe each proposed change:

All changes have been made where appropriate in the study protocol and all consents

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(3). On page 13 of the protocol, we may use the minute ventilation measurement during the exercise procedure but it is not necessary to do the measurement at each interval. Also we determined that the blood pressure measurements may be collected intermittently, mainly because it is not feasible during exercise.

(4). Also included in this modification are two new instruction/information sheets we will give to the volunteers.

2. Is this modification being submitted in response to an unanticipated problem/adverse event or new findings? ___yes __X_no

If yes, explain, including whether these events or findings are relevant to participants' willingness to continue.

3. Do any of the proposed changes increase risk? ___yes _X__no If yes, explain.

IRB study #: 09-1625 **Date:** 6-3-2010

Title of Study: Epigenetic Effects of Diesel Exhaust and Ozone Exposure

Principal Investigator: Melanie Jardim, Ph.D. **Faculty advisor:** (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

_____ _____

Signature of Principal Investigator or designee Date

_____ _____

Signature of Faculty Advisor (if applicable) Date

LAMARCK INSTRUCTIONS

EXPOSURE DAY

1-PLEASE EAT BREAKFAST AND DRINK PLENTY OF FLUIDS

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

3-NO MEDICATIONS (EXCEPT BIRTH CONTROL PILLS) i.e. ADVIL, ASPIRIN ETC. FOR 1 WEEK PRIOR TO STUDY.

4- AVOID SMOKE AND FUMES FOR AT LEAST 48 HOURS BEFORE STUDY.

5- BRING YOUR LUNCH-NOTHING TO HEAT UP

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

7-WEAR COMFORTABLE EXERCISE CLOTHES AND SHOES.

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

9- GUYS- IF YOU HAVE A HAIRY CHEST -YOU WILL HAVE YOUR CHEST SHAVED. YOU CAN SHAVE YOUR OWN CHEST OR WE WILL.

10-NO PAN FRIED OR GRILLED FOODS 24 HOURS PRIOR TO STUDY

11- NO CAFFIENE FOR 12 HOURS PRIOR TO STUDY

BRONCHOSCOPY DAY

1-NOTHING TO EAT OR DRINK AFTER MIDNIGHT
2-NO STRENUOUS EXERCISE FOR 24 HOURS AFTER STUDY.
3-PLAN TO STAY IN THE LOCAL AREA FOR 24 HOURS AFTER BRONCHOSCOPY

Environmental Public Health Division
Chapel Hill, NC 27514

HOLTER MONITOR INSTRUCTIONS

1. Do not remove battery cover, battery or card as this turns off the monitor.

2. The monitor, wires, and electrodes should remain attached to each other and you at all times.

3. Try not to allow the electrodes or wires to be pulled on.

4. **DO NOT DO CAR MAINTENANCE DURING THE TIME OF THE STUDY**.

5. Avoid all electrical equipment including electric blankets, heating pads, shop tools waterbed heater, etc. Computers appear to be okay.

6. **DO NOT GET THE HOLTER WET**.

   - Keep the wires and electrodes dry as well.
   - Do not carry damp or wet objects close to monitor.

7. If an electrode loosens you may apply pressure around the edges for about 30 seconds to try and make it stick again. You should not push on the very center of the electrode. If the electrode falls off or doesn't have good contact, you can replace it with a new one.

. Keep the monitor next to you in the bed while sleeping. Do not place it down outside of your bed.

19. If you experience any severe itching, redness, or swelling of your skin around the electrode sites, remove electrodes.

5/10

What is the purpose of this form?

This application is to seek *initial* IRB approval for a research study.

What parts of this application should you submit?

Answer all questions, or mark "not applicable," when appropriate. Do not alter wording or delete questions from this form.

- For ***all studies***, submit Part A, which consists of these sections:
  - Part A.1. Contact Information, Agreements, and Signatures
  - Part A.2. Summary Checklist
  - Part A.3. Conflict of Interest Questions and Certification
  - Part A.4. Questions Common to All Studies
  - Part A.5. The Consent Process and Consent Documentation (including Waivers)

- For ***studies that involve direct interaction*** with human subjects (any contact with subjects including questionnaires, interviews, focus groups, observation, treatment interventions, etc), submit:
  - Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

- For ***studies*** that use existing data, records or human biological specimens, including for use in identifying potential subjects, submit:
  - Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

Note: You should submit Parts B or C only as applicable. If the study involves *both* direct interaction *and* use of existing materials, use both Parts B and C in addition to Part A.

Who can serve as principal investigator (PI)?

The PI is the person who will personally conduct or supervise this research study. Under most circumstances, this will be a faculty member. For IRB communication purposes, a trainee/student may be listed as PI. However, a faculty advisor must be identified, who holds ultimate responsibility for ensuring that this project complies with all University, regulatory, and fiscal requirements.

→ *See next page for additional instructions*

Complete submission instructions can be found at http://ohre.unc.edu/submission_instructions.php. **All application and consent materials must be copied or printed on one side only**. See the checklist on page 1 of the application itself for items to include and number of copies.

Some applications require additional review prior to the IRB submission. Examples include the Clinical and Translational Research Center (formerly the GCRC and CCCT facilities) http://gcrc.med.unc.edu/investigators/admin/gcrcapp.htm) or the Oncology Protocol Review Committee (PRC; http://cancer.med.unc.edu/research/prc/default.asp). See their web sites for details.

Many schools, departments, centers and institutes in Academic Affairs have local review committees that review before the IRB. See http://ohre.unc.edu/submission_instructions.php for a list of these units or consult your own unit for details.

Address for all Applications and Other Correspondence

IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

Types of Review

There are three levels of IRB Review (full board, expedited, and exempt), determined by the nature of the project, level of potential risk to human subjects, and the subject population. *The type of review applicable to a particular study is determined by the IRB.* Regardless of the kind of review, all applications use the same submission form.

Exempt and expedited review can be given to studies that constitute no more than minimal risk to the human subjects, i.e., the risk one experiences in daily living. These reviews are done in the IRB office on a continual basis.

Full board review is required for studies that involve greater than minimal risk or vulnerable populations that require special protection by the IRB. These require review by the convened IRB. See http://ohre.unc.edu/guide_to_irb.php for additional guidance.

---- Instructions – Do not submit this page with your application ----

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version June 25, 2009

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

Date: June 3, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Name and degrees of Principal Investigator: Melanie J. Jardim, Ph.D.; David Diaz-Sanchez, Ph.D. Kelly Duncan, Ph.D.
Department: US EPA Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514
UNC-CH PID: 715306970 Pager:
Phone #: 919-966-7320 Fax #: 919-966-6271 Email Address: jardim.melanie@epa.gov

For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: David Diaz-Sanchez, Ph.D.
Department: US Epa Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514
Phone #: 919-966-0676 Fax #: 919-966-6271Email Address: diaz-sanchez.david@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any): Michael Schmitt, MSPH
Department: US EPA Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514
Phone #: 919-966-0647 Fax #: 919-966-6271 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 name, location (UNC or specific outside location), role and email address for each person who should receive electronic copies of IRB correspondence to PI.**

Robert Devlin, PhD.; Heidi Hiers, RN; Maryann Bassett, RN; Martin Case, BS; Andrew Ghio MD; Martha Sue Carraway, MD; Robert Truckner, MD;Howard Kehrl, MD; Tracey Montilla, RN; Michael Schmitt, MS.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify):

For external funding, RAMSeS proposal number (from Office of Sponsored Research): The PI is a USEPA scientist and this project is being funded by intramural EPA funds

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

Applications must "stand alone" and should provide all information requested, i.e., complete answers must be contained in the application. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

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 (include DHHS-approved sample, when one exists), fact or information sheets, phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
□	4. All recruitment materials including final copies of printed advertisements, audio/video taped advertisements, scripts, flyers, letters, and 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., Clinical and Translational Research Center (CTRC), Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. For drug studies, Investigator Brochure if one exists. If none, include package insert for previously approved uses..	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	_X_
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_X_
A.2.4. Do you have specific plans 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_
c. Is this research classified (e.g., requires security clearance)?	__	_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?	_X_	__
If yes, *see instructions for* ***Consent for Stored Samples***.		
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?	__	_X_
If yes, *approval by the* ***UNC-CH Institutional Biosafety Committee*** *is required.*		
A.2.16. Does this study involve UNC-CH cancer patients?	__	_X_
If yes, *submit this application directly to the* ***Oncology Protocol Review Committee***.		
A.2.17. Will subjects be studied in the Clinical and Translational Research Center (CTRC) or is the CTRC involved in any other way with this study? If yes, obtain the ***CTRC Addendum*** *and submit completed application (IRB application and Addendum) directly to the CTRC. The CTRC includes facilities located on the 3rd floor of the Main Hospital (formerly GCRC) and Ground floor Burnett-Womack (formerly CCCT).*	__	_X_
A.2.18. Will gadolinium be administered as a contrast agent?	__	__X
A.2.19. Will subjects' Social Security Number (SSN) be collected for:		
a. processing payments greater than $200 per year, to support IRS reporting (see also B.6)?	__	_X_
b. processing payments of any amount through UNC-CH Accounts Payable?	__	_X_
c. use as a unique identifier for study tracking purposes for national registry or database?	__	_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 personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity engaged in the performance of this project as a subcontractor, sub-recipient or vendor?	__ yes	_X_ no
(d) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_X_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit the form, which is accessible online at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

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

_____ _____

Signature of Faculty Advisor Date

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

Complete answers must be provided. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

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 protocol is to compare the genetic and epigenetic effects between diesel exhaust and ozone exposure in healthy individuals and in mild/moderate asthmatics.

Participants: We will recruit up to 30 mild to moderate asthmatics and up to 30 healthy adults to participate in this study.

Procedures (methods): Subjects will be exposed to clean air, to 300 $\mu g/m^3$ of diesel exhaust for 2 hours and to 0.3 ppm of ozone for 2 hours with intermittent exercise in a controlled environment chamber. Primary endpoints will include spirometry and lung cell changes post-exposure. Secondary endpoints will include analysis of blood clotting/coagulation factors, Holter monitoring of cardiac parameters, analysis of soluble factors present in plasma and bronchial lavage and analysis of intracellular factors present in lung tissue obtained from a brush biopsy.

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.

Several epidemiological studies have shown a strong link between exposure to air pollution and adverse cardiopulmonary effects, such as respiratory tract infections, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke(Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005; Lewtas 2007). Ozone and diesel exhaust are ubiquitous in the urban atmosphere making it difficult to identify the exact source of these effects. Although experimental chamber studies have been previously performed with diesel exhaust or ozone, no one has yet looked at the epigenetic changes resultant from acute exposures to these air pollutants.

Numerous studies have shown that healthy subjects exposed to ozone have decreased pulmonary function and increased neutrophils and soluble pro-inflammatory mediators in the lower airways(Peden, Setzer et al. 1995; Bascom 1996). These include markers of increased permeability, decreased macrophage phagocytic ability, and increases in arachidonic acid metabolites, cytokines, fibronectin, lactase dehydrogenase, coagulation factor VII, tissue factor, elastase and plasminogen activator occurred in response to ozone-induced exposures(Devlin, McDonnell et al. 1991; Koren, Devlin et al. 1991; McDonnell, Kehrl et al. 1991; Devlin 1993; McKinnon, Madden et al. 1993). Diesel exhaust fumes contain primarily fine and ultrafine carbonaceous particles generated by the incomplete combustion of fuel(Diaz-Sanchez, Penichet-Garcia et al. 2000; Lewtas 2007). Diesel exhaust particles (DEP) are the largest single source of vehicular emitted airborne PM and can persist in the air, where they are readily inhaled and deposited throughout the respiratory tract. DEP induced toxicity is mainly attributed to its chemical composition, consisting of a carbonaceous core to which organic and inorganic species can become adsorbed (Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005). Healthy human volunteers exposed to 300 $\mu g/m^3$ diesel exhaust for one hour with intermittent exercise resulted in markers of systemic and redox-sensitive pulmonary inflammatory responses, vascular dysfunction and impaired endogenous fibrinolysis. Although there were no adverse health effects associated with the exposure, acute changes in the aforementioned endpoints can be used to extrapolate information in order to begin to understand the underlying biological mechanisms that link long-

term exposure of diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (Salvi, Blomberg et al. 1999; Behndig, Mudway et al. 2006).

Previous studies reported a wide variation in response to air pollution exposure among individuals in each subject group with no clear understanding in reasons (McDonnell 1991). Asthmatics have been identified as a susceptible subpopulation as seen by their heightened sensitivity to air pollutants, although the severity of health responses of asthmatics exposed to diesel exhaust and ozone are not yet fully understood. It is hypothesized that increased susceptibility could be due to increased baseline airway inflammation and airway responsiveness which characterizes asthma. Indeed, respirable particulate matter and ozone exposure are associated with episodes of increased asthma exacerbation (Devlin 1993; Bascom 1996; Diaz-Sanchez and Riedl 2005). Elevated levels of air pollution can affect airway reactivity with decrements in several indices of pulmonary function especially in those patients with preexisting asthma.

Despite a decade of intensive studies, adverse health effects attributed to air pollutant exposure are still not well understood. It is becoming increasingly clear that epigenetics plays a pivotal role in disease development and outcome, which has been extensively studied in cancer. The term epigenetics refers to the study of heritable gene expression changes, which occur without changing the DNA sequence itself. DNA methylation, histone modifications and microRNA expression all regulate gene expression, and changes in these mechanisms can result in an overall change in gene expression patterns. Unlike the static DNA sequence, these mechanisms are regulated by endogenous and exogenous cues, rendering them more likely to change in response to environmental stimuli. It is not currently known how these changes become permanent, or how epigenetic changes control gene expression in response to acute environmental perturbations. Few studies have examined how the environment can modulate epigenetic marks in individuals. In a pioneering study published in 2005, Fraga et. al. showed that as identical twins age, DNA methylation patters change indicating that differential gene expression observed in older identical twins may be attributed to epigenetic changes, further suggesting that these changes may have been molded by the environment (Fraga, Ballestar et al. 2005). Traffic particle exposure has been associated with methylation changes in retrotransposable elements, randomly dispersed throughout the DNA sequence, however the biological consequence of this is not understood as these are not coding sequences (Baccarelli, Wright et al. 2009). In vitro studies in primary human bronchial epithelial cells grown at air liquid interface showed that microRNA expression profiles change rapidly in response to exposure to diesel exhaust particles(Jardim 2009). Animal studies attempting to address these questions have been difficult as animals have very different epigenetic mechanisms making it difficult to draw conclusions from these studies. Long term exposure to cigarette smoke, a source of particulates, lead to changes in microRNA expression in rat lungs, however the contribution of these changes to biological consequences is not currently known (Izzotti, Calin et al. 2009). Another study, showed that an in utero diet rich in methyl donors enhanced allergic airway disease in mice via changes in methylation of several genes, and that these changes were transgenerational (Hollingsworth, Maruoka et al. 2008). While it is difficult to draw biological meaning from epigenetic animal studies, what these studies show is that the environment can play an important role in regulating gene expression. Although the biological and physiological role epigenetics plays in response to environmental pollutants is not yet understood, however we will begin to address this question in this study by performing in depth gene expression analysis, including microarrays, microRNA arrays and DNA methylation assays on samples collected from patients.

In addition to possible epigenetic mechanisms, genetic polymorphisms, particularly polymorphisms in anti-oxidant genes, have been proposed as a factor to explain variability in these responses. The role of genetic background in mediating airway and vascular inflammation following acute exposure to diesel exhaust and ozone in human subjects will be studied in this protocol as a secondary question. As such, bronchial epithelial cells collected from this study will be further evaluated by in vitro exposure to both gaseous and particulate air pollution.

The purpose of this study is to first, make a direct comparison of epigenetic responses (primarily changes in DNA methylation, microRNA expression and chromatin modification) in individuals exposed to ozone and diesel exhaust; second, to assess whether mild to moderate asthmatics exhibit the similar effects and to the same degree as healthy individuals; and third, to understand the contribution of genetic background and epigenetic mechanisms to cardiopulmonary effects observed in pollutant exposed cells. Although many the observed adverse health effects are generally attributed to oxidative stress upon exposure, we still do not know how these result in physiological changes to the respiratory and cardiovascular organ systems. We hypothesize that exposure to diesel exhaust or ozone will result in changes in epigenetic mechanisms, which may be linked to adverse health effects of exposure. If adverse health effects are induced solely due to oxidative stress, then patients should exhibit similar cardiopulmonary and epigenetic responses when exposed to either, diesel exhaust or ozone. However, if differential

responses to these agents are observed, then alternative mechanisms of cellular injury may exist. Therefore, we will employ several methods to assess potentially important physiological changes to these systems, including cutting edge techniques such as micro-array profiling, DNA methylation assays, microRNA expression profiling and assays for indications of chromatin remodeling.

Baccarelli, A., R. O. Wright, et al. (2009). "Rapid DNA methylation changes after exposure to traffic particles." Am J Respir Crit Care Med **179**(7): 572-8.

Bascom, R. (1996). "Environmental factors and respiratory hypersensitivity: the Americas." Toxicol Lett **86**(2-3): 115-30.

Behndig, A. F., I. S. Mudway, et al. (2006). "Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans." Eur Respir J **27**(2): 359-65.

Brunekreef, B. and S. T. Holgate (2002). "Air pollution and health." Lancet **360**(9341): 1233-42.

Devlin, R. B. (1993). "Identification of subpopulations that are sensitive to ozone exposure: use of end points currently available and potential use of laboratory-based end points under development." Environ Health Perspect **101 Suppl 4**: 225-30.

Devlin, R. B., W. F. McDonnell, et al. (1991). "Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung." Am J Respir Cell Mol Biol **4**(1): 72-81.

Diaz-Sanchez, D., M. Penichet-Garcia, et al. (2000). "Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity." J Allergy Clin Immunol **106**(6): 1140-6.

Diaz-Sanchez, D. and M. Riedl (2005). "Diesel effects on human health: a question of stress?" Am J Physiol Lung Cell Mol Physiol **289**(5): L722-3.

Folinsbee, L. J., J. F. Bedi, et al. (1985). "Pulmonary response to threshold levels of sulfur dioxide (1.0 ppm) and ozone (0.3 ppm)." J Appl Physiol **58**(6): 1783-7.

Fraga, M. F., E. Ballestar, et al. (2005). "Epigenetic differences arise during the lifetime of monozygotic twins." Proc Natl Acad Sci U S A **102**(30): 10604-9.

Hollingsworth, J. W., S. Maruoka, et al. (2008). "In utero supplementation with methyl donors enhances allergic airway disease in mice." J Clin Invest **118**(10): 3462-9.

Izzotti, A., G. A. Calin, et al. (2009). "Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke." FASEB J **23**(3): 806-12.

Jardim, M., Fry, RC, Jaspers, I, Dailey, L and Diaz-Sanchez, D (2009). "Disruption of MicroRNA Expression in Human Airway Cells by Diesel Exhaust Particles is Linked to Tumorgigenesis-Associated Pathways." Environmental Health Perspectives **117**(11).

Koren, H. S., R. B. Devlin, et al. (1991). "Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone." Toxicol Pathol **19**(4 Pt 1): 406-11.

Lewtas, J. (2007). "Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects." Mutat Res **636**(1-3): 95-133.

McDonnell, W. F. (1991). "Intersubject variability in human acute ozone responsiveness." Pharmacogenetics **1**(2): 110-3.

McDonnell, W. F., H. R. Kehrl, et al. (1991). "Respiratory response of humans exposed to low levels of ozone for 6.6 hours." Arch Environ Health **46**(3): 145-50.

McKinnon, K. P., M. C. Madden, et al. (1993). "In vitro ozone exposure increases release of arachidonic acid products from a human bronchial epithelial cell line." Toxicol Appl Pharmacol **118**(2): 215-23.

Peden, D. B., R. W. Setzer, Jr., et al. (1995). "Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics." Am J Respir Crit Care Med **151**(5): 1336-45.

Riedl, M. and D. Diaz-Sanchez (2005). "Biology of diesel exhaust effects on respiratory function." J Allergy Clin Immunol **115**(2): 221-8; quiz 229.

Salvi, S., A. Blomberg, et al. (1999). "Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers." Am J Respir Crit Care Med **159**(3): 702-9.

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).

Subjects for this study will be healthy adults, 18-55 years old, (male and female) and, otherwise healthy, 18-55 year old mild to moderate asthmatics (as defined by NHLBI guidelines), who are non-smoking. Subjects will be recruited through the Westat Corporation (see section B1 below). There are no gender or race based restrictions. Pregnant and lactating women will be excluded because of the unknown effects of air pollutants on inflammatory markers during pregnancy and lactation.

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.

Healthy Individuals

Specific Inclusion Criteria

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66).
2. Normal lung function (pre or post albuterol), defined by Knudson 1976/1984 as:
   a. FVC of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   b. FEV_1 of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   c. FEV_1/FVC ratio of $\geq$ 75 % of predicted values.
3. Oxygen saturation of $\geq$ 96 %.
4. Ability to tolerate intervals of moderate exercise

Exclusion Criteria:

1. A history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease, and lung cancer.
2. If the subject is pregnant, attempting to become pregnant or breastfeeding.
3. Allergy to any medications which may be used or prescribed in the course of this study.
4. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM and/or ozone challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
5. Smoking history within 2 years of the study.
6. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
7. Untreated hypertension (> 150 systolic, > 90 diastolic)
8. Dementia.
9. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
10. History of skin allergy to tape or electrodes.
11. Subjects who do not understand or speak English
12. Subjects who are unable to perform moderate exercise

Temporary exclusions:

1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.
4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:

1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin $\geq$ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

Mild to Moderate Asthmatics

Inclusion Criteria:

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66) where diagnosis will be judged by EPA physicians.
2. Mild/Moderate asthmatics as defined by NHLBI guidelines.
3. Positive history of asthma (wheezing, chest tightness, and reversible airway obstruction);
4. Baseline $FEV_1/FVC \geq 60\%$;
5. Oxygen saturation of $\geq$ 94%
6. Ability to tolerate moderate exercise

Exclusion Criteria

1. Use of oral steroid therapy within the past month
2. Physician directed emergency treatment for asthma exacerbation within the preceding 6 months.
3. Abnormal EKG that precludes evaluating heart rate variability.
4. Aside from mild/moderate asthma, a history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma, and cancer (possible exception for history of nonmelanoma skin cancer).
5. If the subject is pregnant, attempting to become pregnant or breastfeeding.
6. Allergy to any medications which may be used or prescribed in the course of this study
7. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
8. Dosing level of an inhaled steroid must be consistent with mild asthma as outlined by the NHLBI NAEPP guidelines. Regular use of oral corticosteroids, or use of inhaled steroid at doses typically used for severe asthma, will result in exclusion of that individual from the protocol.
9. Severe asthmatics as defined by: nighttime symptoms of cough or wheeze greater than 1 time per week at baseline, daily exacerbation of asthma or requirement for albuterol due to asthma symptoms (cough, wheeze, chest tightness, but not to include prophylactic use of albuterol prior to exercise), more than mild interference with normal activity, any episode of physician directed emergency treatment for asthma requiring oral corticosteroid therapy within the past twelve months.
10. Smoking history within 2 years of study.
11. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
12. History of skin allergy to tape or electrodes.
13. History of respiratory diseases other than allergic rhinitis and asthma
14. Untreated hypertension (> 150 systolic, > 90 diastolic)
15. Dementia.
16. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
17. Subjects who do not understand or speak English
18. Subjects who are unable to perform moderate exercise

Allowances: subjects may

1. Use daily theophylline therapy.
2. Use daily inhaled steroids.
3. Use inhaled cromolyn therapy.

Temporary exclusions:

1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.
4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:

1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin $\geq$ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This is a randomized cross-over study with three arms. This study will compare the epigenetic responses of healthy adults and adults with mild asthma to diesel exhaust and ozone exposures. Up to thirty subjects will be recruited into each group. Subjects will be randomly exposed to clean air, diesel exhaust and ozone with exposures separated by a minimum of two weeks. Responses of primary interest will include: 1) genetic and epigenetic changes 2) FEV1 as measured by spirometry, and 3) lung inflammation and cell changes as evaluated by bronchoalveolar lavage. The performance of these tests and procedures are considered essential to conducting the study. Exploratory endpoints will include: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, telemetry, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18-24 hours post-exposure. All medical procedures will be performed by study personnel.

Physical Examination Day:

Prior to recruitment into the study, subjects will undergo a physical exam. During this visit, a short review of the subject's history will be performed and vital signs, height and weight will be assessed (temperature, pulse, respiratory rate, and blood pressure), to determine whether the individual meets any major exclusion criteria for the study. Up to 50 ml of blood may be collected for a CBC/ differential, chemistry and lipid panels, assessment of coagulation function, phagocytic function and cell surface markers. At this time the subject may undergo a physical examination for bronchoscopy or may be scheduled for one at another time.

