OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 19-Feb-2008

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

Date: November 9, 2011

Title of Study: Cardiopulmonary Effects of Exposure of Healthy Older GSTM1 Null and Sufficient Individuals to Concentrated Ambient Air Particles (CAPTAIN)

Name and degrees of Principal Investigator: James M Samet, PhD, MPH

Co- Principal Investigator: Haiyan Tong, MD, PhD

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For trainee-led projects: __ undergraduate __ graduate _ postdoc __ resident __ other X NA

Name of faculty advisor: James M Samet, PhD, MPH
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Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; Ana Rappold, PhD, Susan Steck, PhD, RD; Haibo Zhou, PhD, Heidi Hiers, RN, Michael (Billy) Ray, BS.

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

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

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

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

Check	Item	Total No. of Copies
X	1. This application. One copy must have original PI signatures.	3
X	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
X	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
X	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
□	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. Investigator Brochure if a drug study.	1

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

Signature of Principal Investigator _____ Date _____

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

Signature of Faculty Advisor _____ Date _____

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

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

Signature of Department Chair or designee _____ Date _____

Print Name of Department Chair or designee _____ Department _____

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	X	_ _
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	_X_	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_X_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations: a. UNC-CH students or UNC-CH employees? b. Non-English-speaking? c. Decisionally impaired? d. Patients? e. Prisoners, others involuntarily detained or incarcerated, or parolees? f. Pregnant women? g. Minors (less than 18 years)? ***If yes***, give age range: to years	 _X_ __ __ _ __ __ __	 __ _X_ X__ _X _X_ _X_ _X_
A.2.5. a. Are sites outside UNC-CH engaged in the research? b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study? ***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)	__ __ __ __	_X_ _X_ __ __
A.2.6. Will this study use a data and safety monitoring board or committee? ***If yes:*** UNC-CH School of Medicine DSMB? (must apply separately) Lineberger Cancer Center DSMC? Other? Specify:	__ __ __ __	_X_ __ __ __
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc? b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__ __	_X_ _X_
A.2.8. a. Investigational drugs? (provide **IND #**) b. Approved drugs for "non-FDA-approved" conditions? *All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).*	__ __	_X_ _X_
A.2.9. Placebo(s)?	__	_X_
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_X_
A.2.11. Fetal tissue?	__	_X_
A.2.12. Genetic studies on subjects' specimens?	_X_	__
A.2.13. Storage of subjects' specimens for future research? ***If yes***, *see instructions for* ***Consent for Stored Samples***.	_X_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? ***If yes***, *approval by the* ***UNC-CH Radiation Safety*** ***Committee*** *is required.*	__	_X_
A.2.15. Recombinant DNA or gene transfer to human subjects? ***If yes***, *approval by the* ***UNC-CH Institutional Biosafety*** ***Committee*** *is required.*	__	_X_
A.2.16. Does this study involve UNC-CH cancer patients? ***If yes***, *submit this application directly to the* ***Oncology Protocol Review Committee***.	__	_X_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? ***If yes***, *obtain the* ***GCRC Addendum*** *from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	_ _	X
A.2.18. Will gadolinium be administered as a contrast agent?	.._	.._X_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_X no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_X no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no

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

_____

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

_____ _____

Signature of Principal Investigator Date

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

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

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

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: Advanced age and common genetic polymorphism of the antioxidant enzyme glutathione-s-transferase M1 (GSTM1) appear to confer susceptibility to ambient particulate matter (PM). This study will compare responses to PM exposure in older volunteers with GSTM1 null and sufficient genotypes.

Participants: 30 healthy 50-75 yo subjects.

Procedures (methods): Subjects will be placed on a diet restricted of omega and oleic fatty acids for approximately 42 days prior to undergoing sequential exposures to air (control) and concentrated ambient PM (CAP) for 2 hr. Pulmonary, vascular and cardiac function will be evaluated pre, immediately post and 18 hr post exposure.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to ambient levels of particulate matter (PM) and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated a disproportionate risk among the elderly who are exposed to PM (2). Dietary factors such as intake of omega-3 fatty acids have been linked to human susceptibility to the adverse effects of ambient PM (14).Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern over elevated rates of cardiovascular events related to both short-term and long-term exposure to PM (3). The risk of death from cardiovascular disease (myocardial infarction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs, which is a reflection of autonomic tone on the heart (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9) and acute-phase reactants (10, 11) such as C-reactive protein (12). Animal

studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentiates atherosclerosis (13). Despite a decade of intensive studies, much about the PM health effects problem, especially the cardiovascular effect, is still not well understood. The present study is designed to test the hypothesis that genetic expression of the Phase II metabolizing enzyme GSTM1 alters the outcome of adverse responses to PM exposure.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.
12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Holguin F, Tellez-Rojo MM, Lazo M, et al. Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. *Chest.* Apr 2005;127(4):1102-1107.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female volunteers of any ethnicity and race. The study will enroll both GSTM1 null and sufficient subjects. The fraction of subjects who are GSTM1 null will be targeted to be approximately 40 %, reflecting the prevalence of this polymorphism in the general US population.

