

**EPA CENTER AT HSPH FOR AMBIENT PARTICLE HEALTH EFFECTS  
AT HARVARD SCHOOL OF PUBLIC HEALTH**

**2nd YEAR PROGRESS REPORT**

**Overview**

The EPA Particle Center at HSPH now includes four projects (the 2<sup>nd</sup> of the original 5 was eliminated, as discussed in the 1<sup>st</sup> year progress report, and the projects were re-numbered accordingly): Project 1, with a focus on exploring the pathways of particle toxicity for cardiovascular responses in the Normative Aging Study is being conducted for Years One through Three. Project 2, with a focus on cardiovascular toxicity of concentrated ambient fine, ultrafine and coarse particles in controlled human exposures, is being conducted for Years One through Five. Project 3, with a focus on assessing toxicity of local and transported ambient particles using animal models exposed to CAPs, is being conducted for Years One through Three. Project 4, with a focus on assessing toxicity of vehicular emissions, using animal models, will be conducted for Years Three through Five. This 2<sup>nd</sup> year progress report includes preliminary results for (re-numbered) Projects 1, 2, and 3.

**Date of Report:** August 1, 2007

**EPA Grant Number:** R-832416-010

**Center:** EPA Center at HSPH for Ambient Particle Health Effects

**Center Director:** Professor Petros Koutrakis

**Project Title:** Cardiovascular Responses in the Normative Aging Study: Exploring the Pathways of Particle Toxicity

**Investigators:** Joel Schwartz (PI), Helen Suh (co-PI), Pantel Vokonas, David Sparrow

**Institution:** Harvard School of Public Health

**EPA Project Officer:** Stacey Katz

**Project Period:** October 1, 2005 – September 30, 2010

**Period Covered by the Report:** August 1, 2006 – July 31, 2007

**Objective(s) of the Research Project:** In our original EPA-funded Particle Center, we examined air pollution mediated responses of individuals participating in the Normative Aging Study (NAS), a large prospective cohort living in Eastern Massachusetts. As part of this effort, we collected ECGs and blood samples from each study participant and analyzed these samples for HRV and CRP, respectively. In analyses of these data, we found ambient PM<sub>2.5</sub> and ambient black carbon (BC) concentrations to be associated with decrements in HRV, with these decrements greatest for hypertensive individuals. Ambient BC concentrations were further found to be associated with increased CRP and fibrinogen levels. These results suggest that the PM-mediated autonomic changes may be brought about through pathways involving the autonomic nervous system and systemic inflammation. Definitive identification of PM-mediated biological mechanisms was limited, however, by the lack of other intermediate cardiac and inflammation endpoints, the use of central site monitoring to characterize exposures for the entire cohort, and by the traditional epidemiologic approaches used to examine exposure-effect associations.

In Project 1 of our new Center, we are continuing our analysis of the NAS cohort, with continued ECG, CRP and fibrinogen measurements and, importantly, with additional exposure and health measurements for each NAS participant, to enhance our ability to identify important biological pathways. As proposed, these additional measurements will include ECG, blood inflammatory marker, medication, genotypic, food frequency, and particle exposure measurements for each of the current NAS participants, with measurements of urine oxidative stress markers added to the study in the past year. ECG, blood and urine samples are being analyzed for a variety of measures (HRV, ST segments, QT intervals, CRP, sICAM-1, sVCAM-1, homocysteine, 8-OHdG and creatinine); these measures will be used as intermediate markers of the inflammatory, endothelial, oxidative stress and autonomic pathways. In addition, these measures will be related to individual-specific indoor PM<sub>2.5</sub>, SO<sub>4</sub><sup>2-</sup>, and BC exposures that are being measured for one week prior to the clinic visit and to ambient air pollution (PM<sub>2.5</sub>, PM<sub>10</sub>, PM<sub>2.5-10</sub>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, BC, EC, OC, and PN) concentrations that are being measured at our stationary ambient monitoring (SAM) site. The study will use this data to test three primary hypotheses (with amendments to these hypotheses in *italics*):

Hypothesis 1: Cardiovascular effects of particles (PM) will differ by source and by different source-related components. Specifically, short-term exposures to sulfate and traffic particles will be associated with increases in:

- **acute inflammation and/or endothelial dysfunction**, as measured by increases in CRP, soluble intercellular adhesion molecule 1 (sICAM-1), and soluble vascular cell adhesion molecule 1 (sVCAM-1);
- **autonomic dysfunction**, as measured by reduced heart rate variability (HRV) *and increased arrhythmias*;
- **oxidative stress**, as measured by increases in *8-OHdG and creatinine* and;
- **general cardiovascular responses**, as measured by increases in blood pressure and ECG changes including *ST-segment level and QT-length*.

Hypothesis 2: Effects of PM on these outcomes will be modified by subject characteristics (genetic, dietary, or pharmacological) that influence susceptibility to:

- **oxidative stress, endothelial dysfunction, and/or acute inflammation**, specifically Glutathione-s-transferase (GSTM1) null or the long repeat Hemeoxygenase-1 (HO-1) genotypes; statin, beta blocker, or calcium channel blocker use; dietary intake of Vitamin C or omega-3 (Ω-3) fatty acids;
- **autonomic dysfunction**, specifically beta blocker use, calcium channel blocker use or dietary intake of Ω-3 fatty acids;
- **general cardiovascular disease**, specifically hypertension and;
- **reactive airways disease**, specifically methacholine reactivity.

Hypothesis 3: Long-term exposure to PM from traffic is associated with increased risk of inflammation (e.g., CRP, sICAM-1, sVCAM-1, and homocysteine); autonomic dysfunction (e.g., reduced HRV), and impaired cardiovascular outcomes (e.g., elevated blood pressure). This association is modified by the same factors that modify acute responses.

### **Progress Summary/Accomplishments:**

We have made substantial progress in our NAS study, both in the analysis of data previously collected as part of our original Harvard-EPA Particle Center and in the collection of new data for our current Harvard-EPA Particle Center. This progress is discussed briefly below.

Analysis of Previously Collected Data: In Year Two, we continued our analysis and interpretation of data collected on the NAS participants as part of our original Harvard-EPA Particle Center. These analyses have focused on the relation between ambient pollution and intermediate cardiac (HRV) and pulmonary (pulmonary function), and inflammatory (white blood count, C-reactive protein (CRP), fibrinogen) outcomes and on how these relationships vary with individual-specific factors, such as genetic polymorphisms, diet, and weight. Results from these analyses have helped to identify important biological pathways by which ambient particles may impact health and important susceptible populations. Findings from these analyses have been presented at conferences and have been submitted or published in peer-reviewed journals, with representative papers discussed briefly below.

- In a paper by Park et al. (2006), we investigated the relation between ambient particles and HRV in our NAS subjects and examined whether this relation was mediated by transition metals, such as iron, which have been shown to produce reactive oxygen species when present in high concentrations in humans. The importance of transition metals to particle toxicity was examined using polymorphisms in the hemochromatosis (HFE) gene, two of which (C282Y and H63D) are associated with increased uptake of iron and other transition metals as compared to the wild type genotype. Polymorphisms in the HFE gene were hypothesized to be important, as it was possible that differences in transition metal uptake would lead to corresponding differences in the effects of particle-bound transition metals on cardiac function, as was found with blood and bone levels of the transition metal lead. Results from our analysis showed that the impact of PM<sub>2.5</sub> on HRV was indeed modified by HFE variants, with ambient PM<sub>2.5</sub> negatively associated with the high frequency (HF) component of HRV in individuals with the wild type genotype, but having no association in individuals with the C282Y or H63D variant. These results suggest that the effect of PM<sub>2.5</sub> on cardiac autonomic function is shielded in individuals with greater iron uptake and that PM-associated iron and other transition metals may be important to particle toxicity. Further, results suggest that the oxidative stress and/or inflammatory pathways may be important to PM-mediated toxicity.
- As discussed in Park et al. (2007), we investigated whether the impact of ambient particles on HRV in our NAS subjects differs by air mass origin, as an indicator of important pollution sources. To do so, the paths traveled by the air masses (“back-trajectories”) before arriving in Boston were identified, classified into six clusters and subsequently related to pollution and several HRV measures.