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.
- On the Exposure Day 2, eat a normal breakfast.
- No caffeine for 12 hours prior to all study visits.
- At the end of Exposure Day 2, do not eat or drink anything after midnight in preparation for bronchoscopy on Exposure Day 3.

Training Day

1. Eligible subjects will report to the research lab for an approximate 2-hour training session.
2. Informed consent will be obtained.
3. Assessment of **vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation
4. **Pregnancy tests** will be administered to any women who may have child-bearing potential.
5. Subjects will be trained on the cycle ergometer and the work load to elicit a minute ventilation normalized for body surface of 25L/m^2/BSA will be determined. In most subjects this will be about 50 L/minute (IE. VO^2 of approximately 2.0 L/m). A cycle ergometer work setting of 75 to 100 watts will usually achieve such a physiological response
6. Subjects will perform **spirometry.**
7. Subjects will be shown chamber where exposures will take place

Exposure Day 1

The subjects will be exposed ozone, diesel exhaust and clean air on three separate occasions, with the exposures separated by a minimum of two weeks. .

1. Subjects will be asked to arrive at the study site no sooner than 8 AM and will undergo **assessment of vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation, and **symptom score** assessment.
2. **Pregnancy tests** will be administered to any women who may have child-bearing potential.
3. A **telemetry unit** will be attached for cardiac monitoring.
4. Subject will be fitted with a **Holter monitor** and the subject will be asked to recline in a quiet dark place for 30 minutes. After approximately 20 minutes a time marker will be activated so that heart rate variability frequency readings can be taken over the next 10 minutes. The subject will wear the Holter monitor for the next 24 hrs.
5. **Venipuncture:** Up to 80 ml of blood will be collected for a CBC/ differential, and may be used for assessment of coagulation function and markers, phagocytic function, inflammation, and cell surface markers. A portion of the sample may be used for RNA isolation for microarray analysis and for DNA isolation for genotyping purposes.
6. **Baseline Spirometry**
7. **Exposure sessions**: Exposure to ozone, diesel exhaust or clean air will be conducted in an exposure chamber at the EPA Human Studies Facility on the UNC campus. Each subject will be exposed up to 0.3ppm ozone, diesel exhaust (up to 300 ug/m^3) or clean air for 2 hours. Subjects will begin exercising on an exercise bike. Each exercise session will consist of a 15 minute exercise interval at a level of up to 25 L/m2/BSA followed by a 15 minute rest period. Minute ventilation may be measured during each exercise period. Continuous heart rate and rhythm, and oxygen saturation, will be monitored using telemetry and pulse oximetry respectively. Blood pressure may be monitored intermittently. Subjects will be asked to refrain but not prohibited from using inhaled bronchodilator during exposures; if medication use is necessary subjects will be instructed to maintain consistent medication use across the two exposures. The subjects will be able to end their exposure and exit the chamber at any time. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, ataxia, or a greater than 5 point drop in saturated oxygen (or a drop to lower than 89%). They will be monitored continuously by trained personnel and a physician is on call whenever a subject is undergoing a procedure at the facility. A detailed description of the physical facilities in the Human Studies Division of the U.S. EPA Health Effects Research Laboratory located on the UNC-CH campus have been published as U.S. Government Publication EPA-600/1-78-064, and is on file in the office of the UNC Committee on the Protection of the Rights of Human Subjects. The medical station of the Human Studies Division includes a fully-equipped medical examination room, a cardiac/respiratory emergency cart, a subject recovery room, a nurse's station, a waiting room and conference rooms. The facility is staffed by qualified RNs, and an on-site physician is available to respond to a medical

emergency in a timely manner. The exposure atmosphere will be at approximately 40 ± 10% RH and approximately 22 ± 2 °C. The DE will be generated from a diesel generator used to power a load bank 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 up to 300 $\mu g/m^3$. The monitoring analyzer registers a concentration in real time. Exposures will be terminated at values ≥ 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 1080 μg, which assumes that the subject will inhale 2.7 m^3 during the 120 min exposure. 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 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) 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. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured in real time. Particle size distribution may not be determined during each subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. Ozone exposures will be conducted in a (6 ft x 6 ft x 8 ft) stainless steel chamber with a continuous supply of exposure medium. Ozone will be monitored continuously. **Immediately post exposure**, subjects will again undergo **spirometry, Holter monitor frequency reading, symptom scoring and assessment of vital signs.**

8. **Then,** up to 80 ml of blood will be collected for a CBC/ differential, assessment of coagulation function, blood leukocyte phagocytic function, cytokines, cell surface markers and RNA isolation for microarray analysis.
9. Subjects will then be assessed and discharged by the nursing staff.

Exposure Day 2 (approximately 24 hours after exposure)

The subject will arrive at the U.S. EPA HSF for:

1. **Vital sign monitoring.**
2. **Symptom scoring.**
3. **Venipuncture**: up to 80 ml of blood will be collected for a CBC/ differential, coagulation function, assessment of blood monocyte phagocytic function, inflammation, and cell surface markers, and RNA isolation for microarray analysis.
4. **Holter monitor** frequency reading and removal
5. **Spirometry**
6. **Bronchoscopy.** Subjects will have undergone a physical examination including assessment for suitability for transnasal fiberoptic bronchoscopy at the Human Studies Facility by a board certified or board eligible pulmonologist.

   Prior to the procedure, subjects will perform spirometry (as above); bronchoscopy will not be performed if lung function does not meet the recommended performance level (NIH guidelines; FEV1≥ 60 % predicted). Prior to bronchoscopy, a small tube (IV) will be placed in a vein for potential use in administering fluids. Subjects will receive inhaled albuterol prior to the bronchoscopy at the discretion of the pulmonary physician performing the procedure. Bronchoscopy with bronchoalveolar lavage and brush biopsy will be performed by a licensed physician who is Board certified or board eligible in pulmonary medicine and is experienced in fiberoptic bronchoscopy. The physician will be assisted by at least one R.N experienced in bronchoscopy. The subjects will be monitored closely throughout the procedure in the following manner: 1) Chest electrodes for continuous electrocardiogram, 2) Pulse oximetry of arterial blood, and 3) blood pressure monitoring using an electronic sphygmomanometer. Subjects will be premedicated with IV atropine (0.6 mg) to prevent bradycardia and hypotension that could result from vagal stimulation provoked by passing the bronchoscope through the subject's larynx and to minimize the amount of airways secretions. Nasal oxygen will be administered during the procedure. No sedatives and/or narcotics are administered at any time during bronchoscopy. We have found that premedication with sedatives (midazolam) or with opiates (demerol) is not required for research bronchoscopy subjects. Avoidance of these drugs reduces procedure risk and markedly shortens post-procedure recovery time. Topical lidocaine anesthesia will be used for subject comfort and to prevent cough, with a maximum dose of 15 ml of 2% lidocaine solution (360 mg lidocaine). Electrocardiographic and pulse oximeter will be monitored throughout the procedure. After the airways are visualized, the bronchoscope tip is wedged in a 4th-6th order bronchus (right middle lobe) for bronchial lavage. A volume of 20 cc of sterile saline will be injected and immediately aspirated through the channel in the bronchoscope using a syringe. An additional 30 mls of saline will be injected and aspirated into the same lobe. The lavage fluid aliquots will be put in

polypropylene tubes and kept on ice until processed. Up to 8 brush biopsies will then be obtained from the lower trachea, main, and lobar bronchi after topical application of 1% lidocaine. A cytology brush (Bronchoscope Cytology Brush; Bard, Tewksbury, MA) is applied to the region under direct visualization. For a single brushing, six passes, each with a linear excursion of 2-5 cm, are made with the brush along the endobronchial surface.

Subjects are observed and monitored for a minimum of 1 to 2 hours after the procedure in the on-site medical station by a registered nurse with physician supervision. At discharge subjects will be symptom-free. At least 2 physicians, including the responsible bronchoscopist, will be available (24 hours daily) to subjects who develop any symptoms after discharge. The subject is discharged by a physician and is provided with the names and telephone numbers of the bronchoscopist and one other physician. The subject is contacted 24 hours post-bronchoscopy to ask about any untoward effects.

Subjects will return to the U.S. EPA HSF for an additional exposure sessions and post exposure bronchoscopies (air control, ozone or diesel exhaust) after a minimum time interval of two weeks.

We anticipate performing several clinical procedures during the course of this study which include primary, secondary and exploratory endpoints. However, it is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure, because of a investigator problem (such as but not limited too building/equipment failures, weather delays, or staffing issues), then the patient will be compensated for said procedure(s) and time on that day and may be rescheduled.

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.

There will be no direct benefit to the subject for participating, other than information that may be learned during their physical examination about their overall physical status. The primary benefit from the study will be to add to the scientific knowledge about whether exposure to air pollutants induces epigenetic changes.

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.

Risk and Procedures for Minimizing Risks of Diesel Exhaust Exposure

- Diesel Exhaust Exposure: The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban area. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if

it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City. We have seen in IRB-07-0190 that exposure to 300 $\mu g/m^3$of diesel exhaust does not cause adverse health effects.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. In the pilot phase of study #07-0190 (Haiyan Tong MD, Ph.D, PI), subjects reported "moderate" eye irritation following diesel exhaust exposure and the symptom was resolved within a few hours after the exposure. Subjects will be provided with the choice to wear chemical safety goggles during exposures to prevent or minimize the eye irritation. 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 health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

Risk and Procedures for Minimizing Risks of Ozone Exposure

Potential risks may likely 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. It is likely that most subjects participating in this study will experience some degree of tracheobronchial irritation and cough.

Risks and Procedures for Minimizing Risks of Venipuncture:

Insertion of the needle may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.

Risks and Procedures for Minimizing Risks of Bronchoscopy:

The most significant risk to a volunteer in this protocol involves bronchoscopy. Over 1000 procedures have been performed within the EPA Human Studies Facility over the past 15 years. From January 1, 1986 through December 30, 1995, 736 procedures were performed in normal nonasthmatic subjects, and these have been reported in the Journal of Bronchology 1998; 5:185-194. The overall complication rate was less than 1% (actually less than 0.1%). The most frequent complication was a low grade fever. Bronchoscopy with BAL can be associated with more significant complications, including hypoxemia, cough, wheezing, and post-bronchoscopy fever. These may be increased in asthmatic subjects, and some investigators have found hypoxemia to be more severe in asthmatics

compared to normal subjects (Djukanović, R., J. W. Wilson, C. K. Lai, S. T. Holgate, and P. H. Howarth. 1991. The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am. Rev. Respir. Dis.* 143: 772-777), while others have not (Van Vyve, T., P. Chanez, J. Bousquet, J. Y. Lacoste, F. B. Michel, and P. Godard. 1992. Safety of bronchoalveolar lavage and bronchial biopsies in patients with asthma of variable severity. *Am. Rev. Respir. Dis.* 146: 116-121); Anonymous. Workshop summary and guidelines: investigative use of bronchoscopy, lavage, and bronchial biopsies in asthma and other airway disease. *J Allergy Clin Immunol* 88:808-14, 1991.;
Jarjour NN, Peters SP, Djukanovic R, Calhoun WJ. Investigative use of bronchoscopy in asthma. Am J Respir Crit Care Med 157:692-97, 1998.) Furthermore, hypoxemia is rarely severe enough to require premature termination of the procedure and can usually be treated or prevented by supplemental oxygen (Van Wyre).

Bronchoscopies are performed by experienced physicians who are either Board-certified or Board-eligible in pulmonary medicine. The physician performing the bronchoscopy is always assisted by at least one nurse familiar with the procedure. The subject's vital signs and ECG are continuously monitored during the bronchoscopic procedures and at regular intervals during the recovery phase. The procedure is immediately terminated if the subject experiences any alterations such as tachypnea, depressed respiration, tachycardia, bradycardia, abnormal rhythms or significant changes in blood pressure. As indicated earlier, a fully equipped emergency cart including a defibrillator, endotracheal intubation equipment and emergency medications (atropine, epinephrine) is available at all times. An on-site physician is always available to respond and the UNC Memorial Hospital Emergency Room is within 1/4 mile of Human Studies Facility. Before the subject is sent home, he/she receives a brief physical examination by a physician and is given phone numbers and a pager number with which to contact a physician should he/she experience any problems or have any questions.

The complication rate during medical bronchoscopy is approximately 0.08 to 0.12% for all events, including low oxygen saturation, respiratory embarrassment, myocardial infarction, or death. The medical screening of the subjects is specifically designed to exclude subjects with a history of medical problems which might put them at risk from the particle exposure and bronchoscopic procedures. Medical problems which could arise as a result of bronchoalveolar lavage and brush biopsy include the following:

Discomfort of the nose and throat is a primary risk of bronchoscopy. As outlined, the lidocaine gargle, spray and liquid is used to anesthetize these areas prior to passing the bronchoscope to reach the lower airways. The procedure will be discontinued if adequate anesthesia cannot be achieved by the means described.

Low-grade fever (38-38.5 °C) can occur in approximately 25 % of hospitalized subjects undergoing bronchoscopy. The fever invariably resolves within 18 hours without treatment or with acetaminophen. Our experience at HSD, EPA has shown that this fever and the associated malaise can almost always be prevented by administering ibuprofen following the procedure. The subject will be asked to contact the physician who performed the procedure or one of the nurses at the Medical Station if fever persists or is higher than 38.5 °C. As part of the standard protocol, a nurse or physician will contact the subject by telephone between 24 and 48 hours after the procedure to inquire on the general health of the subject and specifically about the presence of fever.

Cough is a common, albeit minor, problem encountered during bronchoscopy. Cough results from mechanical stimulation of cough receptors in the airway by the bronchoscope or the cytologic brush used for the brush biopsies. In addition to the discomfort, prolonged coughing with the bronchoscope in place can result in mild mechanical trauma to the vocal cords. Lidocaine solution is passed through the bronchoscope channel at the main carina and at the level of the right mainstream bronchus to suppress the cough reflex during the procedure, more can be used as needed. The procedure will be terminated if adequate cough suppression cannot be attained.

Epistaxis is caused by trauma to the nose by the bronchoscope. This condition is expected to be minor and resolve on its own. Small streaks of blood in nasal secretions may be present for up to 12 hours following the procedure. If the bleeding becomes moderate or severe during bronchoscopy, the procedure will be terminated and the bronchoscope removed. The subject's anterior nasal passage will be packed with sterile gauze to stop the bleeding. If the bleeding does not resolve with packing, the subject will be transferred to the UNC Hospital Emergency Room.

Lidocaine use presents a small risk to the subjects, as a variable fraction of the medication is absorbed through the airway mucosa. If a significant amount of lidocaine is absorbed, adverse reactions such as bradycardia, hypotension, urticarial reactions, confusion, lightheadedness, euphoria, tremors and seizures can result. We take particular precautions to avoid the possibility of overdose of topical lidocaine and limit the total amount of lidocaine instilled into the airways to a maximum of 360 mg. Also, subjects will be screened by history prior to the procedure for known allergies to lidocaine or other similar topical anesthetics.

Bronchospasm, manifested as wheezing, chest tightness or dyspnea, is a risk of bronchoscopy that is caused by stimulation of irritant receptors in the airways by the bronchoscope. This risk is minimized by 1) only allowing persons with stable mild to moderate stable asthma to participate in the study, 2) pretreatment with inhaled bronchodilator before the procedure per request of the bronchoscopy physician and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physicians responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. In the unlikely event that the subject requires

additional treatment because the asthma attack continues, they will be transported by ambulance to the emergency room of the UNC Hospital Emergency Room.

Pneumothorax is a risk of bronchoscopic procedures such as transbronchial biopsy, peripheral lung protected brushings or peripheral lung cytology brushings. Because these procedures will not be performed, the risk of pneumothorax in this study is extremely small. There is a very small risk of a pneumothorax resulting from a endobronchial brush biopsy. The symptoms include dyspnea and chest pain. The subjects will be interviewed and examined by the bronchoscopy physician 2 hours after the procedure with these symptoms in mind. If a pneumothorax is suspected, the subject will be transferred to the UNC Hospital Emergency Room by ambulance for further evaluation and treatment.

Bleeding in the lower airway may occur from trauma caused by the bronchoscope or by brush biopsy. Bleeding is typically very minor, in the order of 1/10 cc, is not clinically significant in an otherwise healthy subject and should spontaneously resolve in a matter of minutes. Epinephrine, diluted 1/20,000 fold will be administered through the bronchoscope to the affected site if bleeding is minor to moderate. The maximum dose of epinephrine used will be 2-3 cc to prevent local tissue necrosis. The site will be monitored through the bronchoscope until the bleeding stops. If bleeding fails to resolve with epinephrine or if it is sufficiently severe to cause hemoptysis or hemoglobin desaturation, oxygen supplementation will be provided to keep arterial oxygen saturation above 90% and the subject will be transported to the NC Memorial Hospital Emergency Room by ambulance. If it becomes necessary, the subject can be intubated before transport.

The use of epinephrine to control bleeding can produce systemic effects resulting from mucosal absorption of the drug. These symptoms are transient and include headache, palpitations and tachycardia when the dose is greater than 1 mg and is given intravenously. Two cc of a 1/20, 000 dilution of epinephrine contains 0.10 mg of the drug, a dose that is very unlikely to induce any untoward effects. This is especially true when the drug is applied to the mucosa, since only a fraction is expected to be absorbed.

Bradycardia and/or hypotension may result from increased vagal nerve output provoked by coughing or Valsalva during the passage of the bronchoscope through the vocal cords and trachea. Atropine (0.6 mg iv) is prophylactically administered to the subjects before the start of the procedure to minimize this risk. The primary side effects associated with atropine administration is mild sinus tachycardia which typically resolves within 30 minutes as the drug is cleared from the subject's system. In rare cases (less than 1 percent) the sinus tachycardia is accompanied by hypotension.

Hypoxemia: Arterial blood oxygen saturation is continuously monitored during the procedure with a finger pulse oximeter. Subjects are supplemented with oxygen at a flow rate of 2 l/min via nasal cannula during the procedure. The oxygen flow can be increased to a maximum of 6 l/min if the arterial oxygen saturation falls below 93 %. If oxygen supplementation is not effective, the procedure will be discontinued immediately. Removal of the bronchoscope and treatment with inhaled bronchodilator should be sufficient to allow the subject's arterial oxygen saturation to return to normal. Under normal conditions, oxygen supplementation is ceased once the procedure is completed. However, supplementation will be continued if arterial oxygen saturation is below 93 % on room air. In the event that low oxygen saturation persists, if the subject cannot be weaned from the oxygen supplementation, or if it falls precipitously at any time, an emergency situation will be declared and the subject will be transported immediately by ambulance to the UNC Hospital Emergency Room. These risks are minimized during the research procedure at the Human Studies Facility, in part because premedication with sedatives (midazolam) or with opiates (demerol) (as is used in medical bronchosopy) is not required for healthy research subjects. Avoiding these drugs reduces procedure risk and allows the participants to enjoy a normal day after leaving the EPA Human Studies Facility.

The risk of pneumonia as a result of bronchoalveolar lavage in the lobe involved in the procedure is less than 1%. Symptoms of pneumonia could include fever, dyspnea, persistent cough, productive cough and chest pain. The subject will be instructed to call the physician who performed the procedure or the Medical Station if these symptoms occur. The medical staff will evaluate the subject over the phone between 24 and 48 hours after the procedure for signs of pneumonia.

The subject will be urged to contact the Medical Station or the bronchoscopy physician should he/she experience any of the following symptoms: 1) Epistaxis, 2) Persistent cough, 3) Hemoptysis, 4) Chest pain, 5) Dyspnea, 6) Wheezing, 7) Sputum production, 8) Hoarseness or sore throat.

Confidentiality Risk

Risk of breach of confidentiality is minimal. For the mild asthmatic subject, it is conceivable that an employer or insurance company could learn of the diagnosis if the subject requires medical intervention as a result of participating in the study. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files which may contain the subject's name or screening number are secure in the EPA building which has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, spirometry) will be discussed with the subject and the

subject will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data 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 changes in epigenetic mechanisms in bronchial epithelial cells can be linked to physiological changes in healthy and mild/moderate asthmatic volunteers safely exposed to low levels of O3 and diesel exhaust. The study will follow a randomized, repeated measures design with each subject exposed to clean air, diesel exhaust and ozone. The data analysis will be focused on changes in lung function measurements, primarily FVC and FEV_1, and secondarily, HRV measurements between pre and post 2hr exposure for each exposure and lung cell composition changes. Also blood endpoints include inflammatory factors (eg IL-6), blood clotting factors (eg fibrinogen), and susceptibility factors (eg specific genotypes associated with susceptibility to air pollution).

In evaluating exposure effects of O3 and DE 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 (Folinsbee, Bedi et al. 1985) for a constant acute exposure to 0.3 ppm O_3 versus filtered air. In 22 subjects, the pre exposure FEV_1 was 4.608 ± 0.514 (mean $\pm$ SEM) in FA exposure and 4.656 ± 0.592 in O_3. The post exposure FEV_1 was 4.64 ± 0.476 in FA 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 the required sample size for each group is 30 subjects. We will initially recruit 30 subjects for each group (healthy and mild/moderate asthmatics) for this study to ensure the statistical power that can analyze small differences expected from low concentration exposures.

Other Exploratory Observations:
We hypothesize that subjects harboring polymorphisms in antioxidant genes will have increased responses to diesel exhaust and ozone compared to subjects who do not. In order to address this we will use collected cell samples for in vitro exposure experiments

Exploratory endpoints will include the effect of diesel exhaust and ozone on changes in: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values.

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, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data**. Are the identifiers in A.4.9 above linked or maintained with the research data?

_X_ yes __ no

A.4.11. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in a locked file cabinet in the Medical Station at the U.S. EPA 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 a day for seven days a week

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

_X_ No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission.** Please check all that apply.

For electronic data stored on a desk top computer:
_X_ Secure network _X_ Password access __ Data encryption__ Password protected file(s)
__ Other comparable safeguard (describe):

For portable computing devices/external storage devices (e.g. laptop computer, PDA, CDs, memory sticks):
__ Power-on password __ Automatic log-off __ Data encryption __ Password protected file(s)
__ Other comparable safeguard (describe):

For hardcopy data (including human biological specimens, CDs, tapes, etc.):
__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office _X_ Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials.** Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples. Subjects at any time may request that their samples be no longer stored in the repository. Any analysis in progress at the time of the request or already performed prior to the request being received by the researcher will continue to be used as part of the research study. Once the researcher is notified, the subject's specimens will be destroyed.

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.*

The subject will be given an opportunity to read the consent form. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *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 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 __ yes __ no

Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).*
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Subjects for U.S. EPA Studies (95-EPA-66). Typical methods for recruiting may include mass mailings, fliers and print ads. 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. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of this study will be approximately eighteen months. Subject recruitment and screening is expected to be continuous throughout the study until the intended number of subjects is reached.

Scheduling constraints imposed by concurrent studies in the U.S. EPA Human Studies Division are expected to limit the rate at which subjects can be exposed to 1-2 per week.

The physical examination evaluation may take up to one hour. If the subject is eligible for the study, the individual may have up to nine more visits to the research facility over approximately 6 months. A physical examination for bronchoscopy will be performed. The training day (Day 1) visit will require approximately 2 hours. Exposure day (Day 2) will last approximately 6 hours. Subjects will report to the facility at no sooner than 8 AM. The morning following the exposure, the subject will return for Day 3 which a follow-up visit which may last approximately 4 hours. Subjects will report to the facility at no sooner than 8 AM. Subjects will then return and repeat the Day 2-3 visits with the second and third exposure session. Exposure sessions will be separated by a minimum of two weeks.

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

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators for involuntary reasons after enrollment in the study but prior to completion will be paid for their participation up to that point

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and for any procedures cancelled up to a maximum of $100. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study:
Subjects will be paid approximately $12 per hour for participation in this study. If the subjects complete all three exposures with bronchoscopy, the total compensation including prestudy qualifications will be approximately $2516.

Pre-study qualifications

recruitment screening	$15	Previously paid
physical exam	$15	Previously paid

bronchoscopy physical exam	$20
	Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24
	Training Day Total = $ 44

Subjects will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure
Endobronchial Brush biopsies	$ 25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus	**$100**

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures	**$1548**
TOTAL for completion of THREE exposures (including completion bonus)	**$2422**

If a subject begins but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, they will receive compensation for participation up to that point.

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

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***This section applies even if records are only used to identify potential subjects.***

→ ***If your study does not use existing data, records or specimens for any purpose, 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
If applicant was involved in the original collection, please explain role:

__ 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, do you have permission from the custodians of the data, records or human biological specimens (e.g., pathology dept, tissue bank, original researcher)? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 24 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE (not present)
1 = TRACE/NOTICED (barely detectable)
2 = MILD/LIGHT (present, but not annoying)
3 = MODERATE (present, but somewhat annoying)
4 = SEVERE/HEAVY (present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4

THIS CONSENT FORM IS APPROVED ONLY
BETWEEN 6·3·10 9·27·10
INSTITUTIONAL REVIEW BOARD UNC-CHAPEL HILL

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

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IRB Study # 09-1625
Consent Form Version Date: June 3, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D, David Diaz-Sanchez, Ph.D., and Kelly Duncan
UNC-Chapel Hill Department: US EPA
UNC-Chapel Hill Phone number: 919-966-7320
Email Address: jardim.melanie@epa.gov
Co-Investigators:
Funding Source and/or Sponsor: EPA funds

Study Contact telephone number: 919-966-7320
Study Contact email: jardim.melanie@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 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."