Based on recently completed studies for subjects with the characteristics sought in this study, the expected screening attrition rate is up to 70 %, with a heavy effect of genotype requirement. Post-screening completion rate is up to 50 %. Extrapolating from these rates, we can expect a 15% overall completion yield. Therefore, to obtain 30 subjects, we will enroll up to 200 subjects.

Subjects will be recruited through an-on site contract with the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria:

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- History of bleeding diathesis.
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for approximately 6 weeks before the exposures. Calcium supplements and statins are permitted.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Eye or abdominal surgery (e.g., hernia surgery) within 6 weeks will be exclusionary.
- Active allergies. Seasonal allergies are not exclusive if out of season throughout participation in study.
- A history of chronic illnesses such as diabetes, cancer (non-melanoma skin cancer may be acceptable), rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or asthma. Hypothyroid is acceptable.
- Subjects will be required to avoid taking omega-3 fatty acids for 6 weeks before the exposure day. Subjects who are on prescriptions taking omega-3 fish oil as therapy will be excluded.
- Subjects will be instructed to avoid more than one 4-6 oz/serving of all types of fish and shellfish, as well as all types of nuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil,

soybeans and soy products, omega-3 fortified eggs, and cod liver oil for 6 weeks before their first exposure. Individuals who are unable to comply with these restrictions will be excluded.

- Subjects will be required to limit their use of all cooking oils, dressings, and sauces during the study and to avoid olive oil and other vegetable oils because of their oleic and omega-3 contents. Use of a cooking spray such as PAM will be allowed.
- Male subjects will be asked to refrain from using erectile dysfunction drugs 72 hrs before exposure.
- Because of reported cardioprotective effects of red wine, subjects will be prohibited from drinking red for 6 weeks prior to their first exposure.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year) or smoked more than 5 pack-years in lifetime.
- Because the exposures use a facemask that could trigger headaches in sensitive individuals, subjects who suffer from migraines will be excluded from participation.

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.

Approximately 30 subjects will complete a regimen in which each subject will undergo exposures to clean air and to CAP sequentially on successive days. Each exposure will be 2 hours in duration with the subject at rest in a specialized exposure chamber.
The primary endpoints include HRV, pulmonary function, and biomarkers that include neutrophils, platelets, fibrinogen, C-reactive protein, lactate and plasminogen activator inhibitor. We will also assess endothelial cell function by brachial artery ultrasound. We will carefully monitor the symptoms that subjects may develop during the exposure and over a 24 hour period following exposure. The symptoms include chest pain, dyspnea, pallor, ataxia, and significant heart rhythm anomalies like rapid heartbeat or skipped beats, or arrhythmias noted on EKG. In addition, analyses of blood samples taken after exposure will be scrutinized for abnormalities, including neutrophil counts. Symptoms and/or changes detected in the genotyping blood samples that are considered clinically significant could prompt the study physicians to stop the study. To the extent possible, subjects who fall ill during their participation in the study will be rescheduled.

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for a genetic screening appointment in the medical station in the US EPA Human Studies Facility (HSF). Depending on the need to maintain a representative population of subjects over time, GSTM1 null or sufficient subjects may be enrolled exclusively at various points in the study. At that time a genotype screening informed consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 26, methods of recruiting) will not need genotype screening. Subjects will then undergo a brief medical history screening and blood pressure measurement. Subjects will then have approximately 20 ml of blood collected. A portion of the blood will be used for genetic screening for GSTM1 sufficiency and another portion of the blood will be used for the cholesterol levels, fatty acid analysis, and biochemistry analysis. Information obtained from the blood analyses at this point could be used by the study physicians to stop a subject's participation or end the study. Subjects will be given a copy of the Medical History Form to be filled out and mailed back after they are selected on the genotype.

GSTM1/GSTP1 genotype screening: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1 and other non-selective genotypic markers such as GSTP1. Total DNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene polymorphism.

Physical exam: Subjects who are not excluded during the genetic screening will be scheduled for a physical examination in the HSF. During this visit, subjects will sign an informed consent to undergo the screening procedures and physical examination as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Subjects will then undergo a physical exam, pulse oximetry, and screening/baseline 12-lead electrocardiogram (ECG). A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam and genetic screening will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. Subjects will ask any questions they might have regarding their participation in the study. Subjects will then undergo spirometry for pulmonary function assessment. Subjects will also undergo a min ventilation measurement recorded while sitting at rest. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the training day and on the exposure day if more than 7 days since the last pregnancy test have elapsed.