We found that the effects of black carbon (BC) on all HRV measures were strongest on days with southwest trajectories. Subjects who were examined on days when air parcels came from the west had the strongest associations of HRV with ozone. PM<sub>2.5</sub>, BC, and sulfates were associated with increased LF/HF ratio on days related to local, slow moving air masses. Also significant increases in LF/HF were associated with days when air came from the northwest and west compared to north trajectory days. Results suggest that the effects of ambient particles on HRV differs by the origin of pollutant air masses, providing further evidence that the impacts of ambient particles on autonomic function differs for different particle sources.

- As shown in Zeka et al. (2006), we used the NAS cohort to investigate particle-mediated impacts on several markers of inflammation and thrombotic activity, which may play key roles in the physio-pathological processes leading to cardiovascular disease and mortality. We examined the impacts of ambient particles on inflammation and thrombotic activity using white blood cell counts (WBC), CRP, sediment rate, and fibrinogen and investigated whether these impacts were modified by age, health status, polymorphisms in glutathione-S-transferases (GSTM1: a key factor in cellular defenses against reactive oxygen species), and statin medications. Further, we examined whether these impacts varied with particle component, with specific emphasis on the traffic related components, BC and particle number (PN).

We found both BC and PN to be related to increased inflammation, with associations most consistent for the thrombotic factor fibrinogen and strongest when exposures were averaged over the 4-weeks prior to the measurement. Although no statistically important difference was found for any category of effect modifiers, results suggested that older individuals (>78 years of age) increased the effect of PN on fibrinogen and CRP levels. Similarly, the association between fibrinogen and PN and between BC and CRP was approximately two- and four-fold higher among the obese, respectively. Results also suggested the GSTM1-null subjects and non-statin users had a greater BC-mediated inflammatory effect; however, these differences were not statistically significant. These findings are consistent with an adverse effect of ambient particles on inflammation and thrombotic activity, with these effects strongest for traffic-related particles and possibly in individuals older than 78 years, individuals with chronic pro-inflammatory states (as shown for obese individuals), and individuals with reduced defenses to reactive oxygen species (as shown for people with the GSTM1-null genetic polymorphism or in non-statin users).

- In Gyparis et al. (2007), we presented our GIS-based spatial smoothing model that was developed to predict 24-hr outdoor black carbon concentrations across eastern Massachusetts. This model is based on repeated observations from roughly 100 different locations, derived from State and Federal monitoring networks and from our previous exposure studies funded by our original Harvard-EPA Center. This model extends previous work by including both a regression approach (prediction by variables such as distance to road, etc.) as well as a geospatial smoothing term. In addition, by combining several surrogates of traffic particles (EC, BC, NO<sub>2</sub>), it allows us to obtain an estimate of traffic particle exposure using structural equation modeling that incorporates a measurement error correction. We showed that this model predicts 24-hr BC concentrations extremely well, resulting in an R<sup>2</sup> of 0.81 when 24-hr estimates were regressed on measured concentrations. Model performance is even higher when used to predict annual average BC levels.

This model has subsequently been used to examine the impact of participant specific outdoor BC exposures on mortality within eastern Massachusetts (Maynard et al., 2007) and will also be used to examine the relation between outdoor BC exposures and HRV, inflammatory, oxidative stress, and endothelial function outcomes for our NAS subjects. Prior to this application, however, the model will be revised to include a daily interaction between planetary boundary layer (PBL) and spatial terms to allow for greater temporal-

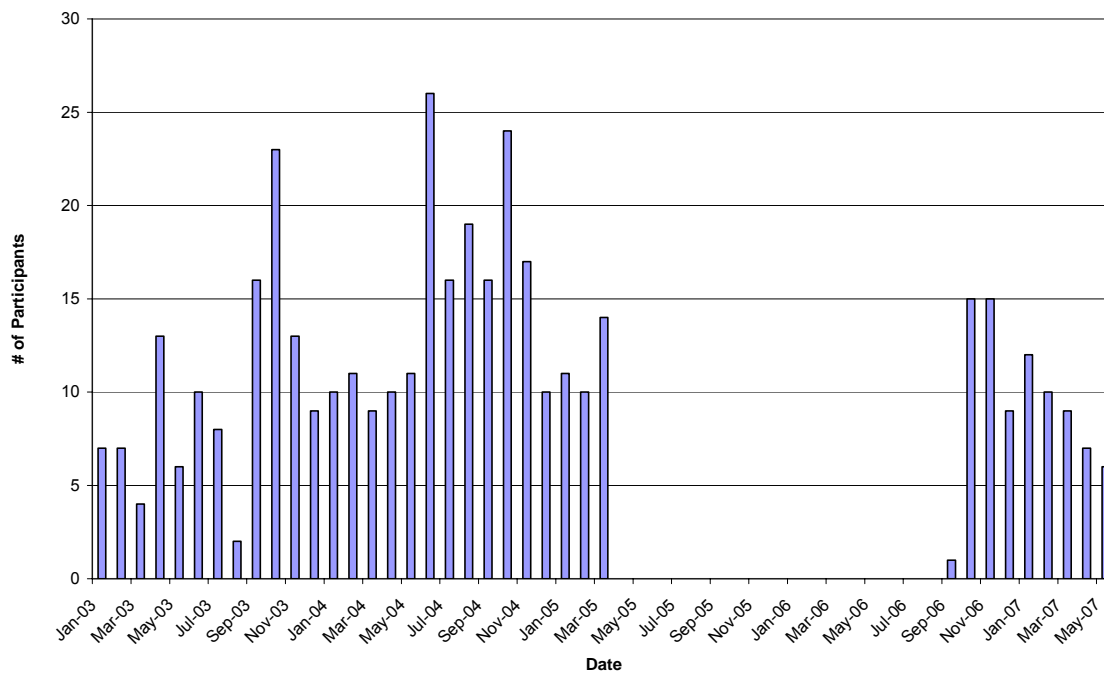
spatial resolution. This revised model will be used to impute more precise short and long term particle exposures for the NAS participants.

Data Collection: In Year Two, we continued to collect information for NAS participants as they came in for their every three-year health exam. At each health exam, we specifically collected ECG, blood inflammatory marker, and daily diet information on the study participants. We supplemented these measurements with those of 8-OHdG and creatinine in urine, which were added to obtain direct measures of oxidative stress in our participants. This addition was possible through the reallocation of funds originally allocated to the project that was eliminated (The Cardiovascular Effects of Particles and Gaseous Co-pollutants from Mobile Sources – the “old” Project 2). In total, we obtained new data for approximately 150 participants, less than anticipated due to attrition from death (73 subjects), sickness (22 subjects), or dropouts (e.g., could participate but decided to miss their health appointment; 7 subjects). Most of the participants were seen in the non-winter months.

In addition to the health measurements, we also collected one-week long indoor PM<sub>2.5</sub> samples inside the homes of 83 NAS participants. These subjects were recruited from the NAS subjects that lived in a non-smoking household and that were going to be at home for the week prior to their health visit. Originally, recruitment also was to be limited to subjects that lived within the greater metropolitan Boston area (defined as within Route 495 highway); however, these criteria were relaxed to allow recruitment of subjects living in southeastern Massachusetts and southern New Hampshire. Of the 53 NAS subjects who did participate in the indoor monitoring portion of the study, fifteen lived outside of our geographic recruitment area, 25 had a health clinic visit prior to the start of indoor monitoring, three lived in a smoking household, five were confused or overwhelmed by the indoor monitoring requirements, and five did not volunteer for the indoor monitoring portion of the study.