The purpose of this particular repository or biobank is to:

- Document changes in lung function (spirometry) and the presence of mild inflammation in the respiratory tract (measured by bronchoscopy to collect cells from the lower respiratory tract) after each exposure.

- Determine if air pollution exposure can affect components of the blood involved in inflammation and clotting.
- See if air pollution particles can cause changes in the ability of the nervous system to correctly regulate one's heart rate.
- Store samples of respiratory cells and blood samples. These collections are part of the protocol described in the accompanying consent form **Consent to Participate in a Research Study** and the components stored represent excess tissues that are not immediately required for the study but may have value for future research.
- Cells from the lung will be used to identify the expression of a panel of inflammation- and oxidative stress-related genes. As new genes are identified that may be associated with adverse health effects of air pollutant exposure, expression of these genes may also be investigated.
- Research reports have suggested that people with particular genes are more susceptible to air pollutants. The epithelial specimens will be genotyped for those specific genes related to adverse health effects of air pollutant exposure.
- Studies of gene expression represent one type of genetic research, thus future investigations involving these specimens are intrinsically of a genetic nature.

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

How will the specimens be collected?
Samples will be collected in the following manner.

- Lung cell collection. Cells will be collected from the airways and airspaces of the lung by bronchoscopy with bronchoalveolar lavage and brush biopsy. (This procedure is explained in separate consent.) Fluid will be collected into specimen tubes and placed on ice. The cells will be separated from the fluid by centrifugation (very fast spinning whereby the cells will separate from the liquid and fall to the bottom of the tube), and both samples may be processed immediately, or frozen for later analysis.
- Blood collection. You will have up to 80 cc (about 5 tablespoons) of blood per sample collected from an arm vein by our trained nursing staff. The blood will be placed into specially designed blood collection tubes which allow separation of the fluid and cell portions of blood. Those samples may be processed immediately, or be frozen for later analysis.

What will happen to the specimens?
Samples will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill, North Carolina. Only project members of the study will have access to the samples. Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the air pollution exposure. Specimens may be released to other investigators if there is a need to analyze samples with equipment that is not located at our facility.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding asthma and how environmental factors affect the disease

What are the possible risks or discomforts involved with the use of your specimens?
This study might involve the following risks and/or discomforts to you:

Risk of breach of confidentiality is minimal. For the mild asthmatic subject, it is conceivable that an employer or insurance company could learn of the diagnosis if you required medical intervention as a result of participating in this study. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

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

Will you receive anything for the use of your specimens?
You will not receive anything for taking part in this research.

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

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

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, U.S. EPA and/or 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.

You will not be identified in any report or publication about research using your specimens. 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 could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

A Federal law called the Genetic Information Nondiscrimination Act (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. GINA does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. GINA also does not protect you against discrimination based on an already-diagnosed genetic condition or disease.

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants. In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive results from research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you personally. There are no plans to re-contact you or other subjects with information about research results.

Can you withdraw the specimens from the research repository?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. It is best to make your request in writing.

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

What will happen if you are injured by this research?

All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

Who is sponsoring this research?

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 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.

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

6-3-10 9-27-10

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study and Consent for Genotype Screening
With Identifying Information
Subjects 18 years of age or older
Biomedical Form

IRB Study:# 09-1625
Consent Form Version Date: June 3, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D., David Diaz-Sanchez, Ph.D. and Kelly Duncan, Ph.D.
Department: United States Environmental Protection Agency
Phone number: 919-966-7320

Co-Investigators: Michael T. Schmitt, MSPH

Sponsor: U.S. Environmental Protection Agency

You are being asked to take part in a research study. The investigators listed above are in charge of the study; other professional persons may help them or act for them.

What are some general things you should know about research studies?

Research studies are designed to gain scientific knowledge that may help other people in the future. You may not receive any direct benefit from participating. There may also be risks associated with participating in research studies.

Your participation is voluntary. You may refuse to participate, or may withdraw your consent to participate in any study at any time, and for any reason, without jeopardizing your ability to participate with EPA-sponsored research.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want to participate. You will be given a copy of this consent form. You are urged to ask the investigators 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 research study is to understand how ozone exposure compares to that of diesel exhaust exposure in order to investigate mechanisms of injury by environmental pollutants. This may be especially important for asthmatics, because studies have shown an association between exposure to air pollution and increased medication usage and emergency room visits in this population. To accomplish this we will:

- Document changes in lung function (spirometry) and the presence of mild inflammation in the respiratory tract (measured by bronchoscopy to collect cells from the lower respiratory tract) after each exposure.
- Determine if air pollution exposure can affect components of the blood involved in inflammation and clotting.
- See if air pollution particles can cause changes in the ability of the nervous system to correctly regulate one's heart rate.
- Cells from your airways will be collected and stored. All specimen collections will be stored throughout the course of this study and then any remaining samples will be stored indefinitely as described in an accompanying consent form ***Consent for Storing Biological Specimens <u>With</u> Identifying Information.***
- The cell samples will be used to identify the expression of a panel of inflammation- and oxidative stress-related genes. As new genes are identified that may be associated with adverse health effects of air pollutant exposure, expression of these genes may also be investigated.
- The cell samples will be used to identify epigenetic changes (mechanisms which control the expression of genes) associated with air pollutant exposure.
- Research reports have suggested that people with particular genes are more susceptible to air pollutants. The cells will be studied for those specific genes related to adverse health effects of air pollutant exposure.

<u>Are there any reasons you should not participate?</u>

You should not participate in this study if:

- You are pregnant, attempting to become pregnant or nursing an infant
- You have a history of significant chronic illness other than asthma, or your asthma is not well-controlled
- You are not 18 to 55 years of age
- You cannot perform intervals of moderate exercise
- You are not fluent with the English language
- You are taking more than 81 mg aspirin regularly
- You are unwilling or unable to refrain from the following medications for the week prior to the study: anti-inflammatory agents such as ibuprofen (Advil), naproxen (Aleve, Naprosyn) or aspirin
- You have skin allergy to tape or electrodes
- You are currently taking or have taken anti-coagulant medication in the week prior to the study
- You are currently smoking or have a smoking history within 2 years of the study
- You have been diagnosed as a severe asthmatic as defined by physician-directed emergency treatment for an asthma exacerbation within the preceding 12 months, use of systemic steroid therapy within the preceding 12 months, nighttime symptoms of cough or wheeze greater than 2 times per month, asthma symptoms (cough, wheeze, chest tightness) more than 2 times per week, requirement for albuterol due to asthma

symptoms more than 2 times per week, (not including prophylactic use of albuterol prior to exercise), have chronic and continuous allergic rhinitis.

- Your dosing level of an inhaled steroid is not consistent with the severity of your asthma as outlined by the NHLBI NAEPP guidelines
- You have a history of fainting in response to pain
- You currently have or have had a viral respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of the study. If this is the case, your visit will be rescheduled
- You currently have or have had seasonal exacerbation of allergic rhinitis symptoms within 1 weeks of the study. If this is the case, your visit will be rescheduled

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

- No over the counter pain medications such as aspirin (aspirin $\leq$ 81 mg/day is allowed), Advil, Aleve or other non-steroidal anti-inflammatory medications for 7 days prior to all visits. Tylenol (acetaminophen) is permitted.
- No vitamin C or E (or multivitamins which contain Vitamins C or E) for 7 days prior to all 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.
- Avoid eating or drinking anything after midnight on the evening of the Exposure Day 1, before the bronchoscopy to take place on Exposure Day 2.
- No caffeine for 12 hours prior to all study visits.
- Avoid use of antihistamines for one week prior to exposures.

How many subjects will participate in this study?

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

How long will your participation last?

Your participation in this study may require up to 9 visits to the research facility over approximately 6 months if you are eligible for the study.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone a general screening visit and physical examination, and a physical exam and pulmonary function test for bronchoscopy to certify that you are a suitable candidate. For the exposure study, we will perform the following procedures:

Training Day

- Report to the EPA medical station at the requested time.
- 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 this consent form. We will give one copy to you. We will check your vital signs (heart rate, respiratory rate, blood pressure, oxygen saturation level and a symptom questionnaire) and do a pregnancy test if applicable(WOMEN ONLY) For the pregnancy test, you will be asked to provide a urine sample. The purpose of this test is to provide evidence that you are not pregnant at the time of the study. The nurse will also ask you when your most recent menstrual cycle began. The results of the pregnancy test will be held in strict confidence. A positive pregnancy test will preclude further participation.
- You will undergo a breathing test. This is known as spirometry, and 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. The information gained from the breathing tests will be used by the investigators to determine whether you qualify for the study.
- You will be trained on the exercise bike and the work load to elicit a minute ventilation normalized for body surface of $25L/m^2/BSA$ will be determined.

Exposure Day 1: You will be asked to eat a normal breakfast and arrive at the EPA medical station no sooner than 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level) and fill out a symptom questionnaire. Have a pregnancy test if applicable.
- Have monitors placed on you so that we can watch your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on for 24 hours. Before exposure, after exposure and the following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate. This will consist of a 20 minute rest period and a 10 minute data collection. The monitor will be removed after the last data collection. These monitors will allow us to determine if air pollutants cause small changes in the ability of your nervous system to regulate how your heart beats.
- Have up to 80 mL blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust or ozone exposure affects the ability of your blood to clot correctly, and to look at changes in proteins and/or blood cells.
- 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.
- Given a Symptom Questionnaire for you to fill out.

- Testing will be postponed if it is determined that your medication history is not within previously established limits, respiratory tract illness has occurred within the previous 6 weeks or if respiratory symptoms are present.

During the exposure, you will:

Undergo a chamber exposure of 2 hours duration, once to clean air, once to diesel exhaust and once to ozone; the amount of pollution you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. You will be blinded as to the exposure condition. The exposure chamber is 6 ft wide by 6. ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. You will exercise for 15 minutes at the previously determined pace and rest for 15 minutes. While exercising, minute ventilation may be measured intermittently. Your blood pressure may also be measured intermittently. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid for your participation up to that point, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Following the exposure, you will:

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have your vital signs checked.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Fill out a symptom score sheet.
- Have blood drawn (up to 80 mL or about 5 tablespoons).
- Be assessed and discharged by the nursing staff.

Exposure Day 2 (24 hour follow up visit): You will come back the next morning and asked to arrive at the EPA medical station at no sooner than 8 am. (approximately 24 hours after exposure) You will:

- Have your vital signs taken.
- Fill out a symptom questionnaire.
- Have blood draw (up to 80 mL or about 5 tablespoons).
- Have spirometry testing.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Have the Holter monitor removed.
- We are asking all subjects to have bronchoscopy on the day following each exposure. Prior to the bronchoscopy you may be pretreated with inhaled bronchodilator (albuterol). The bronchoscopy will not be performed if the baseline lung function shows more than a minimal amount of airway obstruction and this does not resolve

with inhaled bronchodilator. During the bronchoscopy, a fiberoptic scope will be inserted through the nose into one of your airways where a portion of one of your lungs will be washed with sterile saline and a sample of airway lining cells will be obtained with a special brush device.

Bronchoalveolar Lavage (BAL) and Brush Biopsy is frequently used in the diagnosis of lung disease in both adult and pediatric patients. The procedure has also been used in numerous research studies of persons with respiratory disease and in healthy volunteers. The procedure has been used safely many times in studies conducted at our laboratory. The purpose of the BAL procedure is to obtain fluids and cells from the lower regions of the respiratory tract, i.e., the trachea, bronchi and smaller airways. These materials will be analyzed to obtain information about the role of various chemical substances and cells in the pulmonary response to particle exposure of persons with asthma. The bronchoscope is a highly flexible fiberoptic tube that is about two feel long and 1 centimeter in diameter (about the diameter of a pencil). It is an optical device with a light at the end which can be used to transmit images to a camera connected at the other end. Using the bronchoscope, the physician can see into your airways and direct the placement of the bronchoscope. A small channel allows fluid to pass through the bronchoscope.

The BAL procedure will be performed at the laboratory medical station by specially trained experienced pulmonary physicians. You will be expected at the medical station at 8:00 on the morning after the exposure. If you have had anything to drink or eat since midnight the night before the bronchoscopy you will not be allowed to proceed. Before you undergo bronchoscopy, your lung function will be evaluated and you may be treated with inhaled bronchodilator (albuterol). The procedure will not be performed if your lung function does not exceed a predetermined level of function recommended by the National Institute of Health for performing research bronchoscopies in persons with asthma. You may terminate the bronchoscopy procedure at any time. If the physician deems that your are too uncomfortable or anxious, the procedure will be terminated.

A saline lock intravenous catheter will be placed in a vein in your arm to give you medication before the procedure starts (a saline lock is a flexible catheter that stays in your arm for a short time). The saline lock will remain in place so that it can be used to administer medications in case there are any problems during the procedure. You will also be connected to a telemetry monitor that will display heart rate and rhythm, a blood pressure cuff will be placed on your arm, and an oximeter sensor (small band like device will be placed on a finger to allow the medical staff to monitor you during the procedure. No sedatives and/or narcotics will be administered at any time during bronchoscopy.

Before proceeding, the medical staff will again make certain that you have had nothing to eat or drink since midnight the previous night. You will be asked to decongest your nose by administering 2 puffs of neosynephrine nasal spray to each nostril. They will then give you a lidocaine solution and ask you to gargle with it for a few seconds to anesthetize your throat. You will then be asked to inhale (snort) a small amount of lidocaine jelly through one nostril to anesthetize your nose and the back of your throat. A Q-tip with lidocaine jelly will be gently inserted into your nose to ensure

that your nose is completely numb before the bronchoscope is inserted. The procedure will not begin until your nose and throat are well anesthetized. If this cannot be accomplished, the bronchoscopy will not be performed. A tube delivering oxygen will be placed inside your other nostril. Delivery of supplemental oxygen is done as a precaution during all bronchoscopies conducted at our facility.

To start the procedure, the physician will pass the bronchoscope through your anesthetized nostril to the back of your throat and then to above your vocal cords. He will then inject a lidocaine solution to numb your vocal cords before passing the bronchoscope into your trachea (windpipe). More lidocaine, up to a safe maximum dose is injected at various points in your trachea and airways to minimize coughing during the procedure. You will experience some cough during the procedure. This is a normal reflex caused by the presence of the bronchoscope in your airway.

The bronchoscope will be gently wedged in an airway in the right lung and sterile saline will be injected in your lung through a channel in the bronchoscope. The saline will then be gently suctioned from your lung through the channel in the bronchoscope. This sequence will constitute a wash. Up to 2 washes will be performed. A total of 50 cc (about 4 tablespoons) of sterile saline will be used during the BAL procedure. Approximately 75% of the saline injected into your lungs will be recovered by aspiration (suction) through the bronchoscope. The remaining 25% (12.5 cc) is expected to remain in your lungs. The saline left in your lungs will not generally cause you any difficulty breathing or harm you and should be completely absorbed by your lungs within 48 hours.

After the BAL is performed, brush biopsies of your airway epithelium will be taken. A very small brush (3 millimeters in diameter) will be inserted through the channel in the bronchoscope. The brush is visible to the physician performing the procedure through the bronchoscope. Small amounts of surface cells are scraped from the airway by gently brushing the airway several times. Up to 8 brush biopsies are taken at one site in the right lung. After the brush biopsies are obtained, the procedure will be complete and the bronchoscope will be removed from your airway. The total time the bronchoscope will reside in your airways will be 15-30 minutes.

After the procedure the oxygen cannula will be removed if your oxygen saturation is acceptable. The nurses will check your vital sign immediately after the procedure and every half hour thereafter until discharge. During this time you will sit in a recliner at the medical station for an observation period of approximately two hours. You will not have any food or drink during this time. After the recovery period, the nurses will check your gag reflex. Since the gag reflex will be absent due to anesthesia during the procedure, you will not be allowed to ear or drink until the anesthesia wears off. This normally takes about one to one-and-one half hours. Once your gag reflex returns, you will then be given some juice to sip and then some crackers.

The physician who performed the procedure will check you after the recovery period and you will repeat the spirometry lung function test. You will be discharged if your vital signs are stable, chest examination is normal, and lung function is greater than 80% of your pre-procedure value. Prior to discharge you will be requested to take 600 mgm of ibuprofen by mouth; administration of ibuprofen/motrin almost always prevents the post-bronchoscopy malaise and low grade fever that would otherwise

occur in about 25% of persons undergoing the procedure (acetaminophen/tylenol is a less effective alternative medication).

Before going home, you will be give the phone number of the medical station (966-6232) and pager number to the physician who performed the bronchoscopy with instructions to call if you experience any adverse symptoms such as: 1) persistent fever or fever above 101 degrees Fahrenheit, 2) persistent cough, 3) sputum (phlegm) production, 4) chest pain, 5) coughing up any amount of blood, 6) nose bleeds, or 7) shortness of breath

Are there any reasons you should not participate in bronchoscopy?

You should not participate in this study if you have problems with excessive bleeding after minor cuts or abrasions. You should not participate if you have had a recent exacerbation in your asthma that was not controlled with your usual medications. The physician and medical staff will explain other exclusionary condition in detail to you. In addition, during your participation in this study, you will be asked to refrain from, limit, or control taking the following medications:

*No more than 81 mg aspirin or similar medication on a regular basis or during the week of testing.
*Withhold antihistamines for 7 days prior to testing.
*Withhold inhaled bronchodilators 12 hours prior to testing.
You should not participate in this study if you are unwilling or unable to remain in the local area for 24 hours following each bronchosocpy

With your permission, DNA from the collected cells will be genotyped for specific genes related to adverse health effects associated with air pollution exposure. Unwillingness to have samples genotyped will NOT exclude you from participating in the study. If you do not wish for your cells to be used for genotyping but do wish to participate in the study do not sign the section ***Subject's Agreement for Genotype Screening With Identifying Information*** located at the end of this document.

What if we learn about new risks 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.

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. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely. With your permission, we will continue to store any samples that we collect to possibly use for other studies. This allows us to make the best use of the samples we collect from our volunteers. Again, you do not have to allow us to keep these samples if you do not want to.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study. We will share with you any information

that we learn about your lung function or other medical findings. We can also send you the results of some of the blood tests we perform.

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

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

2. 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. Also, the forceful breathing during this test can cause wheezing or shortness of breath to occur.

3. The adhesive patches used as part of the heart monitoring system may cause some mild skin discomfort or discoloration. If this occurs you should notify the nursing staff.

4. During air pollution exposure, you may experience some minor degree of airway irritation, cough, shortness of breath or wheezing. Some studies suggest that persons with asthma may be at risk for developing an asthma attack as a result of exposure to fine air pollution particles. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. Your symptoms will be closely monitored during the exposure and the study terminated if more than modest respiratory symptoms occur during the exposure. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. If you experience a significant drop in your lung function, albuterol will be administered.

Air pollution may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. There is a small possibility that you could have more asthma symptoms for several days, and even less likely, for several weeks, after the particle exposure. This flare in your asthma would be similar to what happens to you and other asthmatics during the allergy season or with a viral infection. Such a flare is considered highly unlikely and the exposure risk is comparable to walking around a major metropolitan area such as Los Angeles or Houston on a smoggy day. If an asthma flare occurs, you will be asked to seek medical treatment from your regular physician who treats you for your asthma.

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber when you are first evaluated for suitability

for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 6 ft in width, 6 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator at all times.

5. Bronchoscopy: As with other medical procedures, there are risks associated with bronchoscopy. However, experience has shown these risks are extremely small in research subjects. The risks associated with bronchoscopy are described in detail below.

A primary risk is bronchoscopic procedures is **discomfort to the nose and throat** brought about by the insertion of the bronchoscope through the nasopharynx. This discomfort should be alleviated or eliminated by the use of local anesthetics such as lidocaine. For this reason, you will be asked to gargle with a lidocaine solution and have lidocaine jelly placed in your nose prior to beginning the procedure. The effectiveness of the anesthesia will be tested by using a cotton swab gently inserted into your nose before inserting the bronchoscope. If you experience pain or discomfort during insertion of the bronchoscope, more lidocaine will be used up to a safe maximum amount. If adequate anesthesia cannot be achieved using this amount of lidocaine, the procedure will not take place.

A second risk of bronchoscopy is **coughing** that results from the irritation of the airway caused by the presence of the bronchoscope in the airway. Coughing is a normal reflex to expel foreign substances from the airways. Because coughing itself can cause mild trauma and discomfort to the airways and vocal cords, lidocaine liquid is sprayed on the airways at various sites to suppress the cough reflex. This technique is usually not completely successful and it is expected that you will have some cough during the procedure, especially during the initial insertion of the bronchoscope into the airway. If coughing is not controllable with the lidocaine or causes you discomfort, the procedure will be terminated.

There is a slight chance that the bronchoscopic procedure will precipitate an **asthma attack**. This risk is minimized by 1) only allowing persons with mild stable asthma to participate in the study, 2) pretreatment with inhaled bronchodilator (albuterol) before the procedure if your lung function is impaired and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physician responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. You will not be discharged from the EPA Medical Station until your lung function has returned to 80% of your pre-procedure performance. . In the unlikely event that you require additional treatment because the asthma attach continues, you will be transported by ambulance to the emergency room of the UNC Hospital, located 1/4 mile away.

Nose bleeds are another risk of bronchoscopy. This is caused by trauma to the nose during the bronchoscopy procedure. Usually, the bleeding is minor and is noticed as a few drops of blood in mucous secretions in the nostril. The bleeding

usually stops on its own within an hour after the bronchoscopy. On very rare occasions, if the bleeding becomes moderate to severe, your nostril will be packed with sterile gauze to absorb the blood and put pressure on the site of bleeding. Should you require additional treatment, you will be transported to the UNC Hospital Emergency Room. Major nose bleeds during bronchoscopy are very rare and generally limited to persons with kidney disease or a blood clotting disorder. It could also be promoted by chronic use of aspirin (>81 mg), ibuprofen or similar medications. You will not be allowed to participate in this study if you are taking such medications. Therefore the probability of getting a major nose bleed is very small.

Major **bleeding of the airways** is another rare (less than one percent) complication of bronchoscopic procedures. Most episodes of major bleeding due to bronchoscopy occur in persons with underlying predisposing conditions and are due to forceps biopsies of the airways mucosa which will **not** be performed in this study. People who suffer from kidney disease, blood clotting disorders or are chronic users of aspirin-like drugs are at increased risk of bleeding during bronchoscopy. In addition, people with lung cancer or with serious lung infection are more likely to suffer from airway bleeding during bronchoscopy. Your screening medical history, physical examination, and blood analysis will reveal most conditions that would increase your risk of bleeding during the bronchoscopy. You will not be allowed to participate if you have any of these conditions. Therefore your chances of incurring a serious airway bleed during bronchoscopy are extremely small. Nevertheless, if you do experience bleeding during bronchoscopy, you will be under the care of an experienced physician who will take steps to stop the bleeding immediately. A special cart with emergency supplies is available to handle medical emergencies. In the event of a major bleeding episode, you will be immediately transported to the Emergency Room of the UNC Hospital.

Pneumothorax or collapsed lung is another possible complication of bronchoscopic procedures. This complication is more likely to occur during tissue biopsy in the lung periphery, not the major central airways which will be the focus of this study. However you will be advised of the symptoms in the event that you experience a pneumothorax. The symptoms include chest pain and shortness of breath. If you experience these symptoms, you should contact the medical station (966-6232), or page the physician who performed the bronchoscopy. The vast majority of pneumothoraces occur within 24-48 hour of the procedure being completed. While a serious condition, a pneumothorax can be readily treated in an emergency room.

The **lidocaine** used to anesthetize your nasopharynx and reduce your cough can pose certain risks as well. A small fraction of the lidocaine applied to your nose and airways will be absorbed into your bloodstream, where it could cause adverse effects. Your risk of suffering side effects are very small, but would be much greater and potentially life-threatening if you are allergic to lidocaine or other topical anesthetics. You will not be allowed to participate in this study if you have drug allergy to topical anesthetics. When used at high doses, lidocaine can also cause symptoms in your central nervous system (confusion, tremor, euphoria, or, rarely, seizures) or heart rate disturbances (very fast or very slow heart rate) if an

excessive dose of medication is used. Finally, a death in a volunteer receiving an overdose (over 1000 milligrams) of lidocaine during bronchoscopy has been reported from Rochester, New York. However, no serious side effects of this medication have been noted at lower doses such as those described in this protocol which uses up to 360 milligrams of lidocaine during the entire procedure. If any problems develop secondary to the use of lidocaine, the physician bronchoscopist and the doctor on duty that day at the Human Studies Division of the EPA will be available to handle these problems.

