EPA Particle Center at Harvard School of Public Health

Novel Exposure Scenarios to Define the Health Effects of Particle Sources

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

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Cardiovascular Toxicity of Concentrated Ambient Fine, Ultrafine and Coarse Particles in Controlled Human Exposures

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Assessing Toxicity of Local and Transported Particles Using Animal Models Exposed to CAPs

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Toxicological Evaluation of Realistic Emission Source Aerosol (TERESA): Investigation of Vehicular Emission

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Title: Harvard/EPA PM Center: Novel Exposure Scenarios to Define the Health Effects of Particle Sources

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OVERVIEW

Investigating the impacts of air pollution on public health requires an intensely multidisciplinary approach. Our Center brings together a diverse group of experts, including atmospheric chemists, engineers, aerosol scientists, toxicologists, pulmonologists, cardiologists, molecular biologists, epidemiologists, statisticians, and exposure assessors. The Center also builds upon the infrastructure and extensive portfolio of other research at the Harvard School of Public Health and allied institutions, including the Universities of Toronto and Michigan. To date, Center research has produced over 170 peer-reviewed publications. Many of these research findings are of public policy significance and were extensively cited by the 2004 EPA Criteria Document and the draft 2008 Integrated Science Assessment report. This valuable scientific productivity depended heavily on the numerous collaborations fostered by the Center over its 10-year lifespan.

Research Program: Our current Center supports four research projects and two cores which share three common objectives: 1) Define mechanisms of response to PM exposures at the molecular, cellular, and integrative pathophysiological levels, using experimental laboratory and population studies; 2) Investigate associations between specific PM characteristics/sources and inflammation, autonomic responses, and vascular dysfunction and; 3) Identify individuals who are more susceptible to PM exposures because of their genetic predisposition and/or health condition. **Project 1** (NAS study) examines the association between PM exposures and intermediate markers of autonomic dysfunction, systemic inflammation, endothelial activation and oxidative stress, in the Normative Aging Study cohort in Eastern Massachusetts. **Project 2** (Human CAPs study) examines the cardiovascular effects of fine, coarse and ultrafine concentrated ambient particles (CAPs) in healthy adults in Toronto. Measurements of cardiovascular and inflammatory responses include brachial artery diameter, flow-mediated dilatation, heart rate variability (HRV), blood pressure (BP), cardiac output and systemic vascular resistance. **Project 3** (Animal CAPs study) investigates the relationship between PM composition and vascular response. Normal and spontaneously hypersensitive rats are exposed to fine CAPs in Boston. Biological outcomes include pulmonary and systemic inflammation, BP, endothelin-1, atrial naturetic peptide, oxidant response in the heart and lung by *in vivo* chemiluminescence, and vascular morphometry of