Dietary Restriction and Recording: As specified above under Subject Exclusions, subjects will be required to restrict their dietary consumption of fish, seafood, oils, nuts, omega-3 fatty acid fortified foods and dietary supplements and vitamins for approximately 42 days. During their training day subjects will receive instruction on foods to avoid and on estimating dietary portions. Subjects will be asked to record their diet for two 24 hr periods, one during the week and one during the weekend, approximately midway during their dietary restriction period.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Refrain from eating pan fried and/or grilled foods after midnight prior to the exposure day.
- Refrain from ingesting caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.
- Bring a lunch to each exposure day. The lunch composition will be specified to minimize potential variations that could affect the arterial diameter measurements. Subjects will be compensated for the cost of the lunch.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (blood pressure, HRV, endothelial cell function by brachial artery ultrasound (BAU), pulmonary function by spirometry and diffusion limited uptake of CO (DLCO), and blood sampling) will be completed.

- ***HRV measurement*** will be done with the use of a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 48 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20-30 minutes in a reclined position.

- ***Brachial artery ultrasound.*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed using a 14 MHz imaging probe interfaced with an Ultrasonix SP or Touch Pharma ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Pulmonary function***: will be measured by spirometry, including measurement of single breath CO diffusing capacity (D_LCO). Cardiac output (Qc or pulmonary capillary flow) will be simultaneously measured from the uptake of inhaled acetylene gas during the same single breath procedure.

- ***Blood sampling:*** approximately 46.2 ml of blood will be collected pre-exposure in the study.

- ***Symptom questionnaire*** before and after exposure may be collected.

- ***Blood Pressure Monitor:*** An ambulatory blood pressure monitoring device will be placed on the subject's arm and begin collecting readings. Subjects will wear the blood pressure monitor and cuff for the rest of the day after leaving the facility but will be instructed to remove it before going to bed.

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Exposure: All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will enter an exposure chamber for the 2-hour exposure to clean air. The next day they will undergo exposure to fine/ultrafine CAP for 2 hours. The CAP exposure day may be rescheduled if the particulate concentrations are too low on the scheduled day.
The actual concentration of CAP will be determined retrospectively by filter gravimetry, with exposures monitored in real time with a tapered element oscillating microbalance (TEOM). Subjects will be monitored continuously by the EPA personnel by closed-circuit camera, blood pressure measurements, pulse oximetry and ECG telemetry. A duty physician will be available at all times. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Blood pressure, heart rate, and oxygen saturation will be measured continuously during the exposure period. A confirmed blood O2 saturation levels less than 89 % will result in a cessation of exposure. Heart rate alarm limits will be set up for each subject and monitored continuously by the console operator and the nursing staff.

CAP Exposures-Concentrated particles will be generated by drawing ambient air from above the roof of the Human Studies Facility and passing the air through a 2 stage aerosol concentrator which produces up to a 20-fold increase in particle number and mass. Particles larger than about 2.5 microns will be excluded by a size-selective inlet from entering the concentrator at the rooftop intake. During the particle concentrating process, ambient air pollution gases will be diluted by a factor of four. Air temperature and humidity will be monitored and maintained to ensure proper operation of the concentrator. An air conditioner in the chamber can be utilized to both heat or cool chamber air for subject comfort. The flow of air into the chamber will be 65 liters per minute, with approximately 15 liters per minute diverted for analytical instrumentation and filter devices attached upstream from the chamber. The remaining approximately 50 L/min will be provided to the subject through a face mask. Since the air will be pulled into the chamber by a suction blower connected downstream of the chamber, the chamber will be slightly below atmospheric pressure.

The concentration of particles delivered to the chamber will vary depending on the levels of naturally occurring particles in the Chapel Hill air. Although 24 hr averages seldom exceed 15-20 ug/m3, peak values in the summer can be as high as 50-60 ug/m^3 with lower values during the rest of the year. A face mask is used to reduce the daily and seasonal variability of PM concentration. Our past experience provides a basis to expect the particle mass delivered to the mask will be up to 600 ug/m^3. The particle burden, on a mass basis presented to the volunteer will not exceed an exposure an individual receives over a 24 hour period while visiting a typical urban center in America on a smoggy day. The particle mass of the outdoor air entering and exiting the aerosol concentrator will be monitored continuously. Filter samples will be obtained from the devices located upstream from the chamber and analyzed for both mass and chemical composition of particles. If it is confirmed that particulate mass levels exceed 600 ug/m^3 for greater than 6 minutes, exposure will be terminated. The process for confirmation may take up to

additional 10 minutes. The shutdown procedure involves venting a valve on the exposure chamber, which markedly reduces the particle exposure, and alerting the investigator with both auditory and visual signals leading to removal of the subject from the chamber. Blood pressure, telemetry, and oxygen saturation will be measured during the exposure period.

Immediate Post-exposure: Subjects will be released while wearing the Holter and blood pressure monitors. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 46.2 ml of blood samples will be collected.