Prior to the start of the bronchoscopy **atropine** may be given though a vein in your arm in order to counteract any changes in blood pressure that would result from the placement of the bronchoscope in your airway and to limit the quantity of secretions in your nose and throat. Atropine can cause dry mouth and nose for 30 to 60 minutes after the procedure is completed. This effect should wear off about an hour after the procedure is completed.

Placing an **intravenous catheter** in your arm carries a small risk as well. The needle stick can be momentarily painful and more rarely can leave a bruise in your arm. The medical staff who will be placing the catheter are very experienced and therefore the risk is minimal. Infection is also a very small risk that can result from placing the catheter. Again, the medical staff is very experienced in the use of sterile technique to prevent infection. If you notice redness or swelling in the area surrounding the catheter site, you should contact the medical station at 966-6232 or the physician on call for treatment.

A low grade fever (less than 101EF) during the 24 hours following the procedure occurs in about 25% of patients with respiratory disease undergoing bronchoscopy and BAL. The fever is benign and is usually effectively prevented by administering ibuprofen following the procedure. However, if you experience fever greater than 101 EF or lasting longer than 24 hours you could have pneumonia and should contact the Medical Station immediately so that you can be examined and treated.

Pneumonia occurs in less than 1 percent of patients undergoing bronchoscopy and BAL. Signs of pneumonia include fever greater than 101 EF, or persisting for more than 24 hours, persistent cough with or without sputum production, coughing up blood, chest pain, and shortness of breath or wheeze. Should you experience these symptoms, it is imperative that you contact the Medical Station to be seen and evaluated by a physician. As a routine precaution the Medical Station Staff will contact you between 24 and 48 hours following the procedure to inquire about any symptoms you may be experiencing.

In addition, there may be uncommon or previously unrecognized risks that might occur.

There are no risks 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 may cause some irritation, itching, or burning in some people. If this occurs you should notify the nursing staff.

The following are policies and procedures to minimize risk during the experiment:
1. During this study, skilled personnel, in an adequately equipped facility, will be observing you for any adverse effects. You may, without penalty, stop the test at any time by telling the monitor.
2. A physician will be on call at all times during the study to deal with any medical problems that may arise.
3. Every test has some degree of risk and there may be some risks associated with this experiment which are unforeseeable. You should not participate in the study if you have an acute illness.
4. You may terminate your participation in the experiment at any time and for any reason that you wish without penalty; you will be paid an amount for participation prorated for the time and procedures in which you took part. You understand that you are not under any obligation to complete any test procedures which you find objectionable or uncomfortable.
5. The investigator may terminate your participation in the experiment at any time. If this happens, you will be entitled to full payment for your time commitment and any experimental activities up to that point.
6. Your participation in the study may be postponed if your pre-exposure lung function shows airflow obstruction or if you develop a cold or asthma exacerbation.

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. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

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, the U.S. EPA and/or UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

What if you want to stop before your part in the study is complete?
You may withdraw from this study at any time. If you choose to withdraw or are unable to complete the study because of not following the instructions in this consent form, you will receive compensation only for your participation up to that point.
The investigators have the right to stop your participation at any time. This could be because you have had an unexpected reaction, have failed to follow instructions, or because the study has been stopped. After you enroll, if you are dismissed by the investigators before completing the study for reasons that are not your fault, you will be paid for your participation up to that point.
We made need to cancel your study activity due to adverse weather conditions, equipment failure, or other unforeseen events. If a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid for the time scheduled and cancelled at the standard hourly rate of $12 per hour plus any cancelled activities up to a maximum of $100. When possible, you will be rescheduled at a later time.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study. Total compensation will be based on the number of exposure sessions that will be required.

1. If you are complete all three exposures sessions of this study, your total compensation including screening will be approximately $2516.

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

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. 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. Compensation for the visits will be as follows:

You will be exposed to diesel exhaust ,ozone and have an air control exposure in three separate sessions. Exposure Days 1-2 will be repeated for the second and third chamber exposure session, but a second training Day is not needed. If you complete three exposures, your compensation including the pre-study qualifications and training day will be approximately $2516.

Pre-study qualifications

recruitment screening	$15	Previously paid
physical exam	$15	Previously paid
bronchoscopy physical exam	$20	Previously paid

Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24

Training Day Total = $ 44

You will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure

time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure
Endobronchial Brush biopsies	$25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus	**$100**

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures (excluding Prestudy qualifications and training day)	**$1548**
TOTAL for completion of THREE exposures (excluding Prestudy qualifications/training day but including completion bonus)	**$2422**

If you begin but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, you will receive compensation for your participation up to that point however in you arrive in the lab for the procedure having not followed instructions (eating food, taking NSAIDs or aspirin, or failing to report cold or flu symptoms the day prior to the bronchoscopy) you will be compensated $15 for your time, but will not receive compensation for the bronchoscopy.

You will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available.

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 or follow directions of the investigators, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and cancelled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during a current visit if cancelation occurs while you are in the facility. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled. The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you

are dismissed by the investigators prior to completion, you will be reimbursed for time and procedures completed up to that 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/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

Who is sponsoring this study?
This research is funded by the United States Environmental Protection Agency. This means that the research team is being compensated by the sponsor for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the product being studied.

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 US Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, 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 the Chairman of the IRB Committee at 919-966-1344 and /or the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What specimens will be collected and stored?
Bronchial epithelial cells collected via bronchoscopy and all blood specimens will be stored with your permission. If you agree to have your specimens stored for future research you will need to sign the accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information***.

- -

IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D., David Diaz-Sanchez, Ph.D., and Kelly Duncan, Ph.D.

Subject's Agreement to Participate in the Research Study with Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I voluntarily **AGREE** to have my provided bronchial epithelial cells genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

Signing here and agreeing to participate in this study DOES NOT allow us to store your samples.

____________________________________ _______________
Signature of Research Subject Date

____
Printed Name of Research Subject

____________________________________ _______________
Signature of Person Obtaining Consent Date

____
Printed Name of Person Obtaining Consent

IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D., David Diaz-Sanchez, Ph.D., and Kelly Duncan, Ph.D.

Subject's Agreement to Participate in the Research Study without Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I **REFUSE** to have my provided bronchial epithelial cells genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

Signature of Research Subject | Date

Printed Name of Research Subject

Signature of Person Obtaining Consent | Date

Printed Name of Person Obtaining Consent

- -

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
X	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
X	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. If adding personnel, include name, location (UNC or specific outside location), role, and email address for each person who should receive electronic copies of IRB correspondence to PI. For those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/) include documentation of required training in human research ethics.	1

1 List and describe each proposed change:

1. On pages 14-15 of the IRB protocol, we edited the bronchoscopy procedure, including the amount of wash fluid to be used and the exact location within the lung where the procedure is to take place. It was determined that more washes are needed and that the amount of total fluid should be left up to the physician performing the procedure at that time. It was also determined that the location within the lung where the procedure is performed should also be at the discretion of the pulmonologist, as one cannot always guarantee that one location is always best.
2. The consent to participate has been edited to reflect changes in the bronchoscopy procedure (page 7).
3. Minor adjustments have also been made to the participation consent form in order to clarify and/or correct certain points. These appear on pages: 5, 6 and 7

2. Is this modification being submitted in response to an unanticipated problem/adverse event or new findings? ___yes _X__no

If yes, explain, including whether these events or findings are relevant to participants' willingness to continue.

3. Do any of the proposed changes increase risk? ___yes _X__no If yes, explain.

IRB study #: 09-1625 **Date:** July 22, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie Jardim, Ph.D. **Faculty advisor:** (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

_____ _____

Signature of Principal Investigator or designee Date

_____ _____

Signature of Faculty Advisor (if applicable) Date

OFFICE OF HUMAN RESEARCH ETHICS -- Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

This application is to seek approval for a modification to a currently approved study. Any proposed changes to previously approved human subjects research must be reviewed and approved by the IRB prior to implementation. This includes modifications to the study, inclusion or exclusion criteria, recruitment methods, research personnel, or *any* new or revised study materials. Approval is required for all modifications whether initiated by the investigator or external sponsor. **This form should not be used to report violations and deviations.**

Instructions for Submitting

Include with your submission the items indicated in the list on the next page, where applicable.

Unless otherwise instructed, submit to the same IRB that previously reviewed this study. Complete submission instructions can be found at http://ohre.unc.edu/submission_instructions.php. ***All application and consent materials should be copied or printed on one side only.***

Special submission instructions apply for studies that require additional review. Examples include the General Clinical Research Center (GCRC; http://gcrc.med.unc.edu/investigators/admin/gcrcapp.htm) or the Oncology Protocol Review Committee (PRC; http://cancer.med.unc.edu/research/prc/default.asp). See their web sites for details.

Address for all Applications and Other Correspondence

IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

---- Instructions – Do not submit this page with your application ----

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: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 7/23/2010
Expiration Date of Approval: 9/27/2010

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Modification
Expedited Category: Minor Change to Previously Approved Research
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure
Sponsors: US Environmental Protection Agency - GRANTS

This submission has been approved by the above IRB for the period indicated. It has been determined that the risk involved in this modification is no more than minimal. Unless otherwise noted, regulatory and other findings made previously for this study continue to be applicable.

Submission Description:
This amendment received 7/22/10 addresses:

1. the amount of wash fluid to be used & the exact location within the lung where the procedure is to take place
2. clarifying changes to consent

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

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC: David Diaz Sanchez, Environmental Protection Agency; Robert Truckner, (EPA), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 7-23-10 AND 9-27-10
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study and Consent for Genotype Screening
With Identifying Information
Subjects 18 years of age or older
Biomedical Form

IRB Study:# 09-1625
Consent Form Version Date: July 21, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D., David Diaz-Sanchez, Ph.D. and Kelly Duncan, Ph.D.
Department: United States Environmental Protection Agency
Phone number: 919-966-7320

Co-Investigators: Michael T. Schmitt, MSPH

Sponsor: U.S. Environmental Protection Agency

You are being asked to take part in a research study. The investigators listed above are in charge of the study; other professional persons may help them or act for them.

What are some general things you should know about research studies?

Research studies are designed to gain scientific knowledge that may help other people in the future. You may not receive any direct benefit from participating. There may also be risks associated with participating in research studies.

Your participation is voluntary. You may refuse to participate, or may withdraw your consent to participate in any study at any time, and for any reason, without jeopardizing your ability to participate with EPA-sponsored research.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want to participate. You will be given a copy of this consent form. You are urged to ask the investigators 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 research study is to understand how ozone exposure compares to that of diesel exhaust exposure in order to investigate mechanisms of injury by environmental pollutants. This may be especially important for asthmatics, because studies have shown an association between exposure to air pollution and increased medication usage and emergency room visits in this population. To accomplish this we will:

- Document changes in lung function (spirometry) and the presence of mild inflammation in the respiratory tract (measured by bronchoscopy to collect cells from the lower respiratory tract) after each exposure.
- Determine if air pollution exposure can affect components of the blood involved in inflammation and clotting.
- See if air pollution particles can cause changes in the ability of the nervous system to correctly regulate one's heart rate.
- Cells from your airways will be collected and stored. All specimen collections will be stored throughout the course of this study and then any remaining samples will be stored indefinitely as described in an accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information.***
- The cell samples will be used to identify the expression of a panel of inflammation- and oxidative stress-related genes. As new genes are identified that may be associated with adverse health effects of air pollutant exposure, expression of these genes may also be investigated.
- The cell samples will be used to identify epigenetic changes (mechanisms which control the expression of genes) associated with air pollutant exposure.
- Research reports have suggested that people with particular genes are more susceptible to air pollutants. The cells will be studied for those specific genes related to adverse health effects of air pollutant exposure.

Are there any reasons you should not participate?

You should not participate in this study if:

- You are pregnant, attempting to become pregnant or nursing an infant
- You have a history of significant chronic illness other than asthma, or your asthma is not well-controlled
- You are not 18 to 55 years of age
- You cannot perform intervals of moderate exercise
- You are not fluent with the English language
- You are taking more than 81 mg aspirin regularly
- You are unwilling or unable to refrain from the following medications for the week prior to the study: anti-inflammatory agents such as ibuprofen (Advil), naproxen (Aleve, Naprosyn) or aspirin
- You have skin allergy to tape or electrodes
- You are currently taking or have taken anti-coagulant medication in the week prior to the study
- You are currently smoking or have a smoking history within 2 years of the study
- You have been diagnosed as a severe asthmatic as defined by physician-directed emergency treatment for an asthma exacerbation within the preceding 12 months, use of systemic steroid therapy within the preceding 12 months, nighttime symptoms of cough or wheeze greater than 2 times per month, asthma symptoms (cough, wheeze, chest tightness) more than 2 times per week, requirement for albuterol due to asthma

symptoms more than 2 times per week, (not including prophylactic use of albuterol prior to exercise), have chronic and continuous allergic rhinitis.

- Your dosing level of an inhaled steroid is not consistent with the severity of your asthma as outlined by the NHLBI NAEPP guidelines
- You have a history of fainting in response to pain
- You currently have or have had a viral respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of the study. If this is the case, your visit will be rescheduled
- You currently have or have had seasonal exacerbation of allergic rhinitis symptoms within 1 weeks of the study. If this is the case, your visit will be rescheduled

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

- No over the counter pain medications such as aspirin (aspirin $\leq$ 81 mg/day is allowed), Advil, Aleve or other non-steroidal anti-inflammatory medications for 7 days prior to all visits. Tylenol (acetaminophen) is permitted.
- No vitamin C or E (or multivitamins which contain Vitamins C or E) for 7 days prior to all 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.
- Avoid eating or drinking anything after midnight on the evening of the Exposure Day 1, before the bronchoscopy to take place on Exposure Day 2.
- No caffeine for 12 hours prior to all study visits.
- Avoid use of antihistamines for one week prior to exposures.

How many subjects will participate in this study?

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

How long will your participation last?

Your participation in this study may require up to 9 visits to the research facility over approximately 6 months if you are eligible for the study.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone a general screening visit and physical examination, and a physical exam and pulmonary function test for bronchoscopy to certify that you are a suitable candidate. For the exposure study, we will perform the following procedures:

Training Day

- Report to the EPA medical station at the requested time.
- 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 this consent form. We will give one copy to you. We will check your vital signs (heart rate, respiratory rate, blood pressure, oxygen saturation level and a symptom questionnaire) and do a pregnancy test if applicable(WOMEN ONLY) For the pregnancy test, you will be asked to provide a urine sample. The purpose of this test is to provide evidence that you are not pregnant at the time of the study. The nurse will also ask you when your most recent menstrual cycle began. The results of the pregnancy test will be held in strict confidence. A positive pregnancy test will preclude further participation.
- You will undergo a breathing test. This is known as spirometry, and 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. The information gained from the breathing tests will be used by the investigators to determine whether you qualify for the study.
- You will be trained on the exercise bike and the work load to elicit a minute ventilation normalized for body surface of $25L/m^2/BSA$ will be determined.

Exposure Day 1: You will be asked to eat a normal breakfast and arrive at the EPA medical station no sooner than 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level) and fill out a symptom questionnaire. Have a pregnancy test if applicable.
- Have monitors placed on you so that we can watch your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on for 24 hours. Before exposure, after exposure and the following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate. This will consist of a 20 minute rest period and a 10 minute data collection. The monitor will be removed after the last data collection. These monitors will allow us to determine if air pollutants cause small changes in the ability of your nervous system to regulate how your heart beats.
- Have up to 80 mL blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust or ozone exposure affects the ability of your blood to clot correctly, and to look at changes in proteins and/or blood cells.
- 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.
- Given a Symptom Questionnaire for you to fill out.

- Testing will be postponed if it is determined that your medication history is not within previously established limits, respiratory tract illness has occurred within the previous 6 weeks or if respiratory symptoms are present.

During the exposure, you will:

Undergo a chamber exposure of 2 hours duration, once to clean air, once to diesel exhaust and once to ozone; the amount of pollution you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. You will be blinded as to the exposure condition. The exposure chamber is 6 ft wide by 6. ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. You will exercise for 15 minutes at the previously determined pace and rest for 15 minutes. While exercising, minute ventilation may be measured intermittently. Your blood pressure may also be measured intermittently. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid for your participation up to that point, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Following the exposure, you will:

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have your vital signs checked.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Fill out a symptom score sheet.
- Have blood drawn (up to 80 mL or about 5 tablespoons).
- Be assessed and discharged by the nursing staff.

Exposure Day 2 (24 hour follow up visit): You will come back the next morning and asked to arrive at the EPA medical station at no sooner than 8 am. (approximately 24 hours after exposure) You will:

- Have your vital signs taken.
- Fill out a symptom questionnaire.
- Have blood draw (up to 80 mL or about 5 tablespoons).
- Have spirometry testing.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Have the Holter monitor removed.
- We are asking all subjects to have bronchoscopy on the day following each exposure. If you are an asthmatic, prior to the bronchoscopy you may be pretreated with inhaled bronchodilator (albuterol). The bronchoscopy will not be performed if the baseline lung function shows more than a minimal amount of airway obstruction and this does

not resolve with inhaled bronchodilator. During the bronchoscopy, a fiberoptic scope will be inserted through the nose into one of your airways where a portion of one of your lungs will be washed with sterile saline and a sample of airway lining cells will be obtained with a special brush device.

Bronchoalveolar Lavage (BAL) and Brush Biopsy is frequently used in the diagnosis of lung disease in both adult and pediatric patients. The procedure has also been used in numerous research studies of persons with respiratory disease and in healthy volunteers. The procedure has been used safely many times in studies conducted at our laboratory. The purpose of the BAL procedure is to obtain fluids and cells from the lower regions of the respiratory tract, i.e., the trachea, bronchi and smaller airways. These materials will be analyzed to obtain information about the role of various chemical substances and cells in the pulmonary response to particle exposure of persons with asthma. The bronchoscope is a flexible fiberoptic tube that is about two feel long and 1 centimeter in diameter (about the diameter of a pencil). It is an optical device with a light at the end which can be used to transmit images to a camera connected at the other end. Using the bronchoscope, the physician can see into your airways and direct the placement of the bronchoscope. A small channel allows fluid to pass through the bronchoscope.

The BAL procedure will be performed at the laboratory medical station by specially trained experienced pulmonary physicians. You will be expected at the medical station at 8:00 on the morning after the exposure. If you have had anything to drink or eat since midnight the night before the bronchoscopy you will not be allowed to proceed. Before you undergo bronchoscopy, your lung function will be evaluated and if you are an asthmatic you may be treated with inhaled bronchodilator (albuterol). The procedure will not be performed if your lung function does not meet a predetermined level of function recommended by the National Institute of Health for performing research bronchoscopies in persons with asthma. You may terminate the bronchoscopy procedure at any time. If the physician determines an increase level of anxiety, the procedure will be terminated

A saline lock intravenous catheter will be placed in a vein in your arm to give you medication before the procedure starts (a saline lock is a flexible catheter that stays in your arm for a short time). The saline lock will remain in place so that it can be used to administer medications in case medications need to e administered during the procedure. You will also be connected to a telemetry monitor that will display heart rate and rhythm, a blood pressure cuff will be placed on your arm, and an oximeter sensor (small band like device will be placed on a finger to allow the medical staff to monitor your oxygen levels during the procedure. No sedatives and/or narcotics will be administered at any time during bronchoscopy.

Before proceeding, the medical staff will again make certain that you have had nothing to eat or drink since midnight the previous night. You will be asked to decongest your nose by administering 2 puffs of neosynephrine nasal spray to each nostril. They will then give you a lidocaine solution and ask you to gargle with it for about two minutes to anesthetize your throat. You will then be asked to inhale (snort) a small amount of lidocaine jelly through both nostril to anesthetize your nose and the back of your throat. A Q-tip with lidocaine jelly will be gently inserted into your nose

to ensure that your nose is completely numb before the bronchoscope is inserted. The procedure will not begin until your nose and throat are well anesthetized. If this cannot be accomplished, the bronchoscopy will not be performed. A tube delivering oxygen will be placed inside your other nostril. Delivery of supplemental oxygen is done as a precaution during all bronchoscopies conducted at our facility.

To start the procedure, the physician will pass the bronchoscope through your anesthetized nostril to the back of your throat and then to above your vocal cords. The pulmonologist will then inject a lidocaine solution to numb your vocal cords before passing the bronchoscope into your trachea (windpipe). More lidocaine, up to a safe maximum dose is injected at various points in your trachea and airways to minimize coughing during the procedure. You will experience some cough during the procedure. This is a normal reflex caused by the presence of the bronchoscope in your airway.

Brush biopsies of your airway epithelium will be taken from the major lower airway passages. A very small brush (3 millimeters in diameter) will be inserted through the channel in the bronchoscope. The brush is visible to the physician performing the procedure through the bronchoscope. Small amounts of surface cells are scraped from the airway by gently brushing the the wall of the airway several times. Up to 8 brush biopsies are taken.

BAL will be performed by gently wedging the bronchoscope in a small airway of the right lung. Sterile saline will be injected in your lung through a channel in the bronchoscope. The saline will then be gently suctioned from your lung through the channel in the bronchoscope. This sequence will constitute a wash. A total of up to 250 cc (about 20 tablespoons) of sterile saline will be used during the BAL procedure. Approximately 75% of the saline injected into your lungs will be recovered by aspiration (suction) through the bronchoscope. The remaining 25% is expected to remain in your lungs. The saline left in your lungs will not generally cause you any difficulty breathing or harm you and should be completely absorbed by your lungs within 48 hours.After the wash and brush biopsies are obtained, the procedure will be complete and the bronchoscope will be removed from your airway. The total time the bronchoscope will reside in your airways will be up to 30 minutes.

After the procedure the oxygen catheter will be removed if your oxygen saturation is acceptable. The nurses will check your vital sign immediately after the procedure and every half hour thereafter until discharge. During this time you will sit in a recliner at the medical station for an observation period of approximately two hours. You will not have any food or drink during this time. After the recovery period, the nurses will check your gag reflex. Since the gag reflex will be absent due to anesthesia during the procedure, you will not be allowed to ear or drink until the anesthesia wears off. This normally takes about one to one-and-one half hours. Once your gag reflex returns, you will then be given some juice to sip and then some food.

The physician who performed the procedure will check you after the recovery period and you will repeat the spirometry lung function test. You will be discharged if your vital signs are stable, chest examination is normal If you are an asthmatic, before you are discharged, you may perform spirometry and your lung function should be equal to or greater than 80% of your pre-procedure value. Prior to discharge you will be requested to take 600 mgm of ibuprofen by mouth; administration of

ibuprofen/motrin almost always prevents the post-bronchoscopy malaise and low grade fever that would otherwise occur in about 25% of persons undergoing the procedure (acetaminophen/tylenol is a less effective alternative medication).

Before going home, you will be give the phone number of the medical station (966-6232) and pager phone numbers for the physician who performed the bronchoscopy with instructions to call if you experience any adverse symptoms such as: 1) persistent fever or fever above 101 degrees Fahrenheit, 2) persistent cough, 3) sputum (phlegm) production, 4) chest pain, 5) coughing up any amount of blood, 6) nose bleeds, or 7) shortness of breath

Are there any reasons you should not participate in bronchoscopy?

You should not participate in this study if you have problems with excessive bleeding after minor cuts or abrasions. You should not participate if you have had a recent exacerbation in your asthma that was not controlled with your usual medications. The physician and medical staff will explain other exclusionary condition in detail to you. In addition, during your participation in this study, you will be asked to refrain from, limit, or control taking the following medications:

*No more than 81 mg aspirin or similar medication on a regular basis or during the week of testing.
*Withhold antihistamines for 7 days prior to testing.
*Withhold inhaled bronchodilators 12 hours prior to testing.
You should not participate in this study if you are unwilling or unable to remain in the local area for 24 hours following each bronchosocpy

With your permission, DNA from the collected cells will be genotyped for specific genes related to adverse health effects associated with air pollution exposure. Unwillingness to have samples genotyped will NOT exclude you from participating in the study. If you do not wish for your cells to be used for genotyping but do wish to participate in the study do not sign the section ***Subject's Agreement for Genotype Screening With Identifying Information*** located at the end of this document.

What if we learn about new risks 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.

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. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely. With your permission, we will continue to store any samples that we collect to possibly use for other studies. This allows us to make the best use of the samples we collect from our volunteers. Again, you do not have to allow us to keep these samples if you do not want to.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. You will not benefit

personally from being in this research study. We will share with you any information that we learn about your lung function or other medical findings. We can also send you the results of some of the blood tests we perform.

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

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

2. 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. Also, the forceful breathing during this test can cause wheezing or shortness of breath to occur.

3. The adhesive patches used as part of the heart monitoring system may cause some mild skin discomfort or discoloration. If this occurs you should notify the nursing staff.