Laboratory Analysis: We continued to analyze collected health data, with laboratory analysis of HRV, blood and urine samples conducted in the appropriate laboratories. To obtain ECG measures in addition to HRV, we re-analyzed ECG tapes using software from Forest Medical for ST segments, arrhythmias, and QT length. This additional analysis, however, does not include ECG tapes for individuals seen between mid-March and mid-September 2006, which were lost due to problems with computer backups (Figure 1). This data loss does not affect the HRV measures. Indoor PM<sub>2.5</sub> samples have been off-weighed, with concentrations processed. The samples are currently scheduled for BC (by reflectance) and sulfate (by ion chromatography) analysis in our laboratory.

**Figure 1.** Number of Currently Available ECG tapes by Month



**QA/QC.** Investigators followed established protocols for this work. Data collection and analysis is currently underway on the environmental samples. An annual audit is scheduled for August 2007, covering data collection and data management. IRB approvals have been updated. These protocols were reviewed on 3/26/2007 and approved for one year until 3/24/2008 by the VA Jamaica Plain IRB. Copies of the IRB approval reports are attached at the end of this report.

**Publications/Presentations:**

Gryparis A, Coull BA, Schwartz J, Suh HH. Semiparametric latent variable regression models for spatiotemporal modeling of mobile source particles in the greater Boston area. *Appl. Statist* 2007, 56(Part 2):183-209.

Maynard D, Coull BA, Gryparis A, Schwartz J. Mortality risk associated with short-term exposure to traffic particles and sulfates. *Environ. Health Perspect*, 2007, 115(5):751-755.

Park SK, O'Neill MS, Stunder BJB, Vokonas PS, Sparrow D, Koutrakis P, Schwartz J. Source location of air pollution and cardiac autonomic function: Trajectory cluster analysis for exposure assessment. *Journal of Exposure Science and Environmental Epidemiology*, 2007, doi:10.1038

Park SK, O'Neill MS, Wright RO, Hu H, Vokonas PS, Sparrow D, Suh H, Schwartz J. HFE genotype, particulate air pollution, and heart rate variability: A gene-environment interaction. *Circulation*, 2006, 114:2798-2805.

Zeka A, Sullivan J, Vokonas PS, Sparrow D, Schwartz J. Inflammatory markers and particulate air pollution: Characterizing the pathway to disease. *Int. J. Epidemiol.* 2006, 35:1347-1354.

## Other Non-NAS, Epidemiologic- and Center-Related Publications

Baccarelli A, Zanobetti A, Martinelli I, Grillo P, Hou L, Giacomini S, Bonzini M, Lazani G, Mannucci PM, Bertazzi PA, Schwartz J. Effects of exposure to air pollution on blood coagulation. *J. Thrombosis and Haemostasis*, 2006, 5:252-260.

Baccarelli A, Zanobetti A, Martinelli I, Grillo P, Hou L, Lanzani G, Mannucci M, Bertazzi A, Schwartz J. Air pollution, smoking and Plasma Homocysteine. *Environ. Health Perspect.* 2007, 115(2):176-181.

Laden F, Schwartz J, Speizer F, Dockery D. Reduction in fine particulate air pollution and mortality. (Extended follow-up of the Harvard Six Cities Study). *Am J Respir Crit Care Med* 2006, 173:667-672.

Median-Ramón M, Zanobetti A, Cavanagh DP, Schwartz J. Extreme temperatures and mortality: Assessing effect modification by personal characteristics and specific cause of death in a multi-city case-only analysis. *Environ. Health Perspect*, 2006, 114(9) 1331-1336.

O'Neill, MS, Veves, A, Jarnat, JA, Zanobetti, A, Gold DR, Economides PA, Horton, ES, Schwartz, J. Air pollution and inflammation in Type 2 Diabetes: A mechanism for susceptibility. *Occup. Environ. Med.* 2007, 64:373-379.

Wellenius GA, Schwartz J, Mittleman MA. Particulate air pollution and hospital admissions for congestive heart failure in seven United States cities. *Am J Cardiol.*, 2006, 97:404-408

Zanobetti A, Schwartz J. Particulate air pollution, progression, and survival after myocardial infarction. *Environ. Health Perspect*, 2007; 115(5):769-775.

**Future Activities:** We are continuing to collect HRV, blood and urine samples at each participant's health visit and to recruit participants for indoor sampling. For Study Year 3, we anticipate that 140 subjects will be seen at their NAS health appointment, with approximately 60% of these individuals participating in the indoor monitoring component of the study. This number is lower than we had previously anticipated, due to attrition from death, illnesses, and general age-related issues. For participants that have moved since our last address geocoding, we will update residential addresses and geocodes.

In addition, we will continue to analyze data in the laboratory using statistical methods. Laboratory analyses will include analysis of ECG, blood and urine markers discussed above. We plan to begin our statistical analyses of PM exposures and ST, arrhythmias, QT length, 8-OHdG, and ICAM and VCAM data. These analyses will include exposures estimated using our GIS-based spatial model and our measured indoor concentrations.

**Supplemental Keywords:** Normative Aging Study, inflammation, autonomic function, oxidative stress

**Relevant Web Sites** <http://www.hsph.harvard.edu/epacenter/>

**Date of Report:** August 1, 2007

**EPA Grant Number:** R-832416-010

**Center:** EPA Center at HSPH for Ambient Particle Health Effects

**Center Director:** Professor Petros Koutrakis

**Project Title:** Cardiovascular Toxicity of Concentrated Ambient Fine, Ultrafine and Coarse Particles in Controlled Human Exposures

**Investigators:** Frances Silverman (PI), Diane Gold (co-PI)

**Institution:** Gage Occupational & Environmental Health Unit (GOEHU), Toronto, Canada

**EPA Project Officer:** Stacey Katz

**Project Period:** October 1, 2005 – September 30, 2010

**Period Covered by the Report:** August 1, 2006 – July 31, 2007

**Objective(s) of The Research Project:** In Project 2, we will examine the acute cardiovascular effects of coarse, fine and ultrafine concentrated ambient particles (CAPs) in healthy adults, using a newly constructed controlled particle exposure facility, in Toronto, ON, Canada. Based on recommendations of the Science Advisory Committee meeting in July 2006, a number of changes were made to improve the study. The proposed design, which included 50 subjects and 4 exposures per subject (filtered air and 3 size fractions of CAPs) would have taken 4 years to complete. In the revised design, we have decided not to include ultrafine CAPs, at least for the first series of studies. Twenty-four subjects will now each receive three 2-hr exposures selected from four possible treatments including: filtered (FA) air, fine CAPs (target  $250 \mu\text{g}/\text{m}^3$ ; max  $500 \mu\text{g}/\text{m}^3$ ), coarse CAPs (target  $200 \mu\text{g}/\text{m}^3$ ; max  $400 \mu\text{g}/\text{m}^3$ ), and a 2<sup>nd</sup> coarse CAPs exposure at the same levels. Exposures are randomized and at least 2-weeks apart to allow for washout. Once all 24 subjects have completed the three exposures, they will be asked if they wish to complete the 4<sup>th</sup> that they did not receive. If all subjects complete the optional 4<sup>th</sup> exposure there will be 48 coarse CAPs exposures, 24 fine CAPs and 24 FA. The 24 fine CAPs results @  $250 \mu\text{g}/\text{m}^3$  will be added to our CLEANAIR fine CAPs+O<sub>3</sub> cardiovascular study (nearing completion) to look at dose-response relationships in common vascular endpoints. An interim analysis of this data has shown a positive association between the 2-hr exposure fine CAPs mass concentration, at levels ranging from 100 to  $200 \mu\text{g}/\text{m}^3$  (gravimetric), and the change in diastolic BP during exposure, with greater BP increases at higher fine CAPs levels. Cardiovascular outcomes will be measured pre-, post- and 24 hrs post-exposure, and will include measures of: i) vascular dysfunction (brachial artery diameter and reactivity) measured by ultrasonography; ii) cardiac output by echocardiography; iii) blood pressure by automated arm cuff and; iv) markers of systemic inflammation (CBCs and blood IL-6, CRP & endothelins). In addition, we will test for susceptibility genes of PM-induced oxidative stress using blood samples. During exposure, we will continuously measure beat-to-beat arterial BP using a Finometer finger cuff (pulse pressure) that includes calculated determinations of cardiac output, stroke volume and systemic vascular resistance. Mortara Holter (ECG) monitors (the same used by other PM Centers doing human studies) will be worn by the subjects from the start of the exposure day for 24-hrs, as a measure of cardiac autonomic dysfunction (heart rate variability analyses). Filter samples will be collected during exposures for both mass and chemical composition (inorganic ions, trace elements, organic and elemental carbon) and biological material including airborne endotoxin (Limulus Amebocyte Lysate test method) and markers of fungi (beta-glucans; GlucateLL kit). On-site daily measures will include meteorological data, TEOM PM<sub>2.5</sub> and PM<sub>10</sub>, as well as pollen counts. Daily stationary central site monitoring data (gaseous and PM criteria pollutants) will also be obtained to statistically adjust for potential affects on baseline pre-exposure data.