Eighteen Hours Post-PM exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function (BAU) measurements. The subject will then be allowed to relax for 20-30 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 46.2 ml of blood will be taken. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery or any type of hernia will not be tested for pulmonary function. Subjects will perform spirometry and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound). Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and by administration of sublingual nitroglycerin, typically 0.4 mg, (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Approximately 50 ml of venous blood will be drawn before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure (Please refer to Table 1 in this section). The site will be prepared with isopropyl alcohol and a tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but are not limited to the following: blood lipid omega-3 fatty acid levels, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

Genotype Screen	Air Exposure	Ozone-Exposure	Follow-up	TOTAL
20	46.2/46.2	46.2/46.2	46.2	251 ml

Table 1. Blood Draws

Our primary endpoints will be HRV measurement, pulmonary function and peripheral venous blood markers. Our secondary endpoints will be arterial wall function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to ambient particulate matter.

For society, this study will provide new information on the effects of ambient particulate matter on lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that National Ambient Air Quality Standards and motor vehicle emission standards can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at the protection of the public from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A qualified physician is available in the building whenever a subject is undergoing any procedure. The exposure will be terminated for any rapid change in symptoms, tachycardia and/or arrhythmia, decline in arterial oxygen saturation, or for any distress of concern to the volunteer or the console operator. Medical staff and medication are immediately available (within the building) should it be necessary. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On days after exposure subjects will be urged to contact the medical station should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses a single breath of 0.3 % acetylene uptake for measurement of pulmonary blood flow (cardiac output). Although large doses of acetylene can cause nausea, vomiting, and headache our subjects will be exposed to a single inhalation of a low

concentration of acetylene for a brief period of time. Thus the risks of these complications to our subjects are extremely low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound:** There are no known risks associated with imaging of the brachial artery. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin used in the brachial artery ultrasound measurement is a potent vasodilator, and may cause headache, flushing, and transient hypotension. These effects are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded from this study during screening. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Blood Pressure Monitoring.** There is a small chance of bruising as a result of wearing the blood pressure monitoring cuff.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a semirecumbent position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable when in the erect position.
- **CAP Exposure .** The subjects in this study will be exposed to an inhaled particle mass that does not exceed what they would encounter over 24 hours in a typical urban environment on a smoggy day. It is expected that particulate exposure levels will be up to 600 ug/m^3 and the exposure will be terminated if the level exceeds 600 ug/m^3 for greater than 6 minutes. Thus while we cannot completely rule out the possibility of an adverse effect, since we are exposing the volunteers to a particulate exposure burden they would likely encounter if visiting a large city, we feel the risk posed to volunteers is very small. Possible health effects of acute exposures to air pollution particles include chest pain, mild dyspnea, headache, cough, and, wheeze. All of these effects would be expected to resolve spontaneously within hours of exposure cessation. The particulate exposure may possibly cause increased airways inflammation. It is also possible that exposure could uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a subject. During the exposures, subjects will be continuously monitored during the entire exposure by direct observation. A physician on duty in the facility will be available when exposures are occurring. Heart rate, continuous electrocardiogram via telemetry, and S_aO_2 by pulse oximetry will also be monitored continuously, and blood pressure will be monitored every 15 minutes. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, or ataxia. Subjects will be aware that they can terminate their exposure for any reason and still

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receive compensation for the entire session. The investigators or duty physician will end the exposure if the subject is found to be suffering from any adverse effect. Full resuscitation equipment will be available at all times during exposures and in the event of an emergency, after initial medical assessment, patients will be transported to UNC Hospitals Emergency Department for continued treatment.

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

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test our hypothesis that CAP causes adverse cardiovascular effects in GSTM1 null older human volunteers, we will measure a number of relevant endpoints , including blood pressure, HRV, brachial arterial blood flow rate, change in blood coagulation factors, platelets, fibrinogen, c-reactive protein, lactate, plasminogen activator inhibitor, ferretin, and α1-antitrypsin. In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non-continuous variables pre- and post-exposure. A *p* value of 0.05 or less will be considered significant.

The sample size was calculated to detect a difference of 10% change in HRV assuming a standard deviation of 9 %. Using GraphPad software, an N of 15 was derived using a power of 0.8 and an α of 0.05. This study will enroll approximately 30 subjects in order to provide sufficient power to all for the examination of differences between GSTM1 null and positive subjects.

The study cohort will be monitored through genetic screening to ensure that individuals with GSTM1 null genotype are represented in a proportion approximately equal to the prevalence in the US population, about 40%.

Based on recently completed studies for subjects with the characteristics sought in this study, the expected screening attrition rate is up to 70 %, with a heavy effect of genotype requirement. Post-screening completion rate is up to 50 %. Extrapolating from these rates, we can expect a 15% overall completion yield. Therefore, to obtain 30 subjects, we will enroll approximately 200 subjects.

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. __ 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 the medical records office at the U.S. EPA Human Studies Facility.

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

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

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

For electronic data:

_X_ Secure network _X_ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_X_ Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

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

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records. Genotyping and consent forms will be administered by nursing staff and/or study team members. The Main Consent Form will be provided to the subject at the time of the Genotypinc Screen visit.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). __ yes __ no

Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). __ yes __ no

Explain.