4. During air pollution exposure, you may experience some minor degree of airway irritation, cough, shortness of breath or wheezing. Some studies suggest that persons with asthma may be at risk for developing an asthma attack as a result of exposure to fine air pollution particles. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. Your symptoms will be closely monitored during the exposure and the study terminated if more than modest respiratory symptoms occur during the exposure. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. If you experience a significant drop in your lung function, albuterol will be administered.

Air pollution may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. There is a small possibility that you could have more asthma symptoms for several days, and even less likely, for several weeks, after the particle exposure. This flare in your asthma would be similar to what happens to you and other asthmatics during the allergy season or with a viral infection. Such a flare is considered highly unlikely and the exposure risk is comparable to walking around a major metropolitan area such as Los Angeles or Houston on a smoggy day. If an asthma flare occurs, you will be asked to seek medical treatment from your regular physician who treats you for your asthma.

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You

will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 6 ft in width, 6 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator at all times.

5. Bronchoscopy: As with other medical procedures, there are risks associated with bronchoscopy. However, experience has shown these risks are extremely small in research subjects. The risks associated with bronchoscopy are described in detail below.

A primary risk is bronchoscopic procedures is **discomfort to the nose and throat** brought about by the insertion of the bronchoscope through the nasopharynx. This discomfort should be alleviated or eliminated by the use of local anesthetics such as lidocaine. For this reason, you will be asked to gargle with a lidocaine solution and have lidocaine jelly placed in your nose prior to beginning the procedure. The effectiveness of the anesthesia will be tested by using a cotton swab gently inserted into your nose before inserting the bronchoscope. If you experience pain or discomfort during insertion of the bronchoscope, more lidocaine will be used up to a safe maximum amount. If adequate anesthesia cannot be achieved using this amount of lidocaine, the procedure will not take place.

A second risk of bronchoscopy is **coughing** that results from the irritation of the airway caused by the presence of the bronchoscope in the airway. Coughing is a normal reflex to expel foreign substances from the airways. Because coughing itself can cause mild trauma and discomfort to the airways and vocal cords, lidocaine liquid is sprayed on the airways at various sites to suppress the cough reflex. This technique is usually not completely successful and it is expected that you will have some cough during the procedure, especially during the initial insertion of the bronchoscope into the airway. If coughing is not controllable with the lidocaine or causes you discomfort, the procedure will be terminated.

There is a slight chance that the bronchoscopic procedure will precipitate an **asthma attack**. This risk is minimized by 1) only allowing persons with mild stable asthma to participate in the study, 2) pretreatment with inhaled bronchodilator (albuterol) before the procedure if your lung function is impaired and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physician responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. You will not be discharged from the EPA Medical Station until your lung function has returned to 80% of your pre-procedure performance. . In the unlikely event that you require additional treatment because the asthma attach continues, you will be transported by ambulance to the emergency room of the UNC Hospital, located 1/4 mile away.

Nose bleeds are another risk of bronchoscopy. This is caused by trauma to the nose during the bronchoscopy procedure. Usually, the bleeding is minor and is

noticed as a few drops of blood in mucous secretions in the nostril. The bleeding usually stops on its own within an hour after the bronchoscopy. On very rare occasions, if the bleeding becomes moderate to severe, your nostril will be packed with sterile gauze to absorb the blood and put pressure on the site of bleeding. Should you require additional treatment, you will be transported to the UNC Hospital Emergency Room. Major nose bleeds during bronchoscopy are very rare and generally limited to persons with kidney disease or a blood clotting disorder. It could also be promoted by chronic use of aspirin (>81 mg), ibuprofen or similar medications. You will not be allowed to participate in this study if you are taking such medications. Therefore the probability of getting a major nose bleed is very small.

Major **bleeding of the airways** is another rare (less than one percent) complication of bronchoscopic procedures. Most episodes of major bleeding due to bronchoscopy occur in persons with underlying predisposing conditions and are due to forceps biopsies of the airways mucosa which will **not** be performed in this study. People who suffer from kidney disease, blood clotting disorders or are chronic users of aspirin-like drugs are at increased risk of bleeding during bronchoscopy. In addition, people with lung cancer or with serious lung infection are more likely to suffer from airway bleeding during bronchoscopy. Your screening medical history, physical examination, and blood analysis will reveal most conditions that would increase your risk of bleeding during the bronchoscopy. You will not be allowed to participate if you have any of these conditions. Therefore your chances of incurring a serious airway bleed during bronchoscopy are extremely small. Nevertheless, if you do experience bleeding during bronchoscopy, you will be under the care of an experienced physician who will take steps to stop the bleeding immediately. A special cart with emergency supplies is available to handle medical emergencies. In the event of a major bleeding episode, you will be immediately transported to the Emergency Room of the UNC Hospital.

Pneumothorax or collapsed lung is another possible complication of bronchoscopic procedures. This complication is more likely to occur during tissue biopsy in the lung periphery, not the major central airways which will be the focus of this study. However you will be advised of the symptoms in the event that you experience a pneumothorax. The symptoms include chest pain and shortness of breath. If you experience these symptoms, you should contact the medical station (966-6232), or page the physician who performed the bronchoscopy. The vast majority of pneumothoraces occur within 24-48 hour of the procedure being completed. While a serious condition, a pneumothorax can be readily treated in an emergency room.

The **lidocaine** used to anesthetize your nasopharynx and reduce your cough can pose certain risks as well. A small fraction of the lidocaine applied to your nose and airways will be absorbed into your bloodstream, where it could cause adverse effects. Your risk of suffering side effects are very small, but would be much greater and potentially life-threatening if you are allergic to lidocaine or other topical anesthetics. You will not be allowed to participate in this study if you have drug allergy to topical anesthetics. When used at high doses, lidocaine can also cause symptoms in your central nervous system (confusion, tremor, euphoria, or,

rarely, seizures) or heart rate disturbances (very fast or very slow heart rate) if an excessive dose of medication is used. Finally, a death in a volunteer receiving an overdose (over 1000 milligrams) of lidocaine during bronchoscopy has been reported from Rochester, New York. However, no serious side effects of this medication have been noted at lower doses such as those described in this protocol which uses up to 360 milligrams of lidocaine during the entire procedure. If any problems develop secondary to the use of lidocaine, the physician bronchoscopist and the doctor on duty that day at the Human Studies Division of the EPA will be available to handle these problems.

Prior to the start of the bronchoscopy **atropine** may be given though a vein in your arm in order to counteract any changes in blood pressure that would result from the placement of the bronchoscope in your airway and to limit the quantity of secretions in your nose and throat. Atropine can cause dry mouth and nose for 30 to 60 minutes after the procedure is completed. This effect should wear off about an hour after the procedure is completed.

Placing an **intravenous catheter** in your arm carries a small risk as well. The needle stick can be momentarily painful and more rarely can leave a bruise in your arm. The medical staff who will be placing the catheter are very experienced and therefore the risk is minimal. Infection is also a very small risk that can result from placing the catheter. Again, the medical staff is very experienced in the use of sterile technique to prevent infection. If you notice redness or swelling in the area surrounding the catheter site, you should contact the medical station at 966-6232 or the physician on call for treatment.

A low grade fever (less than 101EF) during the 24 hours following the procedure occurs in about 25% of patients with respiratory disease undergoing bronchoscopy and BAL. The fever is benign and is usually effectively prevented by administering ibuprofen following the procedure. However, if you experience fever greater than 101 EF or lasting longer than 24 hours you could have pneumonia and should contact the Medical Station immediately so that you can be examined and treated.

Pneumonia occurs in less than 1 percent of patients undergoing bronchoscopy and BAL. Signs of pneumonia include fever greater than 101 EF, or persisting for more than 24 hours, persistent cough with or without sputum production, coughing up blood, chest pain, and shortness of breath or wheeze. Should you experience these symptoms, it is imperative that you contact the Medical Station to be seen and evaluated by a physician. As a routine precaution the Medical Station Staff will contact you between 24 and 48 hours following the procedure to inquire about any symptoms you may be experiencing.

In addition, there may be uncommon or previously unrecognized risks that might occur.

There are no risks 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 may cause some irritation, itching, or burning in some people. If this occurs you should notify the nursing staff.

The following are policies and procedures to minimize risk during the experiment:
1. During this study, skilled personnel, in an adequately equipped facility, will be observing you for any adverse effects. You may, without penalty, stop the test at any time by telling the monitor.
2. A physician will be on call at all times during the study to deal with any medical problems that may arise.
3. Every test has some degree of risk and there may be some risks associated with this experiment which are unforeseeable. You should not participate in the study if you have an acute illness.
4. You may terminate your participation in the experiment at any time and for any reason that you wish without penalty; you will be paid an amount for participation prorated for the time and procedures in which you took part. You understand that you are not under any obligation to complete any test procedures which you find objectionable or uncomfortable.
5. The investigator may terminate your participation in the experiment at any time. If this happens, you will be entitled to full payment for your time commitment and any experimental activities up to that point.
6. Your participation in the study may be postponed if your pre-exposure lung function shows airflow obstruction or if you develop a cold or asthma exacerbation.

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. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

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, the U.S. EPA and/or UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by

representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

What if you want to stop before your part in the study is complete?
You may withdraw from this study at any time. If you choose to withdraw or are unable to complete the study because of not following the instructions in this consent form, you will receive compensation only for your participation up to that point.
The investigators have the right to stop your participation at any time. This could be because you have had an unexpected reaction, have failed to follow instructions, or because the study has been stopped. After you enroll, if you are dismissed by the investigators before completing the study for reasons that are not your fault, you will be paid for your participation up to that point.
We made need to cancel your study activity due to adverse weather conditions, equipment failure, or other unforeseen events. If a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid for the time scheduled and cancelled at the standard hourly rate of $12 per hour plus any cancelled activities up to a maximum of $100. When possible, you will be rescheduled at a later time.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study. Total compensation will be based on the number of exposure sessions that will be required.

1. If you are complete all three exposures sessions of this study, your total compensation including screening will be approximately $2516.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is

also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. 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. Compensation for the visits will be as follows:

You will be exposed to diesel exhaust ,ozone and have an air control exposure in three separate sessions. Exposure Days 1-2 will be repeated for the second and third chamber exposure session, but a second training Day is not needed. If you complete three exposures, your compensation including the pre-study qualifications and training day will be approximately $2516.

Pre-study qualifications

recruitment screening	$15	Previously paid
physical exam	$15	Previously paid
bronchoscopy physical exam	$20	Previously paid

Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24

Training Day Total = $ 44

You will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure
Endobronchial Brush biopsies	$25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus	**$100**
TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures (excluding Prestudy qualifications and training day)	**$1548**
TOTAL for completion of THREE exposures (excluding Prestudy qualifications/training day but including completion bonus)	**$2422**

If you begin but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, you will receive compensation for your participation up to that point however in you arrive in the lab for the procedure having not followed instructions (eating food, taking NSAIDs or aspirin, or failing to report cold or flu symptoms the day prior to the bronchoscopy) you will be compensated $15 for your time, but will not receive compensation for the bronchoscopy.

You will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available.

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 or follow directions of the investigators, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and cancelled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during a current visit if cancelation occurs while you are in the facility. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible,

canceled visits will be rescheduled. The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be reimbursed for time and procedures completed up to that 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/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

Who is sponsoring this study?
This research is funded by the United States Environmental Protection Agency. This means that the research team is being compensated by the sponsor for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the product being studied.

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 US Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, 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 the Chairman of the IRB Committee at 919-966-1344 and /or the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What specimens will be collected and stored?

Bronchial epithelial cells collected via bronchoscopy and all blood specimens will be stored with your permission. If you agree to have your specimens stored for future research you will need to sign the accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information***.

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IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D., David Diaz-Sanchez, Ph.D., and Kelly Duncan, Ph.D.

Subject's Agreement to Participate in the Research Study with Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I voluntarily **AGREE** to have my provided bronchial epithelial cells genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

Signing here and agreeing to participate in this study DOES NOT allow us to store your samples.

__________________________________ ______________

Signature of Research Subject Date

____

Printed Name of Research Subject

__________________________________ ______________

Signature of Person Obtaining Consent Date

____

Printed Name of Person Obtaining Consent

IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D., David Diaz-Sanchez, Ph.D., and Kelly Duncan, Ph.D.

Subject's Agreement to Participate in the Research Study without Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I **REFUSE** to have my provided bronchial epithelial cells genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

____ ________________

Signature of Research Subject Date

____

Printed Name of Research Subject

____ ________________

Signature of Person Obtaining Consent Date

____

Printed Name of Person Obtaining Consent

- -

APPROVED - IRB UNC-CH

JUL 23 2010

SUBJ #_____ DATE_____

SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 24 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE (not present)
1 = TRACE/NOTICED (barely detectable)
2 = MILD/LIGHT (present, but not annoying)
3 = MODERATE (present, but somewhat annoying)
4 = SEVERE/HEAVY (present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4

What is the purpose of this form?

This application is to seek *initial* IRB approval for a research study.

What parts of this application should you submit?

Answer all questions, or mark "not applicable," when appropriate. Do not alter wording or delete questions from this form.

- For ***all studies***, submit Part A, which consists of these sections:
  - Part A.1. Contact Information, Agreements, and Signatures
  - Part A.2. Summary Checklist
  - Part A.3. Conflict of Interest Questions and Certification
  - Part A.4. Questions Common to All Studies
  - Part A.5. The Consent Process and Consent Documentation (including Waivers)

- For ***studies that involve direct interaction*** with human subjects (any contact with subjects including questionnaires, interviews, focus groups, observation, treatment interventions, etc), submit:
  - Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

- For ***studies*** that use existing data, records or human biological specimens, including for use in identifying potential subjects, submit:
  - Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

Note: You should submit Parts B or C only as applicable. If the study involves *both* direct interaction *and* use of existing materials, use both Parts B and C in addition to Part A.

Who can serve as principal investigator (PI)?

The PI is the person who will personally conduct or supervise this research study. Under most circumstances, this will be a faculty member. For IRB communication purposes, a trainee/student may be listed as PI. However, a faculty advisor must be identified, who holds ultimate responsibility for ensuring that this project complies with all University, regulatory, and fiscal requirements.

→ *See next page for additional instructions*

Complete submission instructions can be found at http://ohre.unc.edu/submission_instructions.php. **All application and consent materials must be copied or printed on one side only**. See the checklist on page 1 of the application itself for items to include and number of copies.

Some applications require additional review prior to the IRB submission. Examples include the Clinical and Translational Research Center (formerly the GCRC and CCCT facilities) http://gcrc.med.unc.edu/investigators/admin/gcrcapp.htm) or the Oncology Protocol Review Committee (PRC; http://cancer.med.unc.edu/research/prc/default.asp). See their web sites for details.

Many schools, departments, centers and institutes in Academic Affairs have local review committees that review before the IRB. See http://ohre.unc.edu/submission_instructions.php for a list of these units or consult your own unit for details.

Address for all Applications and Other Correspondence

IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

Types of Review

There are three levels of IRB Review (full board, expedited, and exempt), determined by the nature of the project, level of potential risk to human subjects, and the subject population. *The type of review applicable to a particular study is determined by the IRB.* Regardless of the kind of review, all applications use the same submission form.

Exempt and expedited review can be given to studies that constitute no more than minimal risk to the human subjects, i.e., the risk one experiences in daily living. These reviews are done in the IRB office on a continual basis.

Full board review is required for studies that involve greater than minimal risk or vulnerable populations that require special protection by the IRB. These require review by the convened IRB. See http://ohre.unc.edu/guide_to_irb.php for additional guidance.

---- Instructions – Do not submit this page with your application ----

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version June 25, 2009

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

Date: July 21, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Name and degrees of Principal Investigator: Melanie J. Jardim, Ph.D.; David Diaz-Sanchez, Ph.D. Kelly Duncan, Ph.D.
Department: US EPA Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514

UNC-CH PID: 715306970 Pager:
Phone #: 919-966-7320 Fax #: 919-966-6271 Email Address: jardim.melanie@epa.gov

For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: David Diaz-Sanchez, Ph.D.
Department: US Epa Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514
Phone #: 919-966-0676 Fax #: 919-966-6271Email Address: diaz-sanchez.david@epa.gov

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any): Michael Schmitt, MSPH
Department: US EPA Mailing address/CB #: 104 Mason Farm Road Chapel Hill, NC 27514
Phone #: 919-966-0647 Fax #: 919-966-6271 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 name, location (UNC or specific outside location), role and email address for each person who should receive electronic copies of IRB correspondence to PI.**

Robert Devlin, PhD.; Heidi Hiers, RN; Maryann Bassett, RN; Martin Case, BS; Andrew Ghio MD; Martha Sue Carraway, MD; Robert Truckner, MD;Howard Kehrl, MD; Tracey Montilla, RN; Michael Schmitt, MS.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify):

For external funding, RAMSeS proposal number (from Office of Sponsored Research): The PI is a USEPA scientist and this project is being funded by intramural EPA funds

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

Applications must "stand alone" and should provide all information requested, i.e., complete answers must be contained in the application. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

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 (include DHHS-approved sample, when one exists), fact or information sheets, phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
□	4. All recruitment materials including final copies of printed advertisements, audio/video taped advertisements, scripts, flyers, letters, and 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., Clinical and Translational Research Center (CTRC), Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. For drug studies, Investigator Brochure if one exists. If none, include package insert for previously approved uses..	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	_X_
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_X_
A.2.4. Do you have specific plans 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_
c. Is this research classified (e.g., requires security clearance)?	__	_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 Clinical and Translational Research Center (CTRC) or is the CTRC involved in any other way with this study? If yes, obtain the ***CTRC Addendum*** *and submit completed application (IRB application and Addendum) directly to the CTRC. The CTRC includes facilities located on the 3rd floor of the Main Hospital (formerly GCRC) and Ground floor Burnett-Womack (formerly CCCT).*	__	_X_
A.2.18. Will gadolinium be administered as a contrast agent?	..__	..__X
A.2.19. Will subjects' Social Security Number (SSN) be collected for:		
a. processing payments greater than $200 per year, to support IRS reporting (see also B.6)?	__	_X_
b. processing payments of any amount through UNC-CH Accounts Payable?	__	_X_
c. use as a unique identifier for study tracking purposes for national registry or database?	__	_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 personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity engaged in the performance of this project as a subcontractor, sub-recipient or vendor?	__ yes	_X_ no
(d) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_X_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit the form, which is accessible online at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

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

_____ _____

Signature of Faculty Advisor Date

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

Complete answers must be provided. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

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 protocol is to compare the genetic and epigenetic effects between diesel exhaust and ozone exposure in healthy individuals and in mild/moderate asthmatics.

Participants: We will recruit up to 30 mild to moderate asthmatics and up to 30 healthy adults to participate in this study.

Procedures (methods): Subjects will be exposed to clean air, to 300 $\mu g/m^3$ of diesel exhaust for 2 hours and to 0.3 ppm of ozone for 2 hours with intermittent exercise in a controlled environment chamber. Primary endpoints will include spirometry and lung cell changes post-exposure. Secondary endpoints will include analysis of blood clotting/coagulation factors, Holter monitoring of cardiac parameters, analysis of soluble factors present in plasma and bronchial lavage and analysis of intracellular factors present in lung tissue obtained from a brush biopsy.

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.

Several epidemiological studies have shown a strong link between exposure to air pollution and adverse cardiopulmonary effects, such as respiratory tract infections, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke(Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005; Lewtas 2007). Ozone and diesel exhaust are ubiquitous in the urban atmosphere making it difficult to identify the exact source of these effects. Although experimental chamber studies have been previously performed with diesel exhaust or ozone, no one has yet looked at the epigenetic changes resultant from acute exposures to these air pollutants.

Numerous studies have shown that healthy subjects exposed to ozone have decreased pulmonary function and increased neutrophils and soluble pro-inflammatory mediators in the lower airways(Peden, Setzer et al. 1995; Bascom 1996). These include markers of increased permeability, decreased macrophage phagocytic ability, and increases in arachidonic acid metabolites, cytokines, fibronectin, lactase dehydrogenase, coagulation factor VII, tissue factor, elastase and plasminogen activator occurred in response to ozone-induced exposures(Devlin, McDonnell et al. 1991; Koren, Devlin et al. 1991; McDonnell, Kehrl et al. 1991; Devlin 1993; McKinnon, Madden et al. 1993). Diesel exhaust fumes contain primarily fine and ultrafine carbonaceous particles generated by the incomplete combustion of fuel(Diaz-Sanchez, Penichet-Garcia et al. 2000; Lewtas 2007). Diesel exhaust particles (DEP) are the largest single source of vehicular emitted airborne PM and can persist in the air, where they are readily inhaled and deposited throughout the respiratory tract. DEP induced toxicity is mainly attributed to its chemical composition, consisting of a carbonaceous core to which organic and inorganic species can become adsorbed (Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005). Healthy human volunteers exposed to 300 $\mu g/m^3$ diesel exhaust for one hour with intermittent exercise resulted in markers of systemic and redox-sensitive pulmonary inflammatory responses, vascular dysfunction and impaired endogenous fibrinolysis. Although there were no adverse health effects associated with the exposure, acute changes in the aforementioned endpoints can be used to extrapolate information in order to begin to understand the underlying biological mechanisms that link long-

term exposure of diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (Salvi, Blomberg et al. 1999; Behndig, Mudway et al. 2006).

Previous studies reported a wide variation in response to air pollution exposure among individuals in each subject group with no clear understanding in reasons (McDonnell 1991). Asthmatics have been identified as a susceptible subpopulation as seen by their heightened sensitivity to air pollutants, although the severity of health responses of asthmatics exposed to diesel exhaust and ozone are not yet fully understood. It is hypothesized that increased susceptibility could be due to increased baseline airway inflammation and airway responsiveness which characterizes asthma. Indeed, respirable particulate matter and ozone exposure are associated with episodes of increased asthma exacerbation (Devlin 1993; Bascom 1996; Diaz-Sanchez and Riedl 2005). Elevated levels of air pollution can affect airway reactivity with decrements in several indices of pulmonary function especially in those patients with preexisting asthma.

Despite a decade of intensive studies, adverse health effects attributed to air pollutant exposure are still not well understood. It is becoming increasingly clear that epigenetics plays a pivotal role in disease development and outcome, which has been extensively studied in cancer. The term epigenetics refers to the study of heritable gene expression changes, which occur without changing the DNA sequence itself. DNA methylation, histone modifications and microRNA expression all regulate gene expression, and changes in these mechanisms can result in an overall change in gene expression patterns. Unlike the static DNA sequence, these mechanisms are regulated by endogenous and exogenous cues, rendering them more likely to change in response to environmental stimuli. It is not currently known how these changes become permanent, or how epigenetic changes control gene expression in response to acute environmental perturbations. Few studies have examined how the environment can modulate epigenetic marks in individuals. In a pioneering study published in 2005, Fraga et. al. showed that as identical twins age, DNA methylation patters change indicating that differential gene expression observed in older identical twins may be attributed to epigenetic changes, further suggesting that these changes may have been molded by the environment (Fraga, Ballestar et al. 2005). Traffic particle exposure has been associated with methylation changes in retrotransposable elements, randomly dispersed throughout the DNA sequence, however the biological consequence of this is not understood as these are not coding sequences (Baccarelli, Wright et al. 2009). In vitro studies in primary human bronchial epithelial cells grown at air liquid interface showed that microRNA expression profiles change rapidly in response to exposure to diesel exhaust particles(Jardim 2009). Animal studies attempting to address these questions have been difficult as animals have very different epigenetic mechanisms making it difficult to draw conclusions from these studies. Long term exposure to cigarette smoke, a source of particulates, lead to changes in microRNA expression in rat lungs, however the contribution of these changes to biological consequences is not currently known (Izzotti, Calin et al. 2009). Another study, showed that an in utero diet rich in methyl donors enhanced allergic airway disease in mice via changes in methylation of several genes, and that these changes were transgenerational (Hollingsworth, Maruoka et al. 2008). While it is difficult to draw biological meaning from epigenetic animal studies, what these studies show is that the environment can play an important role in regulating gene expression. Although the biological and physiological role epigenetics plays in response to environmental pollutants is not yet understood, however we will begin to address this question in this study by performing in depth gene expression analysis, including microarrays, microRNA arrays and DNA methylation assays on samples collected from patients.

In addition to possible epigenetic mechanisms, genetic polymorphisms, particularly polymorphisms in anti-oxidant genes, have been proposed as a factor to explain variability in these responses. The role of genetic background in mediating airway and vascular inflammation following acute exposure to diesel exhaust and ozone in human subjects will be studied in this protocol as a secondary question. As such, bronchial epithelial cells collected from this study will be further evaluated by in vitro exposure to both gaseous and particulate air pollution.