The specific hypotheses to be addressed by this project are the following:

- Acute human exposures to CAPs of coarse, fine and ultrafine (UF potentially in the later phase of the study) size fractions result in cardiovascular responses consistent with vascular narrowing, vascular/autonomic dysfunction, inflammation, and/or endothelial activation compared to FA (control) exposures
- Associations between CAPs and cardiovascular responses will differ by particle size fraction and PM composition

### **Progress Summary/Accomplishments:**

Human Ethics Approval: The study protocol was submitted to St. Michael's Hospital (SMH), Human Research Ethics Board (REB), Office of Research Administration in two stages: first for approval of study work in the 1<sup>st</sup> year, not involving human testing (approval date Dec 19, 2005); and second for the entire study, including human testing (approval date April 27, 2006). Annual renewal of approval from SMH REB was obtained. This includes amendments to the protocol. Application to the University of Toronto (U of T) REB was submitted, minor concerns of the board responded to and approval recently received. A point of clarification related the CAPs' maximum and target levels was raised by Stacey Katz, our EPA project officer. In response, the target levels and maxima were clearly specified and a revised protocol sent to SMH REB for expedited review. Subsequent to this, human ethics approval and supporting documentation was forwarded to Harvard for human ethics approval from the U.S. EPA. This approval is pending. No human exposure testing for this project will be carried out until official approval from the U.S. EPA and the human CAPs exposure facility has been fully tested and characterized. It must be pointed out that a considerable amount of time and personnel costs have accumulated due to the ethics approval process required for two REBs in Toronto (SMH & U of T), as well as the EPA IRB. Each protocol amendment/revision has been consequent to discussions with the SAC and within the PM Center. We are now satisfied with the current design and anticipate no further delays.

New Harvard PM Concentrator Facility: Construction of our human CAPs (coarse, fine, ultrafine) exposure facility, funded through a Canadian infrastructure grant (CFI) and the Harvard/EPA Center, began the end of July, 2006. However, numerous meetings/discussions have been held to plan and finalize the facility, including GOEHU, University of Toronto, Harvard and the Southern Ontario Centre for Atmospheric Aerosol Research (SOCAAR). Both the coarse and fine concentrators have been installed and testing initiated. The ultrafine concentrator system has been delivered to the CAPs facility, and installation and operation training will be completed soon. The new CAPs exposure facility is nearing completion, including a 1.8 m<sup>3</sup> plexiglass subject enclosure with facemask delivery of the CAPs. This larger enclosure will now afford us the potential opportunity of exercising subjects (to increase ventilation/inhaled dose) if deemed necessary in future studies. A full characterization of the facility will be carried out before human testing, including the facemask CAPs levels.

Sub-award Agreement: A sub-award agreement between Harvard and SMH was signed July 11, 2006. Subsequent to this, an account was set up for ordering equipment and supplies. As well, a sub-contract was setup (June 2006) between SMH and the University of Michigan (Rob Brook), for analyses/consultation of ultrasound and echocardiography.

Training: Bruce Urch, the study coordinator and Research Assistant has been facilitating all aspects of the new CAPs facility construction with Harvard, Greg Evans (SOCAAR), the University of Toronto and on-site contactors. Steve Ferguson, from Harvard, ran through a 3-day CAPs training/familiarization session with Bruce and Mike Fila who will run the facility. We have been testing the new Finometer BP finger cuff for stability, outcome measures and use during the 2-hr exposure. Mary Speck, the Laboratory Technologist at GOEHU currently doing ultrasound measurements was trained in Michigan to do the echo cardiac measures by an echo technologist under the supervision of Rob Brook and Julie Kovach. Subsequently, Mary has been practicing the echo techniques in order to perfect echo testing using our Terason system, sending echo images of GOEHU staff to Michigan for review. In discussion with Rob Brook, we decided that he would train us to do the software analyses of the ultrasound and echo images in-house. Strict guidelines will be established to ensure quality data. Julie Kovach, the cardiologist working with Rob Brook has recently left and is being replaced by Ted Kolias, a cardiologist specializing in echocardiography.

Collaborations: There have been regular scientific communications/meetings between the study investigators (GOEHU, Michigan & Harvard), mostly by teleconference but also in-person, which have proven to be both fruitful & beneficial to the development/progress of this project, thus will continue throughout to its completion.

New Collaborations: We are in the process of establishing a collaboration with Health Canada on coarse PM human health effects. They would like to collaborate with Harvard and us on our PM Center study by adding a few measures of inflammation & oxidative stress that they would measure in the urine and blood. Specifically in urine, measures will include: Isoprostane-8 & thiobarbituric acid reactive substances (TBARS) for oxidative stress, D-Glucaric acid for liver response & enzyme stimulation, and VEGF an angiogenesis factor. Measures in blood will include: TNF- $\alpha$  for inflammation, oxidative potential, Isoprostane-8, TBARS and conjugated diene for oxidative stress.

AllerGen researcher, Jeremy Scott, is the PI of a study examining: 1) the effect of coarse CAPs exposure in humans with seasonal allergic rhinitis (in and out of season and  $\pm$  pollution exposure; 2) whether specific gene-environment interactions are responsible for the development and/or exacerbation of symptoms; 3) the development of a system to expose nasal/airway epithelial cells to real-world pollutants; and 4) the contribution of specific gene pathways responsible for the pollutant-induced allergic rhinitis in a murine model. Filter samples will be collected during exposures of people, cells and animals for biological material including airborne endotoxin (Limulus Amebocyte Lysate test method) and markers of fungi ( $\beta$ 1 $\rightarrow$ 3 D-glucans; GlucateLL kit). Samples will also be provided to James Scott for characterization of the microbial community in the air.

AllerGen researcher, James Scott, is also the PI of a study that is part of the Canadian Healthy Infant Longitudinal Development study (CHILD- a Canada-wide multidisciplinary, longitudinal, population-based birth-cohort study). The specific objective is to: adapt DGGE and DNA macroarray technologies for the characterization of airborne and dust-borne fungal contaminants; (2) investigate the value of these data in the context of studying indoor and outdoor exposures and health outcome measures. In addition, endotoxin analyses samples will be made available from the CAPs exposures of the Toronto PM Center study. James Scott has analyzed endotoxin

samples collected during the Toronto CLEANAIR fine CAPs+O<sub>3</sub> study starting from April 2006. Results from 31 samples analyzed show up to a 20-fold range in endotoxin levels for the CAPs.