If you checked "yes" to either (and you are not requesting a waiver in section

A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

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

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

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

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one human study conducted at HSF ("Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Air Pollution Particles", Haiyan Tong) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

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

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

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

It is anticipated that the duration of the study will be approximately 18 months. Participant recruitment and screening is expected to be continuous throughout the study until the intended number of participants is reached.

Genotype Screen	Physical Examination	Training Day	Air Exposure Day	Ozone Exposure Day	Follow-up Day
1 hr	2 hrs	3 hr	8 hrs	8 hrs	3 hrs

Table 2. Study Visit Schedule

If the subject is eligible for the study he/she will make up to 6 visits to the HSF over approximately 5-6 weeks. The number of visits will be determined in part by each subject's previous participation in EPHD studies, as repeating genotyping or baseline ECG measurements may not be necessary. The pre-enrollment genotyping visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after genotyping of the blood sample. The physical exam will take approximately 2 hours. The training day will require approximately 3 hours.. Exposure days will last approximately 8 hours. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours.

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

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

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

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $40.00 at one time or cumulatively more than $200.00 per year, collection of subjects' Social Security Number is required (University policy) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

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

A subject who is unable to complete the study for voluntary reasons or is dismissed for failure to comply with eligibility requirements will receive 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 the entire study, excluding completion bonus.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, subjects will be paid $12 per hour for the time scheduled and canceled. Subjects will be paid in full for any procedures that may have been started during the current visit. Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

The 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.

1. If following genotyping of the blood samples on screening day, it is determined that the individual's genotype is not needed for completion of the study, then that subject may be informed that his/her participation in this study is no longer necessary. The subject will be notified by phone and the total compensation for this study will be approximately $30.
2. If the subject is qualified and finishes the entire study, the total compensation for completion of this study will be approximately $1857.

Pre-study qualifications (screening+blood+physical)	**$60**
Training day (3 hours) + Dietary recording	**$200**
Exposure Day Time and Procedures (includes $5 for lunch)	**($603/day)**
Total:	**$1206**
Follow- up Day Time and Procedures	
Total:	**$141**
On-Time Bonus	**$50**
Dietary Compliance	**$100**
Study Completion Bonus	**$100**

Approximate TOTAL for completion of study = $1857

If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

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

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

Part C. Questions for Studies using Data, Records or Human Biological Specimens without Direct Contact with Subjects

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

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

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

__ yes __ no __ not applicable (explain)

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

__ yes __ no If no, explain

SUBJ #_____ DATE_____

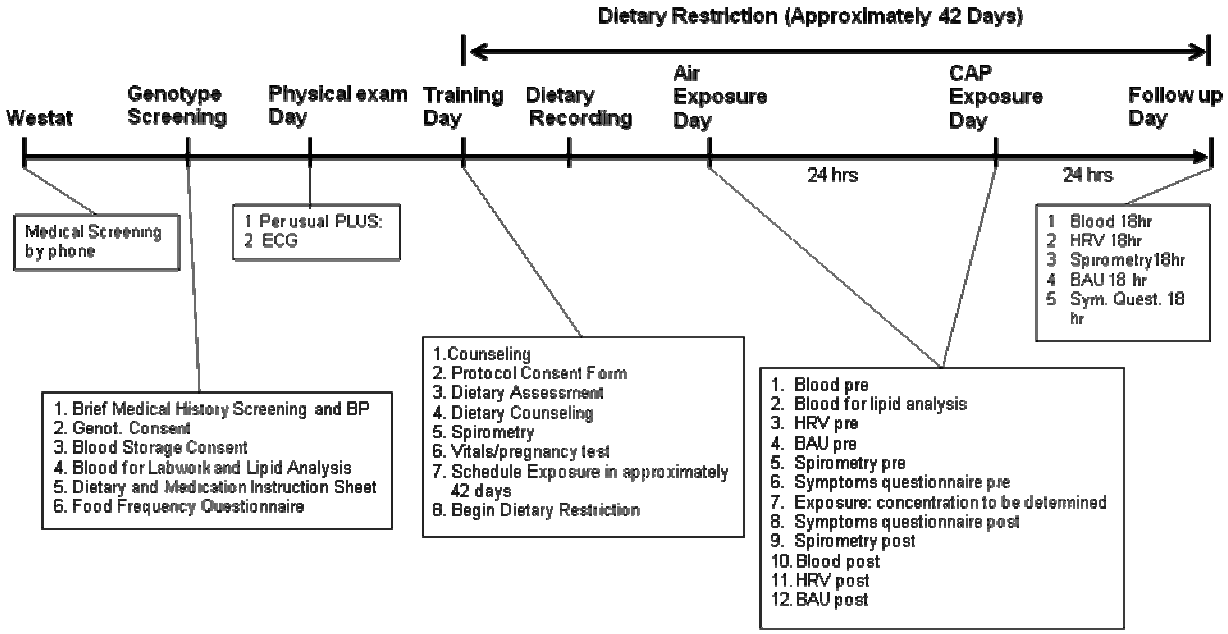
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