The purpose of this study is to first, make a direct comparison of epigenetic responses (primarily changes in DNA methylation, microRNA expression and chromatin modification) in individuals exposed to ozone and diesel exhaust; second, to assess whether mild to moderate asthmatics exhibit the similar effects and to the same degree as healthy individuals; and third, to understand the contribution of genetic background and epigenetic mechanisms to cardiopulmonary effects observed in pollutant exposed cells. Although many the observed adverse health effects are generally attributed to oxidative stress upon exposure, we still do not know how these result in physiological changes to the respiratory and cardiovascular organ systems. We hypothesize that exposure to diesel exhaust or ozone will result in changes in epigenetic mechanisms, which may be linked to adverse health effects of exposure. If adverse health effects are induced solely due to oxidative stress, then patients should exhibit similar cardiopulmonary and epigenetic responses when exposed to either, diesel exhaust or ozone. However, if differential

responses to these agents are observed, then alternative mechanisms of cellular injury may exist. Therefore, we will employ several methods to assess potentially important physiological changes to these systems, including cutting edge techniques such as micro-array profiling, DNA methylation assays, microRNA expression profiling and assays for indications of chromatin remodeling.

Baccarelli, A., R. O. Wright, et al. (2009). "Rapid DNA methylation changes after exposure to traffic particles." Am J Respir Crit Care Med **179**(7): 572-8.

Bascom, R. (1996). "Environmental factors and respiratory hypersensitivity: the Americas." Toxicol Lett **86**(2-3): 115-30.

Behndig, A. F., I. S. Mudway, et al. (2006). "Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans." Eur Respir J **27**(2): 359-65.

Brunekreef, B. and S. T. Holgate (2002). "Air pollution and health." Lancet **360**(9341): 1233-42.

Devlin, R. B. (1993). "Identification of subpopulations that are sensitive to ozone exposure: use of end points currently available and potential use of laboratory-based end points under development." Environ Health Perspect **101 Suppl 4**: 225-30.

Devlin, R. B., W. F. McDonnell, et al. (1991). "Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung." Am J Respir Cell Mol Biol **4**(1): 72-81.

Diaz-Sanchez, D., M. Penichet-Garcia, et al. (2000). "Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity." J Allergy Clin Immunol **106**(6): 1140-6.

Diaz-Sanchez, D. and M. Riedl (2005). "Diesel effects on human health: a question of stress?" Am J Physiol Lung Cell Mol Physiol **289**(5): L722-3.

Folinsbee, L. J., J. F. Bedi, et al. (1985). "Pulmonary response to threshold levels of sulfur dioxide (1.0 ppm) and ozone (0.3 ppm)." J Appl Physiol **58**(6): 1783-7.

Fraga, M. F., E. Ballestar, et al. (2005). "Epigenetic differences arise during the lifetime of monozygotic twins." Proc Natl Acad Sci U S A **102**(30): 10604-9.

Hollingsworth, J. W., S. Maruoka, et al. (2008). "In utero supplementation with methyl donors enhances allergic airway disease in mice." J Clin Invest **118**(10): 3462-9.

Izzotti, A., G. A. Calin, et al. (2009). "Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke." FASEB J **23**(3): 806-12.

Jardim, M., Fry, RC, Jaspers, I, Dailey, L and Diaz-Sanchez, D (2009). "Disruption of MicroRNA Expression in Human Airway Cells by Diesel Exhaust Particles is Linked to Tumorgigenesis-Associated Pathways." Environmental Health Perspectives **117**(11).

Koren, H. S., R. B. Devlin, et al. (1991). "Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone." Toxicol Pathol **19**(4 Pt 1): 406-11.

Lewtas, J. (2007). "Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects." Mutat Res **636**(1-3): 95-133.

McDonnell, W. F. (1991). "Intersubject variability in human acute ozone responsiveness." Pharmacogenetics **1**(2): 110-3.

McDonnell, W. F., H. R. Kehrl, et al. (1991). "Respiratory response of humans exposed to low levels of ozone for 6.6 hours." Arch Environ Health **46**(3): 145-50.

McKinnon, K. P., M. C. Madden, et al. (1993). "In vitro ozone exposure increases release of arachidonic acid products from a human bronchial epithelial cell line." Toxicol Appl Pharmacol **118**(2): 215-23.

Peden, D. B., R. W. Setzer, Jr., et al. (1995). "Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics." Am J Respir Crit Care Med **151**(5): 1336-45.

Riedl, M. and D. Diaz-Sanchez (2005). "Biology of diesel exhaust effects on respiratory function." J Allergy Clin Immunol **115**(2): 221-8; quiz 229.

Salvi, S., A. Blomberg, et al. (1999). "Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers." Am J Respir Crit Care Med **159**(3): 702-9.

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).

Subjects for this study will be healthy adults, 18-55 years old, (male and female) and, otherwise healthy, 18-55 year old mild to moderate asthmatics (as defined by NHLBI guidelines), who are non-smoking. Subjects will be recruited through the Westat Corporation (see section B1 below). There are no gender or race based restrictions. Pregnant and lactating women will be excluded because of the unknown effects of air pollutants on inflammatory markers during pregnancy and lactation.

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.

Healthy Individuals

Specific Inclusion Criteria

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66).
2. Normal lung function (pre or post albuterol), defined by Knudson 1976/1984 as:
   a. FVC of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   b. FEV_1 of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   c. FEV_1/FVC ratio of $\geq$ 75 % of predicted values.
3. Oxygen saturation of $\geq$ 96 %.
4. Ability to tolerate intervals of moderate exercise

Exclusion Criteria:

1. A history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease, and lung cancer.
2. If the subject is pregnant, attempting to become pregnant or breastfeeding.
3. Allergy to any medications which may be used or prescribed in the course of this study.
4. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM and/or ozone challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
5. Smoking history within 2 years of the study.
6. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
7. Untreated hypertension (> 150 systolic, > 90 diastolic)
8. Dementia.
9. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
10. History of skin allergy to tape or electrodes.
11. Subjects who do not understand or speak English
12. Subjects who are unable to perform moderate exercise

Temporary exclusions:

1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.
4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:

1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin $\geq$ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

Mild to Moderate Asthmatics

Inclusion Criteria:

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66) where diagnosis will be judged by EPA physicians.
2. Mild/Moderate asthmatics as defined by NHLBI guidelines.
3. Positive history of asthma (wheezing, chest tightness, and reversible airway obstruction);
4. Baseline $FEV_1/FVC \geq 60\%$;
5. Oxygen saturation of $\geq$ 94%
6. Ability to tolerate moderate exercise

Exclusion Criteria

1. Use of oral steroid therapy within the past month
2. Physician directed emergency treatment for asthma exacerbation within the preceding 6 months.
3. Abnormal EKG that precludes evaluating heart rate variability.
4. Aside from mild/moderate asthma, a history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma, and cancer (possible exception for history of nonmelanoma skin cancer).
5. If the subject is pregnant, attempting to become pregnant or breastfeeding.
6. Allergy to any medications which may be used or prescribed in the course of this study
7. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
8. Dosing level of an inhaled steroid must be consistent with mild asthma as outlined by the NHLBI NAEPP guidelines. Regular use of oral corticosteroids, or use of inhaled steroid at doses typically used for severe asthma, will result in exclusion of that individual from the protocol.
9. Severe asthmatics as defined by: nighttime symptoms of cough or wheeze greater than 1 time per week at baseline, daily exacerbation of asthma or requirement for albuterol due to asthma symptoms (cough, wheeze, chest tightness, but not to include prophylactic use of albuterol prior to exercise), more than mild interference with normal activity, any episode of physician directed emergency treatment for asthma requiring oral corticosteroid therapy within the past twelve months.
10. Smoking history within 2 years of study.
11. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
12. History of skin allergy to tape or electrodes.
13. History of respiratory diseases other than allergic rhinitis and asthma
14. Untreated hypertension (> 150 systolic, > 90 diastolic)
15. Dementia.
16. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
17. Subjects who do not understand or speak English
18. Subjects who are unable to perform moderate exercise

Allowances: subjects may

1. Use daily theophylline therapy.
2. Use daily inhaled steroids.
3. Use inhaled cromolyn therapy.

Temporary exclusions:

1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.
4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:

1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin $\geq$ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This is a randomized cross-over study with three arms. This study will compare the epigenetic responses of healthy adults and adults with mild asthma to diesel exhaust and ozone exposures. Up to thirty subjects will be recruited into each group. Subjects will be randomly exposed to clean air, diesel exhaust and ozone with exposures separated by a minimum of two weeks. Responses of primary interest will include: 1) genetic and epigenetic changes 2) FEV1 as measured by spirometry, and 3) lung inflammation and cell changes as evaluated by bronchoalveolar lavage. The performance of these tests and procedures are considered essential to conducting the study. Exploratory endpoints will include: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, telemetry, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18-24 hours post-exposure. All medical procedures will be performed by study personnel.

Physical Examination Day:

Prior to recruitment into the study, subjects will undergo a physical exam. During this visit, a short review of the subject's history will be performed and vital signs, height and weight will be assessed (temperature, pulse, respiratory rate, and blood pressure), to determine whether the individual meets any major exclusion criteria for the study. Up to 50 ml of blood may be collected for a CBC/ differential, chemistry and lipid panels, assessment of coagulation function, phagocytic function and cell surface markers. At this time the subject may undergo a physical examination for bronchoscopy or may be scheduled for one at another time.

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.
- On the Exposure Day 2, eat a normal breakfast.
- No caffeine for 12 hours prior to all study visits.
- At the end of Exposure Day 2, do not eat or drink anything after midnight in preparation for bronchoscopy on Exposure Day 3.

Training Day

1. Eligible subjects will report to the research lab for an approximate 2-hour training session.
2. Informed consent will be obtained.
3. Assessment of **vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation
4. **Pregnancy tests** will be administered to any women who may have child-bearing potential.
5. Subjects will be trained on the cycle ergometer and the work load to elicit a minute ventilation normalized for body surface of $25L/m^2/BSA$ will be determined. In most subjects this will be about 50 L/minute (IE. VO^2 of approximately 2.0 L/m). A cycle ergometer work setting of 75 to 100 watts will usually achieve such a physiological response
6. Subjects will perform **spirometry.**
7. Subjects will be shown chamber where exposures will take place

Exposure Day 1

The subjects will be exposed ozone, diesel exhaust and clean air on three separate occasions, with the exposures separated by a minimum of two weeks. .

1. Subjects will be asked to arrive at the study site no sooner than 8 AM and will undergo **assessment of vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation, and **symptom score** assessment.
2. **Pregnancy tests** will be administered to any women who may have child-bearing potential.
3. A **telemetry unit** will be attached for cardiac monitoring.
4. Subject will be fitted with a **Holter monitor** and the subject will be asked to recline in a quiet dark place for 30 minutes. After approximately 20 minutes a time marker will be activated so that heart rate variability frequency readings can be taken over the next 10 minutes. The subject will wear the Holter monitor for the next 24 hrs.
5. **Venipuncture:** Up to 80 ml of blood will be collected for a CBC/ differential, and may be used for assessment of coagulation function and markers, phagocytic function, inflammation, and cell surface markers. A portion of the sample may be used for RNA isolation for microarray analysis and for DNA isolation for genotyping purposes.
6. **Baseline Spirometry**
7. **Exposure sessions**: Exposure to ozone, diesel exhaust or clean air will be conducted in an exposure chamber at the EPA Human Studies Facility on the UNC campus. Each subject will be exposed up to 0.3ppm ozone, diesel exhaust (up to 300 ug/m^3) or clean air for 2 hours. Subjects will begin exercising on an exercise bike. Each exercise session will consist of a 15 minute exercise interval at a level of up to 25 L/m2/BSA followed by a 15 minute rest period. Minute ventilation may be measured during each exercise period. Continuous heart rate and rhythm, and oxygen saturation, will be monitored using telemetry and pulse oximetry respectively. Blood pressure may be monitored intermittently. Subjects will be asked to refrain but not prohibited from using inhaled bronchodilator during exposures; if medication use is necessary subjects will be instructed to maintain consistent medication use across the two exposures. The subjects will be able to end their exposure and exit the chamber at any time. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, ataxia, or a greater than 5 point drop in saturated oxygen (or a drop to lower than 89%). They will be monitored continuously by trained personnel and a physician is on call whenever a subject is undergoing a procedure at the facility. A detailed description of the physical facilities in the Human Studies Division of the U.S. EPA Health Effects Research Laboratory located on the UNC-CH campus have been published as U.S. Government Publication EPA-600/1-78-064, and is on file in the office of the UNC Committee on the Protection of the Rights of Human Subjects. The medical station of the Human Studies Division includes a fully-equipped medical examination room, a cardiac/respiratory emergency cart, a subject recovery room, a nurse's station, a waiting room and conference rooms. The facility is staffed by qualified RNs, and an on-site physician is available to respond to a medical

emergency in a timely manner. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. The DE will be generated from a diesel generator used to power a load bank 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 up to 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 1080 µg, which assumes that the subject will inhale 2.7 m^3 during the 120 min exposure. 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 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) 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. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured in real time. Particle size distribution may not be determined during each subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. Ozone exposures will be conducted in a (6 ft x 6 ft x 8 ft) stainless steel chamber with a continuous supply of exposure medium. Ozone will be monitored continuously. **Immediately post exposure**, subjects will again undergo **spirometry, Holter monitor frequency reading, symptom scoring and assessment of vital signs.**

8. **Then,** up to 80 ml of blood will be collected for a CBC/ differential, assessment of coagulation function, blood leukocyte phagocytic function, cytokines, cell surface markers and RNA isolation for microarray analysis.
9. Subjects will then be assessed and discharged by the nursing staff.

Exposure Day 2 (approximately 24 hours after exposure)

The subject will arrive at the U.S. EPA HSF for:

1. **Vital sign monitoring**.
2. **Symptom scoring.**
3. **Venipuncture**: up to 80 ml of blood will be collected for a CBC/ differential, coagulation function, assessment of blood monocyte phagocytic function, inflammation, and cell surface markers, and RNA isolation for microarray analysis.
4. **Holter monitor** frequency reading and removal
5. **Spirometry**
6. **Bronchoscopy.** Subjects will have undergone a physical examination including assessment for suitability for transnasal fiberoptic bronchoscopy at the Human Studies Facility by a board certified or board eligible pulmonologist.

   Prior to the procedure, subjects will perform spirometry (as above); bronchoscopy will not be performed if lung function does not meet the recommended performance level (NIH guidelines; FEV1$\geq$ 60 % predicted). Prior to bronchoscopy, a small tube (IV) will be placed in a vein for potential use in administering fluids. Subjects will receive inhaled albuterol prior to the bronchoscopy at the discretion of the pulmonary physician performing the procedure. Bronchoscopy with bronchoalveolar lavage and brush biopsy will be performed by a licensed physician who is Board certified or board eligible in pulmonary medicine and is experienced in fiberoptic bronchoscopy. The physician will be assisted by at least one R.N experienced in bronchoscopy. The subjects will be monitored closely throughout the procedure in the following manner: 1) Chest electrodes for continuous electrocardiogram, 2) Pulse oximetry of arterial blood, and 3) blood pressure monitoring using an electronic sphygmomanometer. Subjects may be premedicated with IV atropine (0.6 mg) to prevent bradycardia and hypotension that could result from vagal stimulation provoked by passing the bronchoscope through the subject's larynx and to minimize the amount of airways secretions. Nasal oxygen will be administered during the procedure. No sedatives and/or narcotics are administered at any time during bronchoscopy. We have found that premedication with sedatives (midazolam) or with opiates (demerol) is not required for research bronchoscopy subjects. Avoidance of these drugs reduces procedure risk and markedly shortens post-procedure recovery time. Neosynephrine will be used to decongest nasal passages, followed by gargling with a lidocaine solution for a few seconds to anesthetize the throat. The subject will then sniff (snort) a small amount of lidocaine jelly through one nostril to anesthetize the nose and the back of throat. A Q-tip with lidocaine jelly will be gently inserted into the nose to ensure that it is completely numb before the bronchoscope is inserted. Topical lidocaine anesthesia will be administered to the larynx and lower airways through the bronchoscope for subject comfort and to prevent cough, with a maximum dose of 15 ml of 2% lidocaine

solution (360 mg lidocaine) Up to 8 endobronchial brush biopsies will be obtained from the lower trachea and right and left mainstem bronchi. A cytology brush (Bronchoscope Cytology Brush; Bard, Tewksbury, MA) is applied to the region under direct visualization. For a single brushing, six passes, each with a linear excursion of approximately 2-5 cm, are made with the brush along the endobronchial surface. Bronchoalveolar lavage will be performed in the right middle lobe using a total volume of up to 250 cc of sterile 0.9% saline; from one to 5 aliquots of 50 cc each will be injected and immediately aspirated through the channel in the bronchoscope using a syringe.

Subjects are observed and monitored for a minimum of 1 to 2 hours after the procedure in the on-site medical station by a registered nurse with physician supervision. At discharge subjects will be symptom-free. At least 2 physicians, including the responsible bronchoscopist, will be available (24 hours daily) to subjects who develop any symptoms after discharge. The subject is discharged by a physician and is provided with the names and telephone numbers of the bronchoscopist and one other physician. The subject is contacted 24 hours post-bronchoscopy to ask about any untoward effects.

Subjects will return to the U.S. EPA HSF for an additional exposure sessions and post exposure bronchoscopies (air control, ozone or diesel exhaust) after a minimum time interval of two weeks.

We anticipate performing several clinical procedures during the course of this study which include primary, secondary and exploratory endpoints. However, it is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure, because of a investigator problem (such as but not limited too building/equipment failures, weather delays, or staffing issues), then the patient will be compensated for said procedure(s) and time on that day and may be rescheduled.

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.

There will be no direct benefit to the subject for participating, other than information that may be learned during their physical examination about their overall physical status. The primary benefit from the study will be to add to the scientific knowledge about whether exposure to air pollutants induces epigenetic changes.

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.

Risk and Procedures for Minimizing Risks of Diesel Exhaust Exposure

- Diesel Exhaust Exposure: The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban area. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and

with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City. We have seen in IRB-07-0190 that exposure to 300 $\mu g/m^3$of diesel exhaust does not cause adverse health effects.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. In the pilot phase of study #07-0190 (Haiyan Tong MD, Ph.D, PI), subjects reported "moderate" eye irritation following diesel exhaust exposure and the symptom was resolved within a few hours after the exposure. Subjects will be provided with the choice to wear chemical safety goggles during exposures to prevent or minimize the eye irritation. 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 health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

Risk and Procedures for Minimizing Risks of Ozone Exposure

Potential risks may likely 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. It is likely that most subjects participating in this study will experience some degree of tracheobronchial irritation and cough.

Risks and Procedures for Minimizing Risks of Venipuncture:

Insertion of the needle may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.

Risks and Procedures for Minimizing Risks of Bronchoscopy:

The most significant risk to a volunteer in this protocol involves bronchoscopy. Over 1000 procedures have been performed within the EPA Human Studies Facility over the past 15 years. From January 1, 1986 through December 30, 1995, 736 procedures were performed in normal nonasthmatic subjects, and these have been reported in the Journal of Bronchology 1998; 5:185-194. The overall complication rate was less than 1% (actually less than 0.1%). The most frequent complication was a low grade fever. Bronchoscopy with BAL can be associated with more

significant complications, including hypoxemia, cough, wheezing, and post-bronchoscopy fever. These may be increased in asthmatic subjects, and some investigators have found hypoxemia to be more severe in asthmatics compared to normal subjects (Djukanović, R., J. W. Wilson, C. K. Lai, S. T. Holgate, and P. H. Howarth. 1991. The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am. Rev. Respir. Dis.* 143: 772-777), while others have not (Van Vyve, T., P. Chanez, J. Bousquet, J. Y. Lacoste, F. B. Michel, and P. Godard. 1992. Safety of bronchoalveolar lavage and bronchial biopsies in patients with asthma of variable severity. *Am. Rev. Respir. Dis.* 146: 116-121); Anonymous. Workshop summary and guidelines: investigative use of bronchoscopy, lavage, and bronchial biopsies in asthma and other airway disease. *J Allergy Clin Immunol* 88:808-14, 1991.;
Jarjour NN, Peters SP, Djukanovic R, Calhoun WJ. Investigative use of bronchoscopy in asthma. Am J Respir Crit Care Med 157:692-97, 1998.) Furthermore, hypoxemia is rarely severe enough to require premature termination of the procedure and can usually be treated or prevented by supplemental oxygen (Van Wyre).

Bronchoscopies are performed by experienced physicians who are either Board-certified or Board-eligible in pulmonary medicine. The physician performing the bronchoscopy is always assisted by at least one nurse familiar with the procedure. The subject's vital signs and ECG are continuously monitored during the bronchoscopic procedures and at regular intervals during the recovery phase. The procedure is immediately terminated if the subject experiences any alterations such as tachypnea, depressed respiration, tachycardia, bradycardia, abnormal rhythms or significant changes in blood pressure. As indicated earlier, a fully equipped emergency cart including a defibrillator, endotracheal intubation equipment and emergency medications (atropine, epinephrine) is available at all times. An on-site physician is always available to respond and the UNC Memorial Hospital Emergency Room is within 1/4 mile of Human Studies Facility. Before the subject is sent home, he/she receives a brief physical examination by a physician and is given phone numbers and a pager number with which to contact a physician should he/she experience any problems or have any questions.

The complication rate during medical bronchoscopy is approximately 0.08 to 0.12% for all events, including low oxygen saturation, respiratory embarrassment, myocardial infarction, or death. The medical screening of the subjects is specifically designed to exclude subjects with a history of medical problems which might put them at risk from the particle exposure and bronchoscopic procedures. Medical problems which could arise as a result of bronchoalveolar lavage and brush biopsy include the following:

Discomfort of the nose and throat is a primary risk of bronchoscopy. As outlined, the lidocaine gargle, spray and liquid is used to anesthetize these areas prior to passing the bronchoscope to reach the lower airways. The procedure will be discontinued if adequate anesthesia cannot be achieved by the means described.

Low-grade fever (38-38.5 °C) can occur in approximately 25 % of hospitalized subjects undergoing bronchoscopy. The fever invariably resolves within 18 hours without treatment or with acetaminophen. Our experience at HSD, EPA has shown that this fever and the associated malaise can almost always be prevented by administering ibuprofen following the procedure. The subject will be asked to contact the physician who performed the procedure or one of the nurses at the Medical Station if fever persists or is higher than 38.5 °C. As part of the standard protocol, a nurse or physician will contact the subject by telephone between 24 and 48 hours after the procedure to inquire on the general health of the subject and specifically about the presence of fever.

Cough is a common, albeit minor, problem encountered during bronchoscopy. Cough results from mechanical stimulation of cough receptors in the airway by the bronchoscope or the cytologic brush used for the brush biopsies. In addition to the discomfort, prolonged coughing with the bronchoscope in place can result in mild mechanical trauma to the vocal cords. Lidocaine solution is passed through the bronchoscope channel at the main carina and at the level of the right mainstream bronchus to suppress the cough reflex during the procedure, more can be used as needed. The procedure will be terminated if adequate cough suppression cannot be attained.

Epistaxis is caused by trauma to the nose by the bronchoscope. This condition is expected to be minor and resolve on its own. Small streaks of blood in nasal secretions may be present for up to 12 hours following the procedure. If the bleeding becomes moderate or severe during bronchoscopy, the procedure will be terminated and the bronchoscope removed. The subject's anterior nasal passage will be packed with sterile gauze to stop the bleeding. If the bleeding does not resolve with packing, the subject will be transferred to the UNC Hospital Emergency Room.

Lidocaine use presents a small risk to the subjects, as a variable fraction of the medication is absorbed through the airway mucosa. If a significant amount of lidocaine is absorbed, adverse reactions such as bradycardia, hypotension, urticarial reactions, confusion, lightheadedness, euphoria, tremors and seizures can result. We take particular precautions to avoid the possibility of overdose of topical lidocaine and limit the total amount of lidocaine instilled into the airways to a maximum of 360 mg. Also, subjects will be screened by history prior to the procedure for known allergies to lidocaine or other similar topical anesthetics.

Bronchospasm, manifested as wheezing, chest tightness or dyspnea, is a risk of bronchoscopy that is caused by stimulation of irritant receptors in the airways by the bronchoscope. This risk is minimized by 1) only allowing persons with stable mild to moderate stable asthma to participate in the study, 2) pretreatment with inhaled bronchodilator before the procedure per request of the bronchoscopy physician and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the

procedure will be given by the physicians responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. In the unlikely event that the subject requires additional treatment because the asthma attack continues, they will be transported by ambulance to the emergency room of the UNC Hospital Emergency Room.

Pneumothorax is a risk of bronchoscopic procedures such as transbronchial biopsy, peripheral lung protected brushings or peripheral lung cytology brushings. Because these procedures will not be performed, the risk of pneumothorax in this study is extremely small. There is a very small risk of a pneumothorax resulting from a endobronchial brush biopsy. The symptoms include dyspnea and chest pain. The subjects will be interviewed and examined by the bronchoscopy physician 2 hours after the procedure with these symptoms in mind. If a pneumothorax is suspected, the subject will be transferred to the UNC Hospital Emergency Room by ambulance for further evaluation and treatment.