University of Michigan collaborator Rob Brook has written a proposal to: 1) examine vascular dysfunctions following 2-hr coarse CAPs exposures; 2) compare responses to CAPs from both urban and rural sources; 3) compare responses in two races (blacks and whites); and 4) elucidate the CAPs constituents/sources responsible for the cardiovascular responses. This study will be a comprehensive integration of a series of supplemental human experiments with our PM Center study of coarse PM in Toronto. Additional vascular measures will be added to the Toronto study including Endo-PAT (microvascular resistance, artery endothelial function) and SphygmoCor (large arterial compliance, central aortic BP and hemodynamics) devices. Biological constituents will be collected on appropriate filters, with the characterization coordinated by Diane Gold at HSPH for measures of airborne endotoxin (LAL kit) and markers of fungi ( $\beta$ 1 $\rightarrow$ 3 D-glucans; GlucateLL kit).

Evaluation of Autonomic Responses to CAPs in Controlled Exposure Studies: Holter ECG recordings are available for our previous Toronto exposure studies, and have been sent to Boston for heart rate variability (HRV) analyses at no additional cost to the EPA Center. This will inform the new study by enabling the investigators to assess the interrelation between pollution exposures, HRV autonomic responses, vascular, and inflammatory responses.

Evaluation of Baseline Inflammatory Responses to CAPs in Controlled Exposure Studies: Aaron Thompson from the Toronto group will be at HSPH in the Occupational Medicine program for the coming year, and as part of his training will, with the help of Jeff Brook and the rest of the Toronto group, be evaluating the contribution of the prior 24-72 hr ambient pollution to the baseline and post-exposure inflammatory responses to CAPs +/- O<sub>3</sub>.

Summary of Other Findings used to inform the Current Project: Our EPA CLEANAIR fine CAP+O<sub>3</sub> study, with Rob Brook in Michigan, is nearing completion (healthy non-asthmatics, 18-50 yrs). To date, 28 of 38 subjects have completed the Toronto part of the study. Each subject receives 4 exposures, including: 1) FA; 2) fine CAPs (target 150-200  $\mu$ g/m<sup>3</sup>); O<sub>3</sub> (120 ppb); & fine CAPs+O<sub>3</sub>. An interim analysis has shown a small but significant increase in diastolic BP during the 2-hr exposures, more so for the combined CAP+O<sub>3</sub> (3.6 mm Hg increase over 2 hrs; p=0.0003), followed by CAPs alone (2.5 mmHg/2hrs; p=0.055) and O<sub>3</sub> alone (1.8 mmHg/2hrs; p=0.09) with the smallest and non-significant changes for FA (1.6 mmHg/2hrs; p=0.2). A similar exposure trend of about the same size effect was shown for systolic BP. In addition, the magnitude of the BP increase was positively associated with the level of CAPs exposure mass concentration. Blood neutrophils also showed a similar pattern of response to BP, with the greatest pre to post exposure increase shown for CAPs+O<sub>3</sub> (15%; p=0.001), followed by CAPs alone (9%; p=0.04) and O<sub>3</sub> alone (8%; p=0.07), with the smallest and non-significant changes for FA (4%; p=0.3). Data is not as complete for the brachial artery measures, but there appears to be a trend for flow-mediated dilation to decrease post relative to pre-exposure, for CAPs (-1.9%) & CAPs+O<sub>3</sub> (-2.0%), compared to no change for O<sub>3</sub> and an increase of 2.6% for FA.

Rob Brook's part of the CLEANAIR fine CAPs+O<sub>3</sub> study is completed and he will be sending the data to GOEHU for statistical analyses/consultation. This was a randomized cross-over study

with 50 subjects exposed to CAPs+O<sub>3</sub>, pre-treated with 1) placebo (control) vs 2) Bosentan (an anti-endothelin) and 3) vitamin C, an anti-oxidant.

James Scott has analyzed endotoxin samples from the Toronto CLEANAIR fine CAPs+O<sub>3</sub> study for April - December 2006. Results show up to a 20-fold range in endotoxin concentration for CAPs/CAPs+O<sub>3</sub> exposures. This data will be used in regression analyses to test for associations with CAPs exposure-induced changes in BP, brachial diameter and reactivity, HRV and inflammatory measures.

**QA/QC:** Annual renewal of approval from St. Michael's IRB has been obtained. This includes all amendments to the protocol. IRB application to the University of Toronto has been submitted and minor concerns of the board responded to. The final approval letter has been obtained. All IRB documents from both boards have been submitted to the EPA IRB for approval. An annual audit is scheduled for September 2007, covering data collection and data management.

We have weekly internal lab meetings to prepare and review laboratory protocols and QA/QC. In addition, we have regular communications with Harvard and our collaborators in Michigan. The quality management documents QAPP, protocols and SOPs are in preparation, and include all amendments and any additions. EPA will audit the CAPs facility once we have begun to test the first few subjects. Data collection on this project has not begun.

**Publications/Presentations:** At present, there are no direct publications. However, in the meantime, we have been analyzing data and the following manuscripts that will inform the current project, are in preparation. Diane Gold from Harvard will work closely with GOEHU in the preparation of the three manuscripts listed below:

1. "Concentrated Ambient Fine Particles induce an IL-6 inflammatory response in asthmatics and non-asthmatics."
2. Blood endothelins and nitric oxide metabolites (systemic vascular peptides) in response to controlled fine CAPs +/- O<sub>3</sub> in young healthy asthmatics and non-asthmatics and in children.
3. End-tidal CO<sub>2</sub> (capnography) as sensitive marker of ventilation/perfusion changes in response to fine CAPs +/- O<sub>3</sub> in young healthy individuals.

**Future Activities:** The CAPs facility will be completed and exposure characterization carried out. The Mortara Holters were ordered in July 2007 and we will do some ECG measures on staff and send the recordings to Harvard for QA/QC. We will complete training with the echo/ultrasound and Finometer and all other aspects of exposure testing including SOPs. After ethics approval from EPA, we will begin recruiting subjects, after which exposure testing will begin. An interim analysis will be carried out after the 24 subjects have completed the three exposures, to guide subsequent work.

**Supplemental Keywords:** concentrated air particles, acute cardiovascular effects, coarse particles, fine particles, vascular dysfunction

**Relevant Web Sites:** <http://www.hsph.harvard.edu/epacenter/>

**Date of Report:** August 1, 2007

**EPA Grant Number:** R-832416-010

**Center:** EPA Center at HSPH for Ambient Particle Health Effects

**Center Director:** Professor Petros Koutrakis

**Project Title:** Assessing Toxicity of Local and Transported Particles Using Animal Models Exposed to CAPs

**Investigators:** John Godleski (PI), Petros Koutrakis (co-PI)

**Institution:** Harvard School of Public Health

**EPA Project Officer:** Stacey Katz

**Project Period:** October 1, 2005 – September 30, 2010

**Period Covered by the Report:** August 1, 2006 – July 31, 2007

**Objective(s) of The Research Project:** The objective of Project 3 is to differentiate the toxicological effects of locally emitted and transported particles. To do so, short-term 5 hr animal exposures to concentrated ambient fine particles (CAPs) are conducted during the time periods of 5-10 am and 10:30 am-3:30 pm. Starting inhalation exposures at 5 am, before significant vertical mixing takes place, permits us to capture particles predominantly from local sources. We expect that exposures starting about 10:30 am are relatively more enriched in transported particles. Specific biologic outcomes will include: breathing patterns, indicators of pulmonary and systemic inflammation, blood pressure, endothelin-1, endothelial nitric oxide synthase, atrial natriuretic peptide, *in vivo* oxidant responses in the heart and lung, and quantitative morphology of lung and cardiac vessels. To control for circadian variations all outcomes are assessed during both time periods, in relation to those of filtered air (sham) exposures as well as those of positive controls using particles of known toxicity. Animal exposures are characterized using continuous measurements of particle mass, size, number, and black carbon, as well as integrated measurements of particle mass, sulfate, elements, and organics. Strains of rats used include Sprague Dawley (SD), which we have used extensively in previous CAPs studies, Spontaneously Hypertensive Rats (SHR), a sensitive model in many studies, and Wistar Kyoto (WKY) the strain control for SHR rats.