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

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

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



CAPTAIN STUDY FLOW DIAGRAM

Samet Declaration Exhibit 2

OPERATION, MAINTENANCE, AND MODIFICATION
OF THE
HUMAN STUDIES FACILITY

PROTOCOL OPERATIONS PLAN

for

Cardiopulmonary Effects of Exposure of Healthy Older GSTM1 Null and Sufficient Individuals to Concentrated Ambient Air Particles

(**CAPTAIN**)

11-1807

Prepared for
The Environmental Protection Agency
Research Triangle Park, NC 27711

In Response To
Contract EP-D-10-095, EPA Work Order #1202

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1.0 INTRODUCTION

The U.S. Environmental Protection Agency has contracted with TRC Environmental Corporation to operate, maintain, and modify specific components of the EPA's Human Studies Facility located in Chapel Hill, North Carolina. Included are systems associated with human exposure chambers, subject test rooms, and the Medical Station; small scale human exposure systems; and the *in vitro* exposure system. Other tasks include computer operations, data management, safety assurance, quality assurance, and system documentation and preparation of technical reports. Specific TRC responsibilities are described in the work plan and contract statement of work.

This Protocol Operations Plan details the support provided by TRC for the study entitled *Cardiopulmonary Effects of Exposure of Healthy Older GSTM1 Null and Sufficient Individuals to Concentrated Ambient Air Particles* (CAPTAIN), study number 11-1807. Routine activities performed by TRC to support research conducted at the NHEERL are described in the contract Work Plan and are not reiterated here. This document is a supplement to the Work Plan identifying exceptions to the Work Plan and describing additional effort resulting from this specific protocol.

Appendix A contains a summary Subject Activity Schedule for general reference. Appendix B contains a summary of equipment and configuration parameters that will be utilized for this study.

The EPA Principal Investigator (PI) responsible for this study is Dr. James Samet.

This protocol received approval from the UNC Committee on the Protection of Human Subjects on November 21, 2011.

2.0 TASK 1 - SYSTEM MAINTENANCE AND OPERATION

The following paragraphs describe the operational requirements of equipment and systems maintained by TRC and utilized in support of this research study.

Up to 200 test subjects aged 50 – 75 will be recruited to participate in this study, with the goal of completing 30. TRC will monitor the schedule maintained by the subject recruitment contractor (Westat) to determine when to prepare study areas for CAPTAIN activities.

Screening and training periods will precede the exposures. At the training period, subjects will be instructed in day-of-exposure activities, and baseline spirometry, diffusion limited uptake of CO (DLCO), and resting minute ventilation measurements will be obtained. In addition, during the training session, the PI will be responsible for determining the size of face mask required for the subject, and measuring the subject's nose/mouth position while seated in the chamber. A measuring system is available inside and outside Chamber AC89 to enable the PI to determine this vertical position, and forms will be available in a notebook at the console to record the measurement. TRC must make all necessary adjustments prior to starting the aerosol concentrator, which is approximately three hours prior to the scheduled exposure.

Exposures will be conducted in the aerosol exposure chambers located in Room 89, with subjects seated during the entire exposure. Each subject will undergo two two-hour exposures. The first exposure (Tuesday) will be clean air in Chamber PC89. The clean air presented to the subject will be medical grade air that has been humidified to 50 ±10% RH. The second exposure (Wednesday) will be concentrated fine and ultrafine particles in Chamber AC89. Both

clean air and concentrated particles will be presented to the subject through a face mask. Particle exposure sessions can begin no earlier than 10:00 a.m.

On particle exposure days, the TRC Pollutant Control System (PCS) operators will monitor chamber particulate measurements prior to the exposure. If chamber particulate measurements are estimated to be less than 50µg/m^3, TRC will inform the PI, who will determine whether to run or reschedule the exposure.

Training, as well as pre- and post-exposure spirometry and DLCO measurements will be performed in non-exposure Test Room TB87 utilizing a SensorMedics digital plethysmography system. Minute ventilation measurements will be taken utilizing a custom Minute Ventilation System developed in-house. Telemetry ECG will be used to monitor test subjects during all testing. A Holter ECG monitor will be used to monitor subjects during, and for 18 hours after, the exposures. Blood pressure will be monitored periodically throughout the exposure day activities. An oxygen saturation monitor will be available in the chamber during exposure sessions. TRC staff will be responsible for calibrating and maintaining the SensorMedics equipment, telemetry system, ambulatory blood pressure monitor, pulse oximeter, and Holter recorders used during the testing. TRC has no responsibility for the brachial artery ultrasound (BAU) system.