Bleeding in the lower airway may occur from trauma caused by the bronchoscope or by brush biopsy. Bleeding is typically very minor, in the order of 1/10 cc, is not clinically significant in an otherwise healthy subject and should spontaneously resolve in a matter of minutes. Epinephrine, diluted 1/20,000 fold will be administered through the bronchoscope to the affected site if bleeding is minor to moderate. The maximum dose of epinephrine used will be 2-3 cc to prevent local tissue necrosis. The site will be monitored through the bronchoscope until the bleeding stops. If bleeding fails to resolve with epinephrine or if it is sufficiently severe to cause hemoptysis or hemoglobin desaturation, oxygen supplementation will be provided to keep arterial oxygen saturation above 90% and the subject will be transported to the NC Memorial Hospital Emergency Room by ambulance. If it becomes necessary, the subject can be intubated before transport.

The use of epinephrine to control bleeding can produce systemic effects resulting from mucosal absorption of the drug. These symptoms are transient and include headache, palpitations and tachycardia when the dose is greater than 1 mg and is given intravenously. Two cc of a 1/20, 000 dilution of epinephrine contains 0.10 mg of the drug, a dose that is very unlikely to induce any untoward effects. This is especially true when the drug is applied to the mucosa, since only a fraction is expected to be absorbed.

Bradycardia and/or hypotension may result from increased vagal nerve output provoked by coughing or Valsalva during the passage of the bronchoscope through the vocal cords and trachea. Atropine (0.6 mg iv) is prophylactically administered to the subjects before the start of the procedure to minimize this risk. The primary side effects associated with atropine administration is mild sinus tachycardia which typically resolves within 30 minutes as the drug is cleared from the subject's system. In rare cases (less than 1 percent) the sinus tachycardia is accompanied by hypotension.

Hypoxemia: Arterial blood oxygen saturation is continuously monitored during the procedure with a finger pulse oximeter. Subjects are supplemented with oxygen at a flow rate of 2 l/min via nasal cannula during the procedure. The oxygen flow can be increased to a maximum of 6 l/min if the arterial oxygen saturation falls below 93 %. If oxygen supplementation is not effective, the procedure will be discontinued immediately. Removal of the bronchoscope and treatment with inhaled bronchodilator should be sufficient to allow the subject's arterial oxygen saturation to return to normal. Under normal conditions, oxygen supplementation is ceased once the procedure is completed. However, supplementation will be continued if arterial oxygen saturation is below 93 % on room air. In the event that low oxygen saturation persists, if the subject cannot be weaned from the oxygen supplementation, or if it falls precipitously at any time, an emergency situation will be declared and the subject will be transported immediately by ambulance to the UNC Hospital Emergency Room. These risks are minimized during the research procedure at the Human Studies Facility, in part because premedication with sedatives (midazolam) or with opiates (demerol) (as is used in medical bronchosopy) is not required for healthy research subjects. Avoiding these drugs reduces procedure risk and allows the participants to enjoy a normal day after leaving the EPA Human Studies Facility.

The risk of pneumonia as a result of bronchoalveolar lavage in the lobe involved in the procedure is less than 1%. Symptoms of pneumonia could include fever, dyspnea, persistent cough, productive cough and chest pain. The subject will be instructed to call the physician who performed the procedure or the Medical Station if these symptoms occur. The medical staff will evaluate the subject over the phone between 24 and 48 hours after the procedure for signs of pneumonia.

The subject will be urged to contact the Medical Station or the bronchoscopy physician should he/she experience any of the following symptoms: 1) Epistaxis, 2) Persistent cough, 3) Hemoptysis, 4) Chest pain, 5) Dyspnea, 6) Wheezing, 7) Sputum production, 8) Hoarseness or sore throat.

Confidentiality Risk

Risk of breach of confidentiality is minimal. For the mild asthmatic subject, it is conceivable that an employer or insurance company could learn of the diagnosis if the subject requires medical intervention as a result of participating in the study. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files which may

contain the subject's name or screening number are secure in the EPA building which has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, spirometry) will be discussed with the subject and the subject will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data 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 changes in epigenetic mechanisms in bronchial epithelial cells can be linked to physiological changes in healthy and mild/moderate asthmatic volunteers safely exposed to low levels of O3 and diesel exhaust. The study will follow a randomized, repeated measures design with each subject exposed to clean air, diesel exhaust and ozone. The data analysis will be focused on changes in lung function measurements, primarily FVC and FEV_1, and secondarily, HRV measurements between pre and post 2hr exposure for each exposure and lung cell composition changes. Also blood endpoints include inflammatory factors (eg IL-6), blood clotting factors (eg fibrinogen), and susceptibility factors (eg specific genotypes associated with susceptibility to air pollution).

In evaluating exposure effects of O3 and DE 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 (Folinsbee, Bedi et al. 1985) for a constant acute exposure to 0.3 ppm O_3 versus filtered air. In 22 subjects, the pre exposure FEV_1 was 4.608 ± 0.514 (mean $\pm$ SEM) in FA exposure and 4.656 ± 0.592 in O_3. The post exposure FEV_1 was 4.64 ± 0.476 in FA 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 the required sample size for each group is 30 subjects. We will initially recruit 30 subjects for each group (healthy and mild/moderate asthmatics) for this study to ensure the statistical power that can analyze small differences expected from low concentration exposures.

Other Exploratory Observations:
We hypothesize that subjects harboring polymorphisms in antioxidant genes will have increased responses to diesel exhaust and ozone compared to subjects who do not. In order to address this we will use collected cell samples for in vitro exposure experiments

Exploratory endpoints will include the effect of diesel exhaust and ozone on changes in: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values.

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, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data**. Are the identifiers in A.4.9 above linked or maintained with the research data?

_X_ yes __ no

A.4.11. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in a locked file cabinet in the Medical Station at the U.S. EPA 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 a day for seven days a week

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

_X_ No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission**. Please check all that apply.

For electronic data stored on a desk top computer:
_X_ Secure network _X_ Password access __ Data encryption__ Password protected file(s)
__ Other comparable safeguard (describe):

For portable computing devices/external storage devices (e.g. laptop computer, PDA, CDs, memory sticks):
__ Power-on password __ Automatic log-off __ Data encryption __ Password protected file(s)
__ Other comparable safeguard (describe):

For hardcopy data (including human biological specimens, CDs, tapes, etc.):
__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office _X_ Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials**. Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples. Subjects at any time may request that their samples be no longer stored in the repository. Any analysis in progress at the time of the request or already performed prior to the request being received by the researcher will continue to be used as part of the research study. Once the researcher is notified, the subject's specimens will be destroyed.

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.*

The subject will be given an opportunity to read the consent form. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *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 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 __ yes __ no

Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).*
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Subjects for U.S. EPA Studies (95-EPA-66). Typical methods for recruiting may include mass mailings, fliers and print ads. 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. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of this study will be approximately eighteen months. Subject recruitment and screening is expected to be continuous throughout the study until the intended number of subjects is reached.

Scheduling constraints imposed by concurrent studies in the U.S. EPA Human Studies Division are expected to limit the rate at which subjects can be exposed to 1-2 per week.

The physical examination evaluation may take up to one hour. If the subject is eligible for the study, the individual may have up to nine more visits to the research facility over approximately 6 months. A physical examination for bronchoscopy will be performed. The training day (Day 1) visit will require approximately 2 hours. Exposure day (Day 2) will last approximately 6 hours. Subjects will report to the facility at no sooner than 8 AM. The morning following the exposure, the subject will return for Day 3 which a follow-up visit which may last approximately 4 hours. Subjects will report to the facility at no sooner than 8 AM. Subjects will then return and repeat the Day 2-3 visits with the second and third exposure session. Exposure sessions will be separated by a minimum of two weeks.

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

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators for involuntary reasons after enrollment in the study but prior to completion will be paid for their participation up to that point

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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 for any procedures cancelled up to a maximum of $100. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study:
Subjects will be paid approximately $12 per hour for participation in this study. If the subjects complete all three exposures with bronchoscopy, the total compensation including prestudy qualifications will be approximately $2516.

Pre-study qualifications		
recruitment screening	$15	Previously paid
physical exam	$15	Previously paid

bronchoscopy physical exam	$20

Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24

Training Day Total = $ 44

Subjects will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure
Endobronchial Brush biopsies	$ 25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus **$100**

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures	**$1548**
TOTAL for completion of THREE exposures (including completion bonus)	**$2422**

If a subject begins but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, they will receive compensation for participation up to that point.

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

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***This section applies even if records are only used to identify potential subjects.***

→ ***If your study does not use existing data, records or specimens for any purpose, 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
If applicant was involved in the original collection, please explain role:

__ 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, do you have permission from the custodians of the data, records or human biological specimens (e.g., pathology dept, tissue bank, original researcher)? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 24 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE (not present)
1 = TRACE/NOTICED (barely detectable)
2 = MILD/LIGHT (present, but not annoying)
3 = MODERATE (present, but somewhat annoying)
4 = SEVERE/HEAVY (present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

Include the items indicated, where applicable:

- *Check* the relevant items below and include one copy of all checked items 1-5 in the order listed.
- Also include one additional collated set of copies (sorted in the order listed) for items 1 and 2.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
□	1. A concise summary of the requested modification using this form. List and describe each proposed change to aid in IRB review. Add pages as necessary. Provide a concise summary of changes when submitting an updated Investigator Brochure or Master Protocol.	2
□	2. New or revised consent forms, questionnaires, surveys, recruitment materials, advertisements, etc. One copy should have changes highlighted by underlining, and the other clean copy will be used for stamping.	1 highlighted 1 clean
□	3. If you have made substantive changes to the study design or procedures, submit a revised full IRB application with changes highlighted by underlining. If you are making changes only to the first page, just submit that page.	2
□	4. The sponsor's document describing the amendment, if any.	1
□	5. If adding personnel, include name, location (UNC or specific outside location), role, and email address for each person who should receive electronic copies of IRB correspondence to PI. For those study personnel *not* in the online UNC-CH ethics training database (http://cfx3.research.unc.edu/training_comp/) include documentation of required training in human research ethics.	1

1 **List and describe each proposed change:** The purpose of this modification is to request that one of our subjects be allowed to complete one additional exposure as we were unable to collect the primary endpoint, lung epithelial cells via bronchoscopy. As our nurses were looking through some data obtained from the subject's holter monitor, they noticed a small deviation from the pattern. We decided to proceed cautiously and asked a physician expert for an opinion on the matter. We decided to stop all procedures, including the bronchoscopy, until we had clarification of the subject's status. For this reason, the subject was sent home. We then heard back from our expert in the field who said that there was nothing wrong with what was observed on the holter and that the deviation from pattern was within a normal range.

Given this situation, we are submitting for IRB approval, a request to allow an additional exposure session for one subject. We also include with this request, an addendum to the consent form for participation in one additional exposure session, including affiliated procedures.

2. **Is this modification being submitted in response to an unanticipated problem/adverse event or new findings?** ___yes _X__no
If yes, explain, including whether these events or findings are relevant to participants' willingness to continue.

3. **Do any of the proposed changes increase risk?** _X__yes __no If yes, explain.

The risks associated with one extra exposure are minimal. They are the same as one would expect from the exposure as outlined in the original consent form and IRB application. The subject would still only undergo three total bronchoscopies during the study, which is consistent with the original application. Due to study scheduling, the actual time between exposures is four weeks, allowing more than adequate time between exposures.

OFFICE OF HUMAN RESEARCH ETHICS -- Institutional Review Board

MODIFICATION OF APPROVED HUMAN SUBJECTS RESEARCH

Version May 22, 2009

This application is to seek approval for a modification to a currently approved study. Any proposed changes to previously approved human subjects research must be reviewed and approved by the IRB prior to implementation. This includes modifications to the study, inclusion or exclusion criteria, recruitment methods, research personnel, or *any* new or revised study materials. Approval is required for all modifications whether initiated by the investigator or external sponsor. **This form should not be used to report violations and deviations.**

Instructions for Submitting

Include with your submission the items indicated in the list on the next page, where applicable.

Unless otherwise instructed, submit to the same IRB that previously reviewed this study. Complete submission instructions can be found at http://ohre.unc.edu/submission_instructions.php. ***All application and consent materials should be copied or printed on one side only.***

Special submission instructions apply for studies that require additional review. Examples include the General Clinical Research Center (GCRC; http://gcrc.med.unc.edu/investigators/admin/gcrcapp.htm) or the Oncology Protocol Review Committee (PRC; http://cancer.med.unc.edu/research/prc/default.asp). See their web sites for details.

Address for all Applications and Other Correspondence

IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

---- Instructions – Do not submit this page with your application ----

IRB study #: 09-1625 **Date:** August 17, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D.

Faculty advisor: David Diaz-Sanchez, Ph.D. (if applicable)

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

_____ _____

Signature of Principal Investigator or designee Date

_____ _____

Signature of Faculty Advisor (if applicable) Date

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
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: Melanie Jardim
Epa
CB: 7315

From: Biomedical IRB

I. Friedman
Authorized signature on behalf of IRB

Approval Date: 9/20/2010
Expiration Date of Approval: 9/19/2011
RE: Notice of IRB Approval by Full Board Review
Submission Type: Renewal
Study #: 09-1625
Study Title: Epigenetic Effects of Diesel Exhaust and Ozone Exposure
Sponsors: US Environmental Protection Agency - GRANTS

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

Study Description:
Purpose: The purpose of this protocol is to compare the genetic and epigenetic effects between diesel exhaust and ozone exposure in healthy individuals and in mid/moderate asthmatics. Participants: The PI will recruit up to 30 mild to moderate asthmatics and up to 30 healthy adults to participate in this study. Procedures (methods): Subjects will be exposed to clean air, to 300 ug/m3 of diesel exhaust for 2 hours and to 0.3 ppm of ozone for 2 hours with intermittent exercise in a controlled environment chamber. Primary endpoints will include spirometry and lung cell changes post-exposure. Secondary, endpoints will include analysis of soluble factors present in plasma and bronchial lavage and analysis of intracellular factors present in lung tissue obtained from a brush biopsy.

Submission Description:
With this renewal, an amendment dated 08/31/2010 addresses:

1. request to permit one subject to complete one additional exposure due to a prior session which was stopped due to a safety concern which was resolved upon expert physician input.
2. consent addendum for single subject and additional exposure session.

Regulatory and other findings:
Based on the information provided, the IRB has determined that HIPAA does not apply to this study.

Investigator's Responsibilities:
Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval.

Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

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

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Any unanticipated problem involving risks to subjects or others (including adverse events reportable under UNC-Chapel Hill policy) should be reported to the IRB using the web portal at https://irbis.unc.edu/irb.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

CC:
David Diaz Sanchez, Environmental Protection Agency
Robert Truckner, (EPA), Non-IRB Review Contact

THIS CONSENT DOCUMENT SHOULD BE USED ONLY
BETWEEN 9.20.10 AND 9.19.11
APPROVED BY
INSTITUTIONAL REVIEW BOARD, UNC-CHAPEL HILL

University of North Carolina-Chapel Hill

Consent to Participate in a Research Study and Consent for Genotype Screening
With Identifying Information
Subjects 18 years of age or older
Biomedical Form

IRB Study:# 09-1625
Consent Form Version Date: August 26, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D., David Diaz-Sanchez, Ph.D. and Kelly Duncan, Ph.D.
Department: United States Environmental Protection Agency
Phone number: 919-966-7320

Co-Investigators: Michael T. Schmitt, MSPH

Sponsor: U.S. Environmental Protection Agency

You are being asked to take part in a research study. The investigators listed above are in charge of the study; other professional persons may help them or act for them.

What are some general things you should know about research studies?

Research studies are designed to gain scientific knowledge that may help other people in the future. You may not receive any direct benefit from participating. There may also be risks associated with participating in research studies.

Your participation is voluntary. You may refuse to participate, or may withdraw your consent to participate in any study at any time, and for any reason, without jeopardizing your ability to participate with EPA-sponsored research.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want to participate. You will be given a copy of this consent form. You are urged to ask the investigators 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 research study is to understand how ozone exposure compares to that of diesel exhaust exposure in order to investigate mechanisms of injury by environmental pollutants. This may be especially important for asthmatics, because studies have shown an association between exposure to air pollution and increased medication usage and emergency room visits in this population. To accomplish this we will:

- Document changes in lung function (spirometry) and the presence of mild inflammation in the respiratory tract (measured by bronchoscopy to collect cells from the lower respiratory tract) after each exposure.
- Determine if air pollution exposure can affect components of the blood involved in inflammation and clotting.
- See if air pollution particles can cause changes in the ability of the nervous system to correctly regulate one's heart rate.
- Cells from your airways will be collected and stored. All specimen collections will be stored throughout the course of this study and then any remaining samples will be stored indefinitely as described in an accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information.***
- The cell samples will be used to identify the expression of a panel of inflammation- and oxidative stress-related genes. As new genes are identified that may be associated with adverse health effects of air pollutant exposure, expression of these genes may also be investigated.
- The cell samples will be used to identify epigenetic changes (mechanisms which control the expression of genes) associated with air pollutant exposure.
- Research reports have suggested that people with particular genes are more susceptible to air pollutants. The cells will be studied for those specific genes related to adverse health effects of air pollutant exposure.

Are there any reasons you should not participate?

You should not participate in this study if:

- You are pregnant, attempting to become pregnant or nursing an infant
- You have a history of significant chronic illness other than asthma, or your asthma is not well-controlled
- You are not 18 to 55 years of age
- You cannot perform intervals of moderate exercise
- You are not fluent with the English language
- You are taking more than 81 mg aspirin regularly
- You are unwilling or unable to refrain from the following medications for the week prior to the study: anti-inflammatory agents such as ibuprofen (Advil), naproxen (Aleve, Naprosyn) or aspirin
- You have skin allergy to tape or electrodes
- You are currently taking or have taken anti-coagulant medication in the week prior to the study
- You are currently smoking or have a smoking history within 2 years of the study
- You have been diagnosed as a severe asthmatic as defined by physician-directed emergency treatment for an asthma exacerbation within the preceding 12 months, use of systemic steroid therapy within the preceding 12 months, nighttime symptoms of cough or wheeze greater than 2 times per month, asthma symptoms (cough, wheeze, chest tightness) more than 2 times per week, requirement for albuterol due to asthma

symptoms more than 2 times per week, (not including prophylactic use of albuterol prior to exercise), have chronic and continuous allergic rhinitis.

- Your dosing level of an inhaled steroid is not consistent with the severity of your asthma as outlined by the NHLBI NAEPP guidelines
- You have a history of fainting in response to pain
- You currently have or have had a viral respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of the study. If this is the case, your visit will be rescheduled
- You currently have or have had seasonal exacerbation of allergic rhinitis symptoms within 1 weeks of the study. If this is the case, your visit will be rescheduled

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

- No over the counter pain medications such as aspirin (aspirin $\leq$ 81 mg/day is allowed), Advil, Aleve or other non-steroidal anti-inflammatory medications for 7 days prior to all visits. Tylenol (acetaminophen) is permitted.
- No vitamin C or E (or multivitamins which contain Vitamins C or E) for 7 days prior to all 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.
- Avoid eating or drinking anything after midnight on the evening of the Exposure Day 1, before the bronchoscopy to take place on Exposure Day 2.
- No caffeine for 12 hours prior to all study visits.
- Avoid use of antihistamines for one week prior to exposures.

How many subjects will participate in this study?

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

How long will your participation last?

Your participation in this study may require up to 9 visits to the research facility over approximately 6 months if you are eligible for the study.

What will happen if you take part in the study?

Before you agree to participate in this study, you must read the consent form in its entirety. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction.

You should have already undergone a general screening visit and physical examination, and a physical exam and pulmonary function test for bronchoscopy to certify that you are a suitable candidate. For the exposure study, we will perform the following procedures:

Training Day

- Report to the EPA medical station at the requested time.
- 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 this consent form. We will give one copy to you. We will check your vital signs (heart rate, respiratory rate, blood pressure, oxygen saturation level and a symptom questionnaire) and do a pregnancy test if applicable(WOMEN ONLY) For the pregnancy test, you will be asked to provide a urine sample. The purpose of this test is to provide evidence that you are not pregnant at the time of the study. The nurse will also ask you when your most recent menstrual cycle began. The results of the pregnancy test will be held in strict confidence. A positive pregnancy test will preclude further participation.
- You will undergo a breathing test. This is known as spirometry, and 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. The information gained from the breathing tests will be used by the investigators to determine whether you qualify for the study.
- You will be trained on the exercise bike and the work load to elicit a minute ventilation normalized for body surface of $25L/m^2/BSA$ will be determined.

Exposure Day 1: You will be asked to eat a normal breakfast and arrive at the EPA medical station no sooner than 8 am. You will need to wear comfortable clothes and shoes, and bring a lunch.

Prior to exposure, you will:

- Have your vital signs checked (heart rate, respiratory rate, blood pressure, oxygen saturation level) and fill out a symptom questionnaire. Have a pregnancy test if applicable.
- Have monitors placed on you so that we can watch your heart rate and rhythm. One of these monitors will be removed at the end of the day and the other monitor will be kept on for 24 hours. Before exposure, after exposure and the following morning during your next visit to the facility, there will be a 30 minute measurement of your heart rate. This will consist of a 20 minute rest period and a 10 minute data collection. The monitor will be removed after the last data collection. These monitors will allow us to determine if air pollutants cause small changes in the ability of your nervous system to regulate how your heart beats.
- Have up to 80 mL blood drawn (about 5 tablespoons). We will test this blood to see if diesel exhaust or ozone exposure affects the ability of your blood to clot correctly, and to look at changes in proteins and/or blood cells.
- 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.
- Given a Symptom Questionnaire for you to fill out.

- Testing will be postponed if it is determined that your medication history is not within previously established limits, respiratory tract illness has occurred within the previous 6 weeks or if respiratory symptoms are present.

During the exposure, you will:

Undergo a chamber exposure of 2 hours duration, once to clean air, once to diesel exhaust and once to ozone; the amount of pollution you will be exposed to is less than what you would likely encounter over 24 hours on a smoggy day in an urban area. You will be blinded as to the exposure condition. The exposure chamber is 6 ft wide by 6. ft in height, and is 8 ft in length. Chamber conditions will be at a comfortable temperature and relative humidity. A physician or other trained person will be seated outside the chamber observing you at all times. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. You will exercise for 15 minutes at the previously determined pace and rest for 15 minutes. While exercising, minute ventilation may be measured intermittently. Your blood pressure may also be measured intermittently. If it appears you are experiencing significant discomfort, breathing or heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid for your participation up to that point, but will be ineligible for further participation in the study and any payments you would have received for future participation.

Following the exposure, you will:

- Have a breathing test (spirometry). You will breathe through a filter into the machine. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have your vital signs checked.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Fill out a symptom score sheet.
- Have blood drawn (up to 80 mL or about 5 tablespoons).
- Be assessed and discharged by the nursing staff.

Exposure Day 2 (24 hour follow up visit): You will come back the next morning and asked to arrive at the EPA medical station at no sooner than 8 am. (approximately 24 hours after exposure) You will:

- Have your vital signs taken.
- Fill out a symptom questionnaire.
- Have blood draw (up to 80 mL or about 5 tablespoons).
- Have spirometry testing.
- Have a reading of your heart rhythm taken with the Holter monitor.
- Have the Holter monitor removed.
- We are asking all subjects to have bronchoscopy on the day following each exposure. If you are an asthmatic, prior to the bronchoscopy you may be pretreated with inhaled bronchodilator (albuterol). The bronchoscopy will not be performed if the baseline lung function shows more than a minimal amount of airway obstruction and this does

not resolve with inhaled bronchodilator. During the bronchoscopy, a fiberoptic scope will be inserted through the nose into one of your airways where a portion of one of your lungs will be washed with sterile saline and a sample of airway lining cells will be obtained with a special brush device.

Bronchoalveolar Lavage (BAL) and Brush Biopsy is frequently used in the diagnosis of lung disease in both adult and pediatric patients. The procedure has also been used in numerous research studies of persons with respiratory disease and in healthy volunteers. The procedure has been used safely many times in studies conducted at our laboratory. The purpose of the BAL procedure is to obtain fluids and cells from the lower regions of the respiratory tract, i.e., the trachea, bronchi and smaller airways. These materials will be analyzed to obtain information about the role of various chemical substances and cells in the pulmonary response to particle exposure of persons with asthma. The bronchoscope is a flexible fiberoptic tube that is about two feel long and 1 centimeter in diameter (about the diameter of a pencil). It is an optical device with a light at the end which can be used to transmit images to a camera connected at the other end. Using the bronchoscope, the physician can see into your airways and direct the placement of the bronchoscope. A small channel allows fluid to pass through the bronchoscope.