**Progress Summary/Accomplishments:** Table 1 shows the target number of animals for each outcome in the exposure plan for these studies. Because limited numbers of animals can be used in each outcome at a time, 21 repetitions of these experiments were carried out.

**Table 1. Target numbers of outcomes per group of rats with any 5 hr-exposure**

<b>Treatment Group 5-10 AM</b>	<b>Total # Exposed</b>	<b>Blood</b>	<b>Buxco</b>	<b><i>In Vivo</i> Oxidant</b>	<b>RNA, BAL or Morphology</b>
<b>CAPs</b>	<b>5</b>	<b>3</b>	<b>5</b>	<b>2</b>	<b>3</b>
<b>SHAM</b>	<b>5</b>	<b>3</b>	<b>5</b>	<b>2</b>	<b>3</b>

<b>When Assessed</b>		24hrs After Exposure	During Exposure	< 30 min after Exposure	24 hrs After Exposure
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Table 2 lists the number of animals studied for breathing pattern, BAL, and chemiluminescence outcomes, respectively in each treatment group for each strain. The numbers of animals studied provided sufficient power to find differences in each of these groups based on our previous studies.

**Table 2. Numbers of animals of each Strain assessed for breathing pattern data (Buxco), Broncholaveolar Lavage (BAL), and *In Vivo* Chemiluminescence (IVC) either in the early morning (AM) or mid-day and afternoon (PM)**

<b>Outcome Assessed</b>	<b>Group/ Exposure</b>	<b>All AM</b>	<b>All PM</b>	<b>SD AM</b>	<b>SD PM</b>	<b>WKY AM</b>	<b>WKY PM</b>	<b>SHR AM</b>	<b>SHR PM</b>
<b>Buxco</b>	<b>CAPs</b>	105	105	50	50	28	27	27	28
<b>Buxco</b>	<b>Sham</b>	105	105	50	50	28	27	27	28
<b>BAL</b>	<b>CAPs</b>	63	63	30	30	15	18	18	15
<b>BAL</b>	<b>Sham</b>	63	63	30	30	17	16	16	17
<b>IVC</b>	<b>CAPs</b>	21	22	12	11	4	5	5	6
<b>IVC</b>	<b>Sham</b>	21	22	11	11	5	5	5	6

Exposure data from all of these experiments are shown in Table 3. For these experiments the CAPs mass concentrations (Early -  $505.8 \pm 75.8$  and Late  $407.2 \pm 45.7$ ) were slightly higher than previous published studies from our laboratory, but not significantly different from each other using a paired two-tailed t-test. However, it is clear that there are significant differences in black carbon and elemental carbon between the early and late exposure, supporting the premise that the early exposure would be more influenced by local (primarily traffic sources) whereas the exposures later in the same day were more likely to contain transported particles. Since these experiments were not done in the summer, there is no significant difference in sulfur between the morning and afternoon. In these studies, in addition to greater black and elemental carbon in the morning, iron, nickel, and copper levels were also significantly higher in the morning.

When these data were analyzed using the ratio of a specific component to the total fine mass (or fraction of the component) essentially the same findings were observed with more robust p-values. Thus, elemental carbon, copper, and nickel were significantly higher in the morning. In addition, several components were found to be significantly higher in the afternoon. These include sodium, potassium, magnesium, manganese, and silicon. The sulfur fraction was higher in the afternoon than the morning, but this difference was not significant. Therefore, while statistically significant differences were found between AM and PM exposures, the differences were modest.

**Table 3. CAPs mass and component concentrations during the early and late exposure periods (concentrations are reported in micrograms per m<sup>3</sup>)**

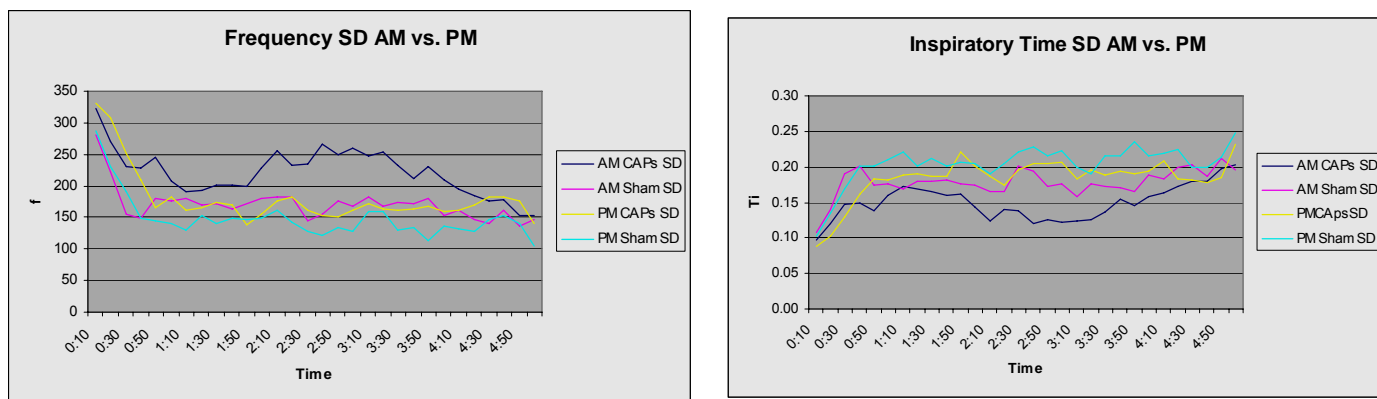
Measures	Early mean ± SE	Late mean ± SE	p =
CAPs Mass	505.8 ± 75.8	407.2 ± 45.7	0.083
<b>*Black Carbon Mass</b>	10.5 ± 0.9	7.3 ± 1.1	<b>0.002</b>
<b>*Elemental Carbon</b>	22.5 ± 2.6	16.5 ± 2.8	<b>0.032</b>
Organic Carbon	72.2 ± 6.9	67.3 ± 6.9	0.505
Total Carbon	94.9 ± 9.0	83.8 ± 9.5	0.261
Sodium	8.9 ± 2.6	10.4 ± 2.9	0.181
Chlorine	9.7 ± 3.6	13.8 ± 6.2	0.174
Silicon	9.5 ± 1.6	8.8 ± 1.1	0.448
Aluminum	3.4 ± 0.6	3.1 ± 0.4	0.437
Sulfur	37.0 ± 5.9	35.7 ± 5.3	0.832
Calcium	6.3 ± 0.9	6.1 ± 0.8	0.761
Titanium	0.33 ± 0.05	0.26 ± 0.04	0.108
Potassium	2.8 ± 0.3	2.7 ± 0.3	0.465
<b>*Iron</b>	13.3 ± 1.9	9.7 ± 1.0	<b>0.035</b>
Zinc	1.0 ± 0.1	1.1 ± 0.1	0.857
<b>*Nickel</b>	0.07 ± 0.015	0.04 ± .007	<b>0.033</b>
Vanadium	0.03 ± 0.01	0.01 ± .009	0.144
Magnesium	1.2 ± 0.3	1.4 ± 0.3	0.293
<b>*Copper</b>	0.4 ± 0.06	0.2 ± 0.03	<b>0.019</b>
Manganese	0.2 ± 0.03	0.3 ± 0.04	0.349
<b>*EC Percent of Mass</b>	5.3 ± 0.5	3.9 ± 0.4	<b>0.007</b>
OC Percent of Mass	17.8 ± 1.7	18.7 ± 1.5	0.586
TC Percent of Mass	23.1 ± 2.1	22.5 ± 1.8	0.753
<b>*Sodium Percent of Mass</b>	2.3 ± 0.7	2.7 ± 0.7	<b>0.026</b>
Chlorine Percent of Mass	2.3 ± 0.9	3.1 ± 1.3	0.180
<b>*Silicon Percent of Mass</b>	2.5 ± 0.4	2.9 ± 0.5	<b>0.040</b>
Aluminum Percent of Mass	0.89 ± 0.17	1.03 ± 0.19	0.107
Sulfur Percent of Mass	7.5 ± 0.7	8.6 ± 0.8	0.123
Calcium Percent of Mass	1.6 ± 0.3	2.0 ± 0.4	0.056
Titanium Percent of Mass	0.09 ± 0.01	0.09 ± 0.02	0.814
<b>*Potassium Percent of Mass</b>	0.68 ± 0.07	0.77 ± 0.08	<b>0.017</b>
Iron Percent of Mass	3.3 ± 0.4	2.9 ± 0.4	0.226
Zinc Percent of Mass	0.25 ± 0.03	0.29 ± 0.04	0.429
<b>*Nickel Percent of Mass</b>	0.019 ± 0.004	0.009 ± 0.001	<b>0.025</b>
Vanadium Percent of Mass	0.007 ± 0.002	0.003 ± 0.002	0.074
<b>*Magnesium Percent of Mass</b>	0.29 ± 0.07	0.39 ± 0.07	<b>0.048</b>
<b>*Copper Percent of Mass</b>	0.09 ± 0.01	0.07 ± 0.01	<b>0.043</b>
<b>*Manganese Percent of Mass</b>	0.06 ± 0.01	0.08 ± 0.01	<b>0.040</b>

