

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

**1<sup>st</sup> YEAR PROGRESS REPORT**

**Overview**

The EPA Particle Center at HSPH originally planned to include five projects: Project 1, with a focus on exploring the pathways of particle toxicity for cardiovascular responses in the Normative Aging Study; Project 2, with a focus on the cardiovascular effects of particles and gaseous co-pollutants from mobile sources; Project 3, with a focus on cardiovascular toxicity of concentrated ambient fine, ultrafine and coarse particles, using controlled human exposures; Project 4, with a focus on assessing toxicity of local and transported ambient particles using animal models exposed to CAPs, and; Project 5, with a focus on assessing toxicity of vehicular emissions, using animal models.

However, after careful consideration of the questions and concerns raised by our Science Advisory Committee during our July 2006 meeting, we decided to eliminate Project 2 and redirect funds to extend Project 1 by continuing health and pollution measurements for our NAS cohort for the duration of the Center. Project 3 will be conducted during Years One through Five, Project 4 Years One through Three, and Project 5 Years Three through Five. This 1<sup>st</sup> year progress report includes preliminary results for Projects 1, 3, and 4.

**Date of Report:** September 2006

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

**Center Number and Internal Number:**

**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

**Institutions of PIs:** Harvard School of Public Health, Boston, MA 02115

**Research Category:** Epidemiology

**Project Period:** October 2005 – September 2010

**Objective or Research:** 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. These additional measurements will include ECG, blood inflammatory marker, medication, genotypic, food frequency, and particle exposure measurements for each of the current NAS participants. ECG and blood samples are being analyzed for a variety of measures (HRV, ST segments, QT intervals, CRP, sICAM-1, sVCAM-1, and homocysteine); these measures will be used as intermediate markers of the inflammatory, endothelial, and autonomic pathways. In addition, they 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 PC) concentrations that are being measured at our stationary ambient monitoring (SAM) site. The study will use this data to test three primary hypotheses:

**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;
- **general cardiovascular responses**, as measured by increases in blood pressure and ECG changes including ST-segment level and QT-interval.

**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:** In Year One, we have continued to collect ECG measurements on the Normative Aging Study participants. In addition, we have begun to collect

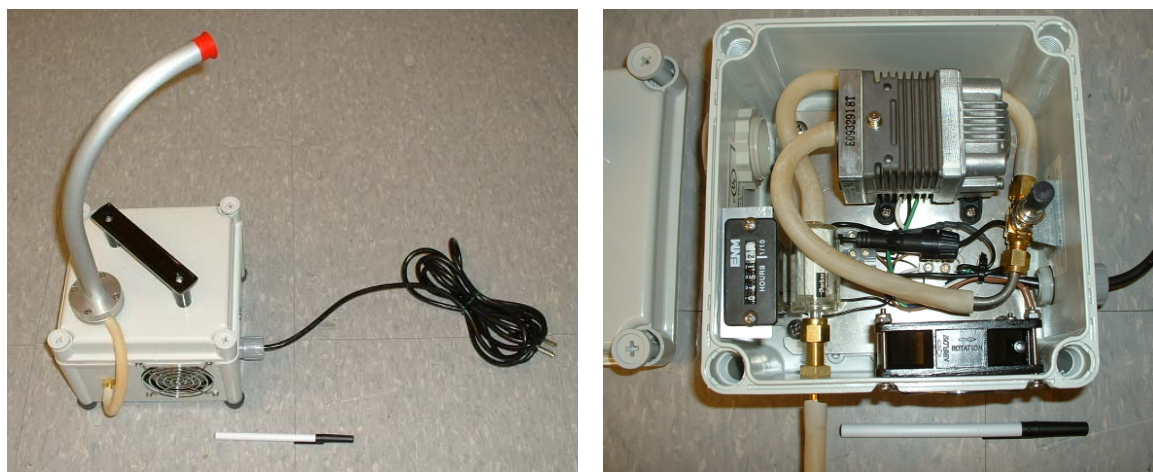
daily diet information during each clinic visit using a validated semi-quantitative food frequency questionnaire. To date, we already have data from enough participants to begin fitting longitudinal models. During Year Two of the Center, we expect to continue these analyses using data for an additional 100 participants. We have also genotyped the participants for the micro-satellite repeat of HMOX-1, and have started to look at this data.

Analyses are underway to examine the modifying effect of lead burden on the association between PM and HRV, the modifying effects of HFE genotypes on that association, and the association between air pollution and homocysteine concentrations. Other analyses of ambient air pollution and health for the NAS cohort have been completed and are currently in press in peer-reviewed journals.

We have constructed and validated an integrated particle sampler to measure one-week long indoor particle concentrations (Figure 1). Correspondingly, participant-friendly mailers, sampling instructions, and sampling logs were developed to allow these samplers to be mailed to study participants, in order for participants to install, operate, and return these samplers to the health clinic.

We are currently recruiting participants for exposure sampling. By September 2006, twenty NAS participants have received and completed indoor particle exposure sampling. These individuals were identified through telephone calls made approximately two weeks prior to their clinic visit. Sampling units were prepared in the laboratory, with the installation of a pre-weighed Teflon® filter and an initial flow measurement. Participants volunteering for indoor exposure monitoring were mailed samplers, together with corresponding instructions and sampling logs, for receipt one-week prior to their clinic visit. After receipt, participants removed samplers from the boxes and plugged samplers into an electrical outlet in the main activity room of their home. Participants unplugged samplers and brought samplers with them to the health visit. Samplers were then returned to the central laboratory for analysis, whereupon a final flow measurement was taken, and the filter was removed for future analysis (gravimetric, reflectance, and ion chromatographic analysis).

**Figure 1.** Indoor Particle Exposure Sampler -- Outside and Inside Views



## QA/QC

Protocols and SOP's, for the portion of the Normative Aging Study pertaining to the work of this project are in place and on file at the Veteran's Affairs Medical Center Jamaica Plain. Recruitment for participants for this project has been at the end of Year 1. The project has IRB approval from the Veteran's Affairs Medical Center IRB. The approval is in the form of an amendment request to the ongoing Normative Aging Study, under the previous EPA Center. The IRB approval will expire in April of 2007. The Veteran's Affairs Medical Center Researchers will file a renewal application in March 2007 to obtain approval for the coming year. Researchers at HSPH do not have access to any personal identifiers of the data and have been granted an exemption by the HSPH IRB. The USEPA's IRB official has reviewed all IRB material and has granted approval for the project to proceed

**Publications/Presentations:** Our paper on air pollution and inflammatory markers is coming out in the International Journal of Epidemiology. Our paper fitting a spatio-temporal model predicting traffic particles at addresses, which will be part of our examination of chronic effects in the NAS cohort, has been accepted in JRSSA.

**Future Activities:** Health and exposure data collection are on-going, as is laboratory and statistical analyses of collected samples. HRV tapes are currently being analyzed for additional HRV measures, including QT length. Using funds redirected from Project 2, health and pollution measurements for our NAS cohort will continue for the duration of the Center. In addition, we will supplement these measurements with 8-OHdG in urine, a marker for oxidative stress.

**Supplemental Keywords:** air pollution, indoor monitoring, inflammation, heart rate variability, spatio-temporal air pollution modeling

**Relevant Web Sites:** [www.hsph.harvard.edu/EPACenter](http://www.hsph.harvard.edu/EPACenter)

**Date of Report:** September 2006

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

**Center Number and Internal Number:**

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

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

**Institutions of PIs:** Gage Occupational & Environmental Health Unit (GOEHU), Toronto, Canada

**Research Category:** Controlled Human Exposure Study

**Project Period:** October 2005 – September 2010

**Objective or Research:** The proposed project will examine the acute cardiovascular effects of fine, ultrafine and coarse CAPs in healthy adults, using a controlled particle exposure facility, in Toronto. Fifty subjects will each receive three CAPs exposures (3 size fractions) and a particle-free air exposure, in random order. Cardiac vascular/physiologic outcomes will be measured both pre-, post- and 24 hrs post-exposure, and will include measures of: i) vascular dysfunction (brachial artery diameter, flow- and nitroglycerin-mediated dilatation by ultrasonography; stroke volume and cardiac output by Doppler, 2-D echocardiography); ii) blood pressure and; iii) markers of systemic inflammation (CBCs, IL-6, CRP, endothelins) in blood taken pre-, post- and 24 hrs post-exposure. We will also perform continuous (in-exposure) measurements of: beat-to-beat arterial BP using a Finometer monitor, including calculated determinations of cardiac output, stroke volume and systemic vascular resistance (SVR) and; measures of cardiac autonomic dysfunction (HRV using 24 hr Holter monitoring). The specific hypotheses to be addressed by this proposed research are the following:

- Acute human exposures to CAPs of ultrafine, fine and coarse size fractions result in cardiovascular responses consistent with vascular narrowing, vascular/autonomic dysfunction, inflammation, and/or endothelial activation compared to filtered air (control) exposures.
- Associations between CAPs and cardiovascular responses differ by particle size fraction and composition.

**Progress Summary/Accomplishments:** *Human Ethics Approval:* The study protocol was submitted to St. Michael's Hospital, Human Research Ethics Board, 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). 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.

*New Harvard PM Concentrator Facility:* Construction of our human CAPs (fine, ultrafine, coarse) exposure facility, funded through a Canadian infrastructure grant (CFI), began the end of July, 2006. However, over the previous year numerous meetings/discussions have been held to

plan the facility, including GOEHU, University of Toronto, Harvard and the Southern Ontario Centre for Atmospheric Aerosol Research. The facility is due to be completed in 3 months. After this, it will be tested and characterized before human studies start.

*Subaward Agreement:* A subaward agreement between Harvard and St. Michael's Hospital was signed July 11, 2006. Subsequent to this an account was set up for ordering equipment and supplies. As well, a sub-contract is being setup between St. Michael's Hospital and the University of Michigan (Rob Brook), for analyses of ultrasound and echocardiography.

*Training:* Bruce Urch, the study coordinator and Research Assistant, travelled to Rochester in May, 2006 for training/familiarization with the use of the Harvard ultrafine concentrator (at the Rochester PM Centre). In June, 2006 he also had training on the Finometer at a local University research lab using the same device for a cardiac nutrition study. Mary Speck, the Laboratory Technologist at GOEHU currently doing ultrasound measurements will be trained in Michigan to do the echo cardiac measures. She has been in contact with the Michigan group and will travel there to be trained by an echo technologist under the supervision of Drs. Robert Brook and Julie Kovach. Subsequently, she will perfect echo testing using our Terason system, sending echo images of GOEHU staff to Michigan for review.

*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.

## **QA/QC**

Approval from St. Michael's IRB has been obtained. We will be submitting an IRB application to the University of Toronto IRB and an amendment request to St. Michael's Hospital IRB containing changes to the project as a result of recommendations from the annual advisory committee, meeting. Once final IRB approval is obtained, the IRB documents will be submitted to the EPA IRB official for approval. The quality management documents QAPP, protocols and SOPs are in preparation. Data collection on this project has not begun.

**Publications/Presentations:** None

**Future Activities:** Two pieces of major equipment will be purchased for this study including echocardiography (echo) and a Finometer. The echo is a separate module of our current Terason ultrasound system, which will be upgraded to the Terason 3000 system to facilitate operation of both the ultrasound and echo. Quotes have been obtained and the echo & Finometer will be ordered in August, 2006.

Also, final modifications to the study design and protocols will be made. Installation and testing of the particle concentrator will be completed, as will study questionnaires. Once completed, study participant recruitment and screening will begin.

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

**Relevant Web Sites:** [www.hsph.harvard.edu/EPAcenter](http://www.hsph.harvard.edu/EPAcenter)

**Date of Report:** September 2006

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

**Center Number and Internal Number:**

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

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

**Institutions of PIs:** Harvard School of Public Health, Boston, MA 02115

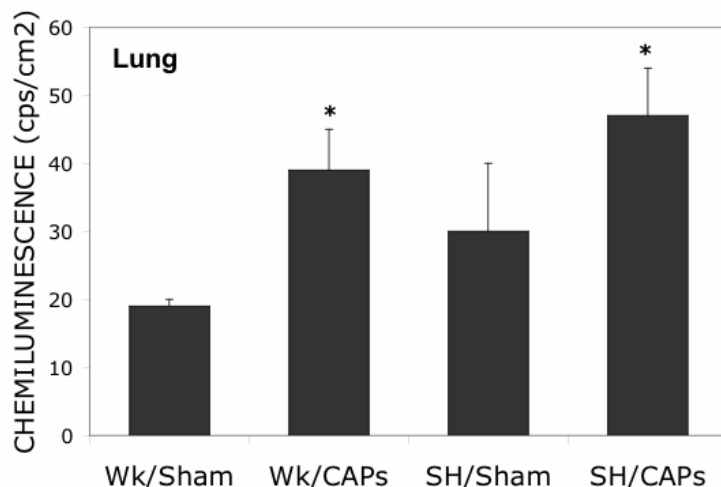
**Research Category:** Toxicology

**Project Period:** October 2005 – April 2008

**Objective or Research:** The Project is intended to differentiate the toxicological effects of locally emitted and transported particles. To do so, short-term animal exposures to CAPs will be conducted during the time periods of 6-10am and 11am-3pm. Starting inhalation exposures at 6am before significant vertical mixing takes place will allow us to capture particles predominantly from local sources. We expect that exposures starting at 11am will be 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. All outcomes will be assessed in relation to those of filtered air (sham) exposures as well as those of positive controls using particles of known toxicity at both time periods to control for circadian variations. Animal exposures will be characterized using continuous measurements of particle mass, size, number, and black carbon, as well as integrated measurements of particle mass, sulfate, elements, and organics.

**Progress Summary/Accomplishments:** We had not previously done studies assessing lung and heart *in vivo* oxidant responses in the compromised model that we plan to use, i.e. spontaneously hypertensive rats and their strain matched controls, Wistar Kyoto rats. These studies were done in the first year of this grant and show increased responses for both the heart and lungs in the compromised population. Lung data are illustrated in Figure 2 below.

**Figure 2.** In Vivo Oxidant Responses with Spontaneously Hypertensive Rats



CAPs:  $260 \pm 60 \mu\text{g}/\text{m}^3$ , n=4, \* p<0.05

Table 1 shows the target number of studies for each outcome in the exposure plan for these studies. Because limited numbers of animals can be used in each outcome at a time, multiple repetitions of these experiments will be needed.

**Table 1.** Target numbers of outcomes per group of rats in each 4 hr-exposure experiment

<b>Treat ment 6-10AM SD Rats</b>	<b>Total # Exposed</b>	<b>Blood</b>	<b>Buxco</b>	<b><i>In Vivo</i> Oxidants</b>	<b>BAL</b>	<b>RNA/ Morph ology</b>
<b>CAPs</b>	<b>8</b>	<b>6</b>	<b>8</b>	<b>2</b>	<b>3</b>	<b>3</b>
<b>Sham</b>	<b>8</b>	<b>6</b>	<b>8</b>	<b>2</b>	<b>3</b>	<b>3</b>
<b>When Assessed</b>		<b>24 hrs before 24 hrs after exposure</b>	<b>During Expos- ure</b>	<b>Immed- iately after exposure</b>	<b>24 hrs after Expos- ure</b>	<b>24 hrs after Expos- ure</b>

Tables 2 and 3 list the number of studies completed and the number of animals studied to date for breathing pattern and chemiluminescence outcomes, respectively. Note that we performed six morning and six afternoon exposures during Year One of the Center.

**Table 2.** Actual number of animals assessed to date: breathing pattern data

	ALL	ALL AM	ALL PM	SD AM	SD PM	WKY AM	WKY PM	SHR AM	SHR PM
CAPS	40	20	20	10	10	5	5	5	5
SHAM	40	20	20	10	10	5	5	5	5
EXPOSURES AM+PM	12	6	6	2	2	4	4	4	4

**Table 3.** Number of animals assessed to date: chemiluminescence data

	ALL	ALL AM	ALL PM	SD AM	SD PM	WKY AM	WKY PM	SHR AM	SHR PM
CAPS	17	8	9	6	5	2	0	2	2
SHAM	17	8	9	5	5	1	2	2	2
EXPOSURES	16	8	8	4	4	4	4	4	4

Data analyses are not complete. However, it is clear that there are no AM/PM differences in sham controls. Therefore, within the time frame of these experiments, diurnal variations do not appear to be of concern for any outcomes measured.

#### 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. In combination with the existing SOP the project proposal serves as the QAPP for this project. This project does not involve human subjects.

**Publications/Presentations:** None

**Future Activities:** We expect to complete these exposure sets by December 2006, 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.

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

**Relevant Web Sites:** [www.hsph.harvard.edu/EPACenter](http://www.hsph.harvard.edu/EPACenter)