The BAL procedure will be performed at the laboratory medical station by specially trained experienced pulmonary physicians. You will be expected at the medical station at 8:00 on the morning after the exposure. If you have had anything to drink or eat since midnight the night before the bronchoscopy you will not be allowed to proceed. Before you undergo bronchoscopy, your lung function will be evaluated and if you are an asthmatic you may be treated with inhaled bronchodilator (albuterol). The procedure will not be performed if your lung function does not meet a predetermined level of function recommended by the National Institute of Health for performing research bronchoscopies in persons with asthma. You may terminate the bronchoscopy procedure at any time. If the physician determines an increase level of anxiety, the procedure will be terminated

A saline lock intravenous catheter will be placed in a vein in your arm to give you medication before the procedure starts (a saline lock is a flexible catheter that stays in your arm for a short time). The saline lock will remain in place so that it can be used to administer medications in case medications need to e administered during the procedure. You will also be connected to a telemetry monitor that will display heart rate and rhythm, a blood pressure cuff will be placed on your arm, and an oximeter sensor (small band like device will be placed on a finger to allow the medical staff to monitor your oxygen levels during the procedure. No sedatives and/or narcotics will be administered at any time during bronchoscopy.

Before proceeding, the medical staff will again make certain that you have had nothing to eat or drink since midnight the previous night. You will be asked to decongest your nose by administering 2 puffs of neosynephrine nasal spray to each nostril. They will then give you a lidocaine solution and ask you to gargle with it for about two minutes to anesthetize your throat. You will then be asked to inhale (snort) a small amount of lidocaine jelly through both nostril to anesthetize your nose and the back of your throat. A Q-tip with lidocaine jelly will be gently inserted into your nose

to ensure that your nose is completely numb before the bronchoscope is inserted. The procedure will not begin until your nose and throat are well anesthetized. If this cannot be accomplished, the bronchoscopy will not be performed. A tube delivering oxygen will be placed inside your other nostril. Delivery of supplemental oxygen is done as a precaution during all bronchoscopies conducted at our facility.

To start the procedure, the physician will pass the bronchoscope through your anesthetized nostril to the back of your throat and then to above your vocal cords. The pulmonologist will then inject a lidocaine solution to numb your vocal cords before passing the bronchoscope into your trachea (windpipe). More lidocaine, up to a safe maximum dose is injected at various points in your trachea and airways to minimize coughing during the procedure. You will experience some cough during the procedure. This is a normal reflex caused by the presence of the bronchoscope in your airway.

Brush biopsies of your airway epithelium will be taken from the major lower airway passages. A very small brush (3 millimeters in diameter) will be inserted through the channel in the bronchoscope. The brush is visible to the physician performing the procedure through the bronchoscope. Small amounts of surface cells are scraped from the airway by gently brushing the the wall of the airway several times. Up to 8 brush biopsies are taken.

BAL will be performed by gently wedging the bronchoscope in a small airway of the right lung. Sterile saline will be injected in your lung through a channel in the bronchoscope. The saline will then be gently suctioned from your lung through the channel in the bronchoscope. This sequence will constitute a wash. A total of up to 250 cc (about 20 tablespoons) of sterile saline will be used during the BAL procedure. Approximately 75% of the saline injected into your lungs will be recovered by aspiration (suction) through the bronchoscope. The remaining 25% is expected to remain in your lungs. The saline left in your lungs will not generally cause you any difficulty breathing or harm you and should be completely absorbed by your lungs within 48 hours.After the wash and brush biopsies are obtained, the procedure will be complete and the bronchoscope will be removed from your airway. The total time the bronchoscope will reside in your airways will be up to 30 minutes.

After the procedure the oxygen catheter will be removed if your oxygen saturation is acceptable. The nurses will check your vital sign immediately after the procedure and every half hour thereafter until discharge. During this time you will sit in a recliner at the medical station for an observation period of approximately two hours. You will not have any food or drink during this time. After the recovery period, the nurses will check your gag reflex. Since the gag reflex will be absent due to anesthesia during the procedure, you will not be allowed to ear or drink until the anesthesia wears off. This normally takes about one to one-and-one half hours. Once your gag reflex returns, you will then be given some juice to sip and then some food.

The physician who performed the procedure will check you after the recovery period and you will repeat the spirometry lung function test. You will be discharged if your vital signs are stable, chest examination is normal If you are an asthmatic, before you are discharged, you may perform spirometry and your lung function should be equal to or greater than 80% of your pre-procedure value. Prior to discharge you will be requested to take 600 mgm of ibuprofen by mouth; administration of

ibuprofen/motrin almost always prevents the post-bronchoscopy malaise and low grade fever that would otherwise occur in about 25% of persons undergoing the procedure (acetaminophen/tylenol is a less effective alternative medication).

Before going home, you will be give the phone number of the medical station (966-6232) and pager phone numbers for the physician who performed the bronchoscopy with instructions to call if you experience any adverse symptoms such as: 1) persistent fever or fever above 101 degrees Fahrenheit, 2) persistent cough, 3) sputum (phlegm) production, 4) chest pain, 5) coughing up any amount of blood, 6) nose bleeds, or 7) shortness of breath

Are there any reasons you should not participate in bronchoscopy?

You should not participate in this study if you have problems with excessive bleeding after minor cuts or abrasions. You should not participate if you have had a recent exacerbation in your asthma that was not controlled with your usual medications. The physician and medical staff will explain other exclusionary condition in detail to you. In addition, during your participation in this study, you will be asked to refrain from, limit, or control taking the following medications:

*No more than 81 mg aspirin or similar medication on a regular basis or during the week of testing.
*Withhold antihistamines for 7 days prior to testing.
*Withhold inhaled bronchodilators 12 hours prior to testing.
You should not participate in this study if you are unwilling or unable to remain in the local area for 24 hours following each bronchosocpy

With your permission, DNA from the collected cells will be genotyped for specific genes related to adverse health effects associated with air pollution exposure. Unwillingness to have samples genotyped will NOT exclude you from participating in the study. If you do not wish for your cells to be used for genotyping but do wish to participate in the study do not sign the section ***Subject's Agreement for Genotype Screening With Identifying Information*** located at the end of this document.

What if we learn about new risks 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.

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. You will be given a separate consent form for this storage, and you do not have to allow your samples to be stored indefinitely. With your permission, we will continue to store any samples that we collect to possibly use for other studies. This allows us to make the best use of the samples we collect from our volunteers. Again, you do not have to allow us to keep these samples if you do not want to.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. You will not benefit

personally from being in this research study. We will share with you any information that we learn about your lung function or other medical findings. We can also send you the results of some of the blood tests we perform.

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

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

2. 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. Also, the forceful breathing during this test can cause wheezing or shortness of breath to occur.

3. The adhesive patches used as part of the heart monitoring system may cause some mild skin discomfort or discoloration. If this occurs you should notify the nursing staff.

4. During air pollution exposure, you may experience some minor degree of airway irritation, cough, shortness of breath or wheezing. Some studies suggest that persons with asthma may be at risk for developing an asthma attack as a result of exposure to fine air pollution particles. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. Your symptoms will be closely monitored during the exposure and the study terminated if more than modest respiratory symptoms occur during the exposure. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. If you experience a significant drop in your lung function, albuterol will be administered.

Air pollution may induce an inflammatory reaction that can last for 24 hours after exposure and may increase the chance of you catching a cold. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period. There is a small possibility that you could have more asthma symptoms for several days, and even less likely, for several weeks, after the particle exposure. This flare in your asthma would be similar to what happens to you and other asthmatics during the allergy season or with a viral infection. Such a flare is considered highly unlikely and the exposure risk is comparable to walking around a major metropolitan area such as Los Angeles or Houston on a smoggy day. If an asthma flare occurs, you will be asked to seek medical treatment from your regular physician who treats you for your asthma.

If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You

will be taken to the exposure chamber when you are first evaluated for suitability for the study to allow you an opportunity to see where you will sit and what the chamber looks like. The chamber dimensions are 6 ft in width, 6 ft in height, and 8 ft in length. Although the chamber is somewhat small, it has multiple windows and you will be in constant visual contact with the investigator who will be monitoring you during the exposure. You also will be able to verbally communicate with the investigator at all times.

5. Bronchoscopy: As with other medical procedures, there are risks associated with bronchoscopy. However, experience has shown these risks are extremely small in research subjects. The risks associated with bronchoscopy are described in detail below.

A primary risk is bronchoscopic procedures is **discomfort to the nose and throat** brought about by the insertion of the bronchoscope through the nasopharynx. This discomfort should be alleviated or eliminated by the use of local anesthetics such as lidocaine. For this reason, you will be asked to gargle with a lidocaine solution and have lidocaine jelly placed in your nose prior to beginning the procedure. The effectiveness of the anesthesia will be tested by using a cotton swab gently inserted into your nose before inserting the bronchoscope. If you experience pain or discomfort during insertion of the bronchoscope, more lidocaine will be used up to a safe maximum amount. If adequate anesthesia cannot be achieved using this amount of lidocaine, the procedure will not take place.

A second risk of bronchoscopy is **coughing** that results from the irritation of the airway caused by the presence of the bronchoscope in the airway. Coughing is a normal reflex to expel foreign substances from the airways. Because coughing itself can cause mild trauma and discomfort to the airways and vocal cords, lidocaine liquid is sprayed on the airways at various sites to suppress the cough reflex. This technique is usually not completely successful and it is expected that you will have some cough during the procedure, especially during the initial insertion of the bronchoscope into the airway. If coughing is not controllable with the lidocaine or causes you discomfort, the procedure will be terminated.

There is a slight chance that the bronchoscopic procedure will precipitate an **asthma attack**. This risk is minimized by 1) only allowing persons with mild stable asthma to participate in the study, 2) pretreatment with inhaled bronchodilator (albuterol) before the procedure if your lung function is impaired and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physician responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. You will not be discharged from the EPA Medical Station until your lung function has returned to 80% of your pre-procedure performance. . In the unlikely event that you require additional treatment because the asthma attach continues, you will be transported by ambulance to the emergency room of the UNC Hospital, located 1/4 mile away.

Nose bleeds are another risk of bronchoscopy. This is caused by trauma to the nose during the bronchoscopy procedure. Usually, the bleeding is minor and is

noticed as a few drops of blood in mucous secretions in the nostril. The bleeding usually stops on its own within an hour after the bronchoscopy. On very rare occasions, if the bleeding becomes moderate to severe, your nostril will be packed with sterile gauze to absorb the blood and put pressure on the site of bleeding. Should you require additional treatment, you will be transported to the UNC Hospital Emergency Room. Major nose bleeds during bronchoscopy are very rare and generally limited to persons with kidney disease or a blood clotting disorder. It could also be promoted by chronic use of aspirin (>81 mg), ibuprofen or similar medications. You will not be allowed to participate in this study if you are taking such medications. Therefore the probability of getting a major nose bleed is very small.

Major **bleeding of the airways** is another rare (less than one percent) complication of bronchoscopic procedures. Most episodes of major bleeding due to bronchoscopy occur in persons with underlying predisposing conditions and are due to forceps biopsies of the airways mucosa which will **not** be performed in this study. People who suffer from kidney disease, blood clotting disorders or are chronic users of aspirin-like drugs are at increased risk of bleeding during bronchoscopy. In addition, people with lung cancer or with serious lung infection are more likely to suffer from airway bleeding during bronchoscopy. Your screening medical history, physical examination, and blood analysis will reveal most conditions that would increase your risk of bleeding during the bronchoscopy. You will not be allowed to participate if you have any of these conditions. Therefore your chances of incurring a serious airway bleed during bronchoscopy are extremely small. Nevertheless, if you do experience bleeding during bronchoscopy, you will be under the care of an experienced physician who will take steps to stop the bleeding immediately. A special cart with emergency supplies is available to handle medical emergencies. In the event of a major bleeding episode, you will be immediately transported to the Emergency Room of the UNC Hospital.

Pneumothorax or collapsed lung is another possible complication of bronchoscopic procedures. This complication is more likely to occur during tissue biopsy in the lung periphery, not the major central airways which will be the focus of this study. However you will be advised of the symptoms in the event that you experience a pneumothorax. The symptoms include chest pain and shortness of breath. If you experience these symptoms, you should contact the medical station (966-6232), or page the physician who performed the bronchoscopy. The vast majority of pneumothoraces occur within 24-48 hour of the procedure being completed. While a serious condition, a pneumothorax can be readily treated in an emergency room.

The **lidocaine** used to anesthetize your nasopharynx and reduce your cough can pose certain risks as well. A small fraction of the lidocaine applied to your nose and airways will be absorbed into your bloodstream, where it could cause adverse effects. Your risk of suffering side effects are very small, but would be much greater and potentially life-threatening if you are allergic to lidocaine or other topical anesthetics. You will not be allowed to participate in this study if you have drug allergy to topical anesthetics. When used at high doses, lidocaine can also cause symptoms in your central nervous system (confusion, tremor, euphoria, or,

rarely, seizures) or heart rate disturbances (very fast or very slow heart rate) if an excessive dose of medication is used. Finally, a death in a volunteer receiving an overdose (over 1000 milligrams) of lidocaine during bronchoscopy has been reported from Rochester, New York. However, no serious side effects of this medication have been noted at lower doses such as those described in this protocol which uses up to 360 milligrams of lidocaine during the entire procedure. If any problems develop secondary to the use of lidocaine, the physician bronchoscopist and the doctor on duty that day at the Human Studies Division of the EPA will be available to handle these problems.

Prior to the start of the bronchoscopy **atropine** may be given though a vein in your arm in order to counteract any changes in blood pressure that would result from the placement of the bronchoscope in your airway and to limit the quantity of secretions in your nose and throat. Atropine can cause dry mouth and nose for 30 to 60 minutes after the procedure is completed. This effect should wear off about an hour after the procedure is completed.

Placing an **intravenous catheter** in your arm carries a small risk as well. The needle stick can be momentarily painful and more rarely can leave a bruise in your arm. The medical staff who will be placing the catheter are very experienced and therefore the risk is minimal. Infection is also a very small risk that can result from placing the catheter. Again, the medical staff is very experienced in the use of sterile technique to prevent infection. If you notice redness or swelling in the area surrounding the catheter site, you should contact the medical station at 966-6232 or the physician on call for treatment.

A low grade fever (less than 101EF) during the 24 hours following the procedure occurs in about 25% of patients with respiratory disease undergoing bronchoscopy and BAL. The fever is benign and is usually effectively prevented by administering ibuprofen following the procedure. However, if you experience fever greater than 101 EF or lasting longer than 24 hours you could have pneumonia and should contact the Medical Station immediately so that you can be examined and treated.

Pneumonia occurs in less than 1 percent of patients undergoing bronchoscopy and BAL. Signs of pneumonia include fever greater than 101 EF, or persisting for more than 24 hours, persistent cough with or without sputum production, coughing up blood, chest pain, and shortness of breath or wheeze. Should you experience these symptoms, it is imperative that you contact the Medical Station to be seen and evaluated by a physician. As a routine precaution the Medical Station Staff will contact you between 24 and 48 hours following the procedure to inquire about any symptoms you may be experiencing.

In addition, there may be uncommon or previously unrecognized risks that might occur.

There are no risks 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 may cause some irritation, itching, or burning in some people. If this occurs you should notify the nursing staff.

The following are policies and procedures to minimize risk during the experiment:
1. During this study, skilled personnel, in an adequately equipped facility, will be observing you for any adverse effects. You may, without penalty, stop the test at any time by telling the monitor.
2. A physician will be on call at all times during the study to deal with any medical problems that may arise.
3. Every test has some degree of risk and there may be some risks associated with this experiment which are unforeseeable. You should not participate in the study if you have an acute illness.
4. You may terminate your participation in the experiment at any time and for any reason that you wish without penalty; you will be paid an amount for participation prorated for the time and procedures in which you took part. You understand that you are not under any obligation to complete any test procedures which you find objectionable or uncomfortable.
5. The investigator may terminate your participation in the experiment at any time. If this happens, you will be entitled to full payment for your time commitment and any experimental activities up to that point.
6. Your participation in the study may be postponed if your pre-exposure lung function shows airflow obstruction or if you develop a cold or asthma exacerbation.

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. Samples used for genetic analysis will be stored at the EPA Human Studies Facility.

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, the U.S. EPA and/or UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by

representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?
All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 et. seq., provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research related injury or illness occurs, you should contact the Director of the EPA NHEERL Human Research Protocol Office at 919.966.6217.

What if you want to stop before your part in the study is complete?
You may withdraw from this study at any time. If you choose to withdraw or are unable to complete the study because of not following the instructions in this consent form, you will receive compensation only for your participation up to that point.
The investigators have the right to stop your participation at any time. This could be because you have had an unexpected reaction, have failed to follow instructions, or because the study has been stopped. After you enroll, if you are dismissed by the investigators before completing the study for reasons that are not your fault, you will be paid for your participation up to that point.
We made need to cancel your study activity due to adverse weather conditions, equipment failure, or other unforeseen events. If a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid for the time scheduled and cancelled at the standard hourly rate of $12 per hour plus any cancelled activities up to a maximum of $100. When possible, you will be rescheduled at a later time.

Will you receive anything for being in this study?
You will be paid approximately $12 per hour for your participation in this study. Total compensation will be based on the number of exposure sessions that will be required.

1. If you are complete all three exposures sessions of this study, your total compensation including screening will be approximately $2516.

We anticipate performing several tests on you during the course of this study. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent us from performing a specific test on you. If we are unable to perform a specific test on you which is a primary endpoint for us, you will be compensated for all tests and time completed on that day and rescheduled. If this test is a secondary endpoint for us and is

also a source of compensation, you will be paid for that test, but not rescheduled to make up the procedure.

In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service as required by law. 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. Compensation for the visits will be as follows:

You will be exposed to diesel exhaust ,ozone and have an air control exposure in three separate sessions. Exposure Days 1-2 will be repeated for the second and third chamber exposure session, but a second training Day is not needed. If you complete three exposures, your compensation including the pre-study qualifications and training day will be approximately $2516.

Pre-study qualifications

recruitment screening	$15	Previously paid
physical exam	$15	Previously paid
bronchoscopy physical exam	$20	Previously paid

Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24

Training Day Total = $ 44

You will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure
Endobronchial Brush biopsies	$25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus	**$100**

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day)	**$774**
TOTAL for completion of TWO exposures (excluding Prestudy qualifications and training day)	**$1548**
TOTAL for completion of THREE exposures (excluding Prestudy qualifications/training day but including completion bonus)	**$2422**

If you begin but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, you will receive compensation for your participation up to that point however in you arrive in the lab for the procedure having not followed instructions (eating food, taking NSAIDs or aspirin, or failing to report cold or flu symptoms the day prior to the bronchoscopy) you will be compensated $15 for your time, but will not receive compensation for the bronchoscopy.

You will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available.

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 or follow directions of the investigators, you will be paid for that portion of the study that has been completed. In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, you will be paid $12 per hour for the time scheduled and cancelled, and 50% of the reimbursement amount for procedures that are canceled up to a total maximum of $100 for all procedures. You will be paid in full for any procedures that may have been started during a current visit if cancelation occurs while you are in the facility. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible,

canceled visits will be rescheduled. The investigators also have the right to stop your participation in the study at any time. This could be because you have had an unexpected reaction, or because the entire study has been stopped, or for some other reason. If you are dismissed by the investigators prior to completion, you will be reimbursed for time and procedures completed up to that 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/Carrboro you will be reimbursed for mileage at the US Government mileage rate in effect at the time.

Who is sponsoring this study?
This research is funded by the United States Environmental Protection Agency. This means that the research team is being compensated by the sponsor for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the product being studied.

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 US Environmental Protection Agency. This means that the research team is being paid by the sponsor for doing the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, 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 the Chairman of the IRB Committee at 919-966-1344 and /or the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

What specimens will be collected and stored?

Bronchial epithelial cells collected via bronchoscopy and all blood specimens will be stored with your permission. If you agree to have your specimens stored for future research you will need to sign the accompanying consent form ***Consent for Storing Biological Specimens With Identifying Information***.

IRB Study:# 09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D., David Diaz-Sanchez, Ph.D., and Kelly Duncan, Ph.D.

Subject's Agreement to Participate in the Research Study with Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I voluntarily **AGREE** to have my provided bronchial epithelial cells genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

Signing here and agreeing to participate in this study DOES NOT allow us to store your samples.

_______________________________ _______________
Signature of Research Subject Date

____
Printed Name of Research Subject

_______________________________ _______________
Signature of Person Obtaining Consent Date

____
Printed Name of Person Obtaining Consent

IRB Study:# 09-1625

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Subject's Agreement to Participate in the Research Study without Genotyping Consent:

I have read the information provided above. I voluntarily **AGREE** to participate in this study and I **REFUSE** to have my provided bronchial epithelial cells genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

______________________________ ______________

Signature of Research Subject Date

____

Printed Name of Research Subject

______________________________ ______________

Signature of Person Obtaining Consent Date

____

Printed Name of Person Obtaining Consent

- -

THIS CONSENT DOCUMENT SHOULD BE USED
BETWEEN 9.20.10 AND 9.19.11
BY
INSTITUTIONAL REVIEW BOARD, UNC CHAPEL HILL

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

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IRB Study # 09-1625
Consent Form Version Date: August 26, 2010

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Principal Investigator: Melanie J. Jardim, Ph.D, David Diaz-Sanchez, Ph.D., and Kelly Duncan
UNC-Chapel Hill Department: US EPA
UNC-Chapel Hill Phone number: 919-966-7320
Email Address: jardim.melanie@epa.gov
Co-Investigators:
Funding Source and/or Sponsor: EPA funds

Study Contact telephone number: 919-966-7320
Study Contact email: jardim.melanie@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 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."

The purpose of this particular repository or biobank is to:

- Document changes in lung function (spirometry) and the presence of mild inflammation in the respiratory tract (measured by bronchoscopy to collect cells from the lower respiratory tract) after each exposure.

- Determine if air pollution exposure can affect components of the blood involved in inflammation and clotting.
- See if air pollution particles can cause changes in the ability of the nervous system to correctly regulate one's heart rate.
- Store samples of respiratory cells and blood samples. These collections are part of the protocol described in the accompanying consent form **Consent to Participate in a Research Study** and the components stored represent excess tissues that are not immediately required for the study but may have value for future research.
- Cells from the lung will be used to identify the expression of a panel of inflammation- and oxidative stress-related genes. As new genes are identified that may be associated with adverse health effects of air pollutant exposure, expression of these genes may also be investigated.
- Research reports have suggested that people with particular genes are more susceptible to air pollutants. The epithelial specimens will be genotyped for those specific genes related to adverse health effects of air pollutant exposure.
- Studies of gene expression represent one type of genetic research, thus future investigations involving these specimens are intrinsically of a genetic nature.

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

How will the specimens be collected?
Samples will be collected in the following manner.

- Lung cell collection. Cells will be collected from the airways and airspaces of the lung by bronchoscopy with bronchoalveolar lavage and brush biopsy. (This procedure is explained in separate consent.) Fluid will be collected into specimen tubes and placed on ice. The cells will be separated from the fluid by centrifugation (very fast spinning whereby the cells will separate from the liquid and fall to the bottom of the tube), and both samples may be processed immediately, or frozen for later analysis.
- Blood collection. You will have up to 80 cc (about 5 tablespoons) of blood per sample collected from an arm vein by our trained nursing staff. The blood will be placed into specially designed blood collection tubes which allow separation of the fluid and cell portions of blood. Those samples may be processed immediately, or be frozen for later analysis.

What will happen to the specimens?
Samples will be stored at the U.S. Environmental Protection Agency Human Studies Facility located in Chapel Hill, North Carolina. Only project members of the study will have access to the samples. Blood samples will be collected in order to look at but not limited to, indicators of inflammation due to the air pollution exposure. Specimens may be released to other investigators if there is a need to analyze samples with equipment that is not located at our facility.

What are the possible benefits to you?
Benefits to you are unlikely. These studies (current and future) may provide additional information that will be helpful in understanding asthma and how environmental factors affect the disease

What are the possible risks or discomforts involved with the use of your specimens?
This study might involve the following risks and/or discomforts to you:

Risk of breach of confidentiality is minimal. For the mild asthmatic subject, it is conceivable that an employer or insurance company could learn of the diagnosis if you required medical intervention as a result of participating in this study. You will be assigned a study number which will be used for data – not your name. The study number is all that will be entered into computer databases. All paper files which may contain your name or screening number are secure in the EPA building which has limited access 24 hours/day. A numeric coding system will be used to ensure that you cannot be directly identified from the samples alone.

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

Will you receive anything for the use of your specimens?
You will not receive anything for taking part in this research.

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

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

No one will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, U.S. EPA and/or 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.

You will not be identified in any report or publication about research using your specimens. 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 could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

A Federal law called the Genetic Information Nondiscrimination Act (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. GINA does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. GINA also does not protect you against discrimination based on an already-diagnosed genetic condition or disease.

Will researchers seek approval from you to do future studies involving the specimens?
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants. In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

Will you receive results from research involving your specimens?
Most research with your specimens is not expected to yield new information that would be meaningful to share with you personally. There are no plans to re-contact you or other subjects with information about research results.

Can you withdraw the specimens from the research repository?
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. It is best to make your request in writing.

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

What will happen if you are injured by this research?