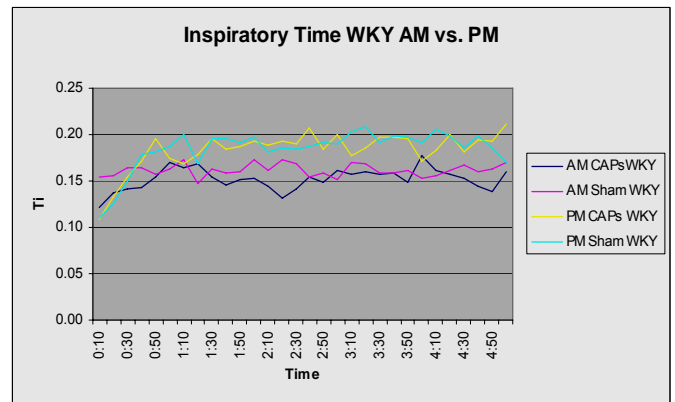
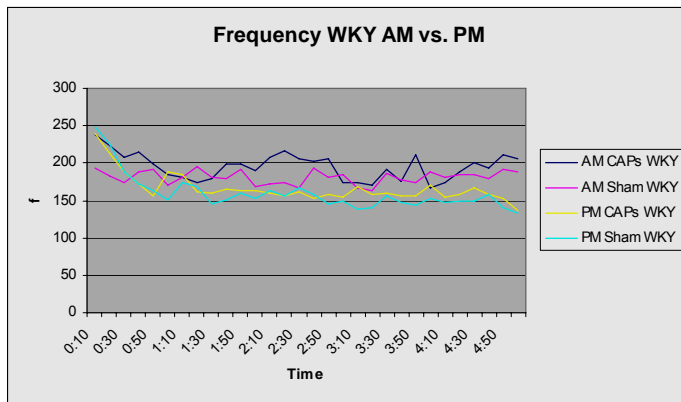
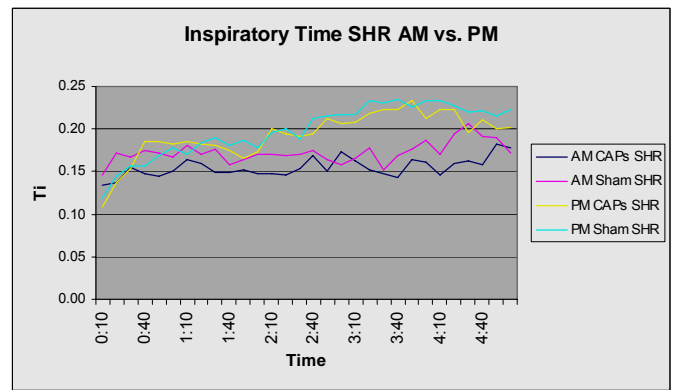
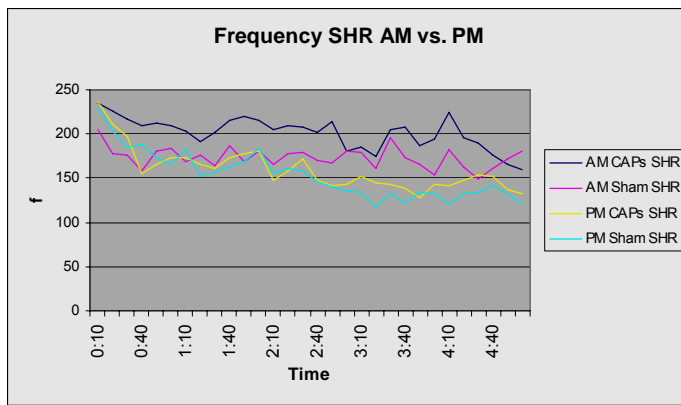
For all rat strains, breathing pattern and *in vivo* chemiluminescence studies show significant differences between CAPs and Sham exposures, as we have previously reported. With breathing

pattern there is an increase in respiratory frequency with concomitant shortening of the time of inspiration and expiration. Statistical modeling was used to assess the size and strength of association between CAPs or Sham exposure and each respiratory outcome. Additive mixed models were applied to 10-minute averaged data collected from all CAPs and Sham animals during AM or PM exposure. A form of the repeated measures model for longitudinal data, additive mixed models represent an extension of linear regression models that (1) estimate potentially non-linear effects of independent variables, and (2) include random effects as independent variables in order to account for clustering of observations that result from repeated measurements being taken on the same animal during the same exposure period. For each outcome, additive mixed models were fit using as independent variables (1) a general nonlinear mean trend for sham animals over the exposure period, (2) an exposure indicator, which implies a constant shift in the mean trend due to the exposure, and (3) random animal effects reflecting animal-to-animal heterogeneity that results in correlation among 10-minute averages taken on the same animal over time. All models were fit using the gamm function in R software (R Development Core Team 2004). Finally, a more general model that relaxed the assumption of a constant shift due to exposure was also fit to the data. This model specified distinct mean trends over the exposure period for the sham and exposed animals, again including random animal effects to account for the repeated measurements taken on each animal. The difference between these estimated trends represents the time-varying effect over the exposure period.

All three strains show an increase in respiratory frequency with CAPs exposure, with slight but significant strain differences. The differences between the AM CAPs and Sham exposures and the PM CAPs and Sham exposures are similar. These data are shown in Figure 2 in which the mean respiratory response for the number of animals per group listed in Table 2 is plotted over the entire exposure period. To simplify the data presentation, only the means are shown for frequency and inspiratory time.

**Figure 2. Respiratory breathing patterns illustrating frequency and inspiratory time**





Analyses of BAL data did not show significant differences between morning and afternoon exposures for any BAL parameter.

Data from the in vivo chemiluminescence studies are presented in Table 4. These studies show that in these exposures, the lung had significant effects of CAPs exposures whereas chemiluminescence changes in the heart did not reach significance. There were significant changes with both early and late exposures and analyses showed that these were not significantly different.

**Table 4. In vivo chemiluminescence of the heart and lungs with early and late exposures**

Sites	Effect	CAPs – Sham Difference Mean ± SE	P value
Heart	All Exposures	5.47 ± 4.2	0.197
	Testing whether CAPs effects are different in the 3 species		0.644
	Testing whether early and late CAPs effects are different		0.366

<b>Lung</b>	All Exposures	10.28 ± 3.07	<b>0.001</b>
	Testing whether CAPs effects are different in the 3 species		0.640
	Early Exposure	12.28 ± 4.41	<b>0.007</b>
	Late Exposure	8.38 ± 4.31	<b>0.055</b>
	Testing whether early and late CAPs effects are different		0.529

Because the CAPs effects from the different rat strains were not significantly different from one another, and the CAPs effects during early and late exposures were also not significantly different, component analyses with the heart and lung data estimated overall concentration slopes not segregated by strain or early/late exposures. In these analyses, no cardiac effects were found using univariate or multivariate analyses. Table 5 illustrates many components with significant univariate relationships to lung chemiluminescence, but none of these were significant in multivariate analyses.

**Table 5. Effects of specific exposure components on in vivo lung chemiluminescence. using univariate analyses**

<b>Element/ Component</b>	<b>Estimate ± SE</b>	<b>P value</b>
<b>CAPs Mass</b>	0.012 ± 0.005	<b>0.027</b>
<b>Organic Carbon</b>	0.106 ± 0.038	<b>0.0064</b>
<b>Elemental Carbon</b>	0.298 ± 0.121	<b>0.016</b>
<b>Al</b>	1.573 ± 0.739	<b>0.036</b>
<b>Si</b>	0.577 ± 0.267	<b>0.034</b>
<b>S</b>	0.132 ± 0.061	<b>0.033</b>
<b>Fe</b>	0.509 ± 0.218	<b>0.022</b>

Overall, the analyses thus far largely confirm our earlier studies and findings with CAPs exposures in Boston. It is of particular interest that, apparently, despite statistically significant differences in the composition of early and late exposures on given days, there is no significant difference in toxicity. It seems that our studies have adequate statistical power, since we were able to detect significant diurnal differences with respiratory patterns, significant strain differences, as well as CAPs vs Sham differences. Since both early and late exposures show significant toxicity, with no significant difference in the biological outcomes between the two

exposure periods, these results do not suggest any difference in the toxic potential of local and transported sources.

**Future Activities:** We are doing exposures of WKY and SH rats, monitoring blood pressure, electrocardiogram, and blood parameters. We expect to complete these exposure sets by December 2007, and have much of the exposure data analyzed. In the coming year we expect to complete analyses of all the outcomes. This project is scheduled to be finished within the first two and a half years of the grant.

**QA/QC:** Quality Management documents Protocol's and SOP's are on file at HSPH for this project. This project uses established animal experiments with Standard Operating Procedures for each specific animal exposure. An annual audit is scheduled for August 2007, covering data collection and data management. In combination with the existing SOP, the project proposal serves as the QAPP for this project. This project does not involve human subjects. These studies are all done with sham exposure controls for both the early morning exposure and the mid-day exposure. Exposure analyses follow the QA/QC standards applied to all exposure data. For all biological outcome determinations, analyses are done by well-trained personnel, and reviewed in detail by senior investigators. In addition, for all biological outcomes, individual animals are randomly chosen representing at least 1% of all outcome data for QA. The data on these animals are reviewed by senior personnel. This includes breathing pattern data, BAL, blood analyses, and chemiluminescence. For breathing pattern, the data are reanalyzed; and for BAL, cell differential determinations are redone. For all other data in which the primary measurement is no longer available (such as chemiluminescence) the specific determination is checked for recording, consistency with other measurements in that animal, and verified by the senior investigator. To pass QA/QC, data must not deviate from the initial determination by more than 10% for any parameter. Finding data outside this standard initiates a complete review of the data set.

**Publications/Presentations:** None

**Supplemental Keywords:** concentrated air particles, acute cardiovascular effects, coarse particles, fine particles, vascular dysfunction

**Relevant Web Sites:** <http://www.hsph.harvard.edu/epacenter>

#### **ATTACHEMENTS FOR PROJECT 1:**

- VA Boston Healthcare System, Research and Development Committee, Report of Committee Action
- VA Boston Healthcare System, Human Subjects Subcommittee (IRB), Report of Committee Action

**VA Boston Healthcare System  
Research and Development Committee  
Report of Committee Action**


Date of Action:	April 4, 2007
Principal Investigator:	Pantel S. Vokonas, M.D.
Title of Submission:	Ambient Particle Health Effects: Exposure, Susceptibility, and Biological Mechanisms/Dosimetry
Protocol Number:	IRB# 1212
Species:	Human
Type of Submission:	Continuing Review

<input checked="" type="checkbox"/>	<b>APPROVED</b>
	<b>APPROVED</b> under administrative review by
	<b>CHANGES REQUIRED:</b> Based on Committee review, the changes or actions noted below are stipulated as required for review and approval by the chairperson. Compliance with these stipulations may be confirmed under Committee procedures for administrative review and approval.
	<b>DEFERRED:</b> The item has been deferred pending changes or clarifications noted below. The proposal will be reconsidered at the next meeting after the requested information or changes are submitted.
	<b>DISAPPROVED:</b> The proposal was disapproved for the reasons noted below. Please consult with the ACOS for Research of the Committee Chairperson before resubmitting.
	<b>APPROVED FOR SUBMISSION TO FUNDING AGENCIES ONLY:</b> This proposal is approved to be submitted as a 'just in time' submission ONLY. If funded, the protocol must be approved by applicable subcommittee(s), and R&D Committee before subjects may be enrolled or data collection has begun.
	<b>NOTED</b>

Note: For 'Changes Required' and 'deferred', responses must be received from the principal investigator within 60 days. After 60 days a new submission and full review are required.

COMMENTS: 5EE

IRB Approved on March 26, 2007

  
 Ronald Goldstein, M.D. or Ann Hendricks, Ph.D.  
 Co-Chair, Research and Development Committee

April 4, 2007  
 Date

**VA BOSTON HEALTHCARE SYSTEM  
HUMAN STUDIES SUBCOMMITTEE (IRB)**

**REPORT OF COMMITTEE ACTION**

Version December 1, 2004

Date of Action:	March 26, 2007
Principal Investigator:	Pantel Vokonas, M.D.
Title of Submission:	"Ambient Particle Health Effects: Exposure, Susceptibility, and Biological Mechanisms/Dosimetry"
Protocol Number:	IRB #1212
Type of Submission & Item Description:	Request for Continued Approval of Human Studies
Human Subject Enrollment:	Yes: <input checked="" type="checkbox"/> No: <input type="checkbox"/>
Vulnerable Population:	Yes: <input type="checkbox"/> No: <input checked="" type="checkbox"/> Category: <input type="checkbox"/> Entire Study: <input type="checkbox"/> Sub-Population: <input type="checkbox"/>
<input checked="" type="checkbox"/>	<b>APPROVED</b> at IRB meeting
<input type="checkbox"/>	<b>APPROVED</b> under procedures for administrative review by
<input type="checkbox"/>	<b>CHANGES REQUIRED:</b> Based on Committee review, the changes or actions noted below are stipulated as required for approval. Compliance with these stipulations may be confirmed under Committee procedures for administrative review.
<input type="checkbox"/>	<b>DEFERRED:</b> The item has been deferred pending changes or clarifications noted below. The proposal will be reconsidered at the next Committee meeting after the requested information or changes are submitted.
<input type="checkbox"/>	<b>DISAPPROVED:</b> The proposal was disapproved for the reasons noted below. Please consult with the ACCOS for Research or the Committee Chairperson before resubmitting.
<input type="checkbox"/>	<b>NOTED</b>

**Note:** For 'Changes Required' and 'deferred', responses must be received from the principal investigator within 60 days. After 60 days a new submission and full review are required.

**COMMENTS (ITEM 3G):**

- (a) Three subjects were lost to follow-up and three subjects withdrew from the study (subjects withdrew at their own request).
- (b) The minimal risk of this study makes it eligible for low intensity adverse event reporting and one year approval.
- (c) Approval dates: 03/26/07 - 03/24/08

  
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 KENNETH BAUER, M.D.  
 Chair, Human Studies Subcommittee