TRC staff will be responsible for maintaining and operating the aerosol concentrator, aerosol chambers, and particulate measurement devices (*i.e.*, filter sampler, Condensation Particle Counter (CPC), Scanning Mobile Particle Sizers (SMPSs), DataRAMs™, Tapered Element Oscillating Microbalance (TEOM®) Particulate Mass Monitor, and a total filter monitoring the chamber environment. Specifically, the following describes TRC activities for all subject particle exposures conducted in Chamber AC89:

- The concentrator will be configured with the size selective inlet but without the size selective outlet. In this configuration, the concentrator is configured to remove particles larger than 2.5µm. However, because the size selective inlet does not have an infinitely sharp cutoff point, some coarse particles (larger than 2.5µm) will enter the chamber.
- The concentrator will be configured to utilize the air stream dryers (Harvard dryers) that were part of the original system. The Harvard dryers will be used with the maximum flow of chilled water, and the exit air temperature will not be controlled.
- A single total Teflo filter will be configured to sample delivery air to the chamber.
- One CPC, one DataRAM™, one SMPS, and one TEOM® will be configured to sample delivery air to the chamber.
- The chamber exhaust flow will be adjusted to keep the total flow through the system at approximately 65 l/min, which will provide approximately 50 l/min to the subject. The remainder of the air flow is required by the sampling equipment.
- The chamber inlet will be configured such that the face mask, when attached, will accommodate the seated subject's nose/mouth area as determined by the PI during training. This position measurement must be communicated to TRC at least three hours prior to the exposure.
- The temperature of the conditioning air will be adjusted as necessary to maximize the performance of the concentrator. A standalone air conditioner in the chamber will be available for use in establishing and maintaining a comfortable environment for the subject.
- The concentrator system will be started two to three hours before the subject is scheduled to enter the chamber to establish exposure conditions.
- PCS will be configured to dilute the particle concentration presented to the subject such that measurements do not exceed 600µg/m^3. Specifically, the PCS operator will monitor the real-time TEOM® and DataRAM™ measurements once the aerosol concentrator system has stabilized. Historically, the average of TEOM® measurements over an entire exposure session has matched the final filter concentration fairly closely, but the

instrument reacts rather slowly to changes in the environment. The relative accuracy of the DataRAM™, on the other hand, has been fairly inconsistent from one exposure to another but the instrument is much faster to react to changes in the environment. Therefore, once the concentrator system has stabilized, the PCS operator will observe the approximate relative accuracy of DataRAM™ to TEOM® measurements and will configure the dilution system, which is based upon DataRAM™ measurements, appropriately. Note that the dilution system will be configured such that dilution begins when the concentration as measured by the TEOM® is approximately 500µg/m^3.

- If for any reason the dilution system fails to perform properly, the PCS operator will use the particle concentration as measured by the DataRAM™ and TEOM® to determine if the exposure should be terminated due to excessively high concentrations. Specifically, the PCS software will issue an audible alarm if the two-minute average concentration measured by the DataRAM™ exceeds 20% of the dilution point. At that time, the PCS operator will initiate continuous monitoring of the two instruments' readings. The PCS operator will carefully monitor the concentration as reported by the TEOM® if the two-minute average DataRAM™ concentrations remain higher than the alarm limit for more than three consecutive average periods. If the TEOM® concentration shows a reasonably steep increase in concentration over the last several minutes or if the TEOM® concentration exceeds 600µg/m^3, the PCS operator will initiate removal of the subject from the chamber. TRC will make a decision within 14 minutes of the first alarm and the PI will be notified if the decision is to abort the exposure.
- Exposure chamber temperature and relative humidity data, as well as the temperature and relative humidity at the inlet to the face mask, will be collected by the PCS.
- Filter media from the sampler will be removed and placed in cold storage at the conclusion of each subject exposure. When sufficient samples are available, TRC will weigh the filters.

For clean air exposures in Chamber PC89, TRC will configure one DataRAM™ to sample delivery air to the chamber. The chamber inlet will be configured such that the face mask, when attached, will accommodate the seated subject's nose/mouth area as determined by the PI during training. In addition, the incoming medical-grade air (approximately 50 l/min) will be humidified to approximately 50% RH. The temperature and relative humidity at the inlet to the face mask will be collected by the PCS.

TRC will maintain Subject Entry/Exit Logs for Chambers AC89 and PC89. The study console operator will be required to enter the date and time that the subject entered and exited the chamber for each exposure session. The study operator will also be required to communicate with the PCS operator to verify that the chamber is ready for subject exposure, and to coordinate with the PCS operator when the subject enters the chamber. At that time, the PCS operator will begin filter sampling. At the end of the exposure period, the study console operator will be required to inform the PCS operator when the subject is leaving the chamber. The telephone number of the PCS control room is posted on the study console.

3.0 TASK 2 - COMPUTER OPERATION AND DATA PROCESSING

An automated symptom questionnaire will be available on the spirometry computers in the Medical Station and in Room TB87. TRC will ensure that the questionnaire data, which will be stored in an Excel spreadsheet, will be saved in a study-specific file on a network drive for convenient access. No modification of the existing OMEGACON questionnaire is planned, and TRC will have no other responsibility for the collection or maintenance of questionnaire data.

Because there are no exposure activities other than blood pressure measurements that will be pre-programmed for the ambulatory blood pressure monitor, there will be no automated study timer established for the CAPTAIN study at the Chamber AC89 or PC89 consoles.

The following data processing activities will be conducted for this study:

- Subject chamber entry and exit times will be manually recorded by the investigator(s)/operator(s). TRC personnel will enter the data into the database and perform a verification of the input.
- Roof weather station data for approximately the 24-hour period preceding the exposure will be printed and saved with other operational data.
- TRC staff will analyze filter weight data from Chamber AC89 to determine exposure conditions (*e.g.*, mass concentration). A spreadsheet with these calculations will be available on the EPA network.
- Data will be obtained from Internet sources and used in a trajectory model to show the geographical origins of Chapel Hill particulates. Model reports will be printed and saved with other operational data.
- All raw data and analysis spreadsheets will be archived. Data obtained in hard copy format will be delivered periodically to the Agency.

Computer systems involved in the collection of medical data will be connected to the Agency network such that data collected using those systems can be stored on a network drive for more convenient access. TRC has no other responsibility for medical data.

4.0 TASK 3 - QUALITY CONTROL

Standard TRC QA/QC procedures will be implemented for this protocol. A Protocol Performance Report and other protocol-specific QA/QC reports will be prepared unless otherwise directed by the Project Officer. Periodically, TRC staff will verify the calculations used to determine summary exposure conditions from filter data, which are provided as part of routine filter analysis. Any discrepancies will be reported immediately to the PI.

5.0 TASK 4 - SAFETY

No special safety measures are required for this study.

6.0 TASK 5 - UPGRADING OF SYSTEMS

No system upgrades are required for this study.

7.0 TASK 6 - SYSTEM DOCUMENTATION AND TECHNICAL REPORTS

No special system documentation or technical reports are anticipated as a result of this study.

APPENDIX A

CAPTAIN SUBJECT ACTIVITY SCHEDULE[1]

TRAINING (Medical Station, Test Room TB87, Chamber AC89)

- Medical screening
- Explain study and obtain informed consent
- Training – PFT, minute ventilation

EXPOSURE

Time[2]	Procedure	Measurements	Location
-2:15	Check-in	Vital signs	Medical Station
	Review exclusion criteria		
	Venipuncture; collect blood sample		
	Apply electrodes and begin ECG monitoring	HRV	
	Brachial artery ultrasound (BAU)		Room 7
	Complete symptom questionnaire	Spirometry	Test Room TB87
		DLCO	
00:00	Begin chamber exposure	SPO_2	Chamber AC89 or
		BP	Chamber PC89
02:00	Leave chamber		
	Complete symptom questionnaire	Spirometry	Test Room TB87
		DLCO	
	Collect blood	HRV	Medical Station
	BAU		Room 7

EIGHTEEN HOURS POST PARTICULATE EXPOSURE (Test Room TB87, Medical Station, Room 7)

- Medical screening
- Blood sample; HRV; spirometry; DLCO; BAU; symptom questionnaire

[1]Schedule provided for general reference. Activities identified are not performed by TRC staff.

[2]Times listed are relative to start of exposure.

APPENDIX B

CAPTAIN EQUIPMENT AND CONFIGURATION

Study Summary

Number of subjects:	200 recruited/30 complete
Locations:	Chamber AC89, Chamber PC89, Test Room TB87, Medical Station
Exposures per subject:	Two 2-hour exposures using face mask – first to clean air; second to concentrated fine/ultrafine particles (preferably >50 µg/m^3)
Study blind status:	N/A

Real-Time Controlled and Monitored Parameters

Parameter	Status	Device	Target Concentration	Warning Limit	Shutdown Limit[3]
Mass concentration	Controlled[4]	DataRAM™	>50 µg/m^3	20%[5]	[4]
Mass concentration	Monitored[6]	TEOM®	N/A	N/A	600 µg/m^3
Particle size distribution	Monitored[6]	SMPS™	N/A	N/A	N/A
Particle count	Monitored[6]	CPC	N/A	N/A	N/A
Temperature	Monitored	Rotronics temperature/RH sensor	N/A	N/A	N/A
Relative humidity	Controlled[7]	Rotronics temperature/RH sensor	50% RH	N/A	N/A

TRC-Maintained Biomedical Equipment Requirements

Equipment	Room TB87	Chamber AC89	Chamber PC89	Medical Station
Plethysmograph	✓			
Minute ventilation	✓			
Telemetry ECG	✓	✓	✓	✓
Holter monitor				✓
Pulse oximeter		✓	✓	✓
Ambulatory BP monitor				✓
Symptom questionnaire	✓			✓

[3]Shutdown concentration reflects instrument readings, not actual subject exposure conditions. Manual shutdown occurs after confirming that concentrations exceed limits for six consecutive minutes. See text for details.
[4]Controlled during particle exposures only; monitored during clean air exposures. Note that dilution point is determined immediately prior to exposure based on relative accuracy of DataRAM™ to TEOM® measurements. See text for details.
[5]Percentage above dilution point
[6]Not monitored during clean air exposures.
[7]Controlled during clean air exposures only; monitored during particle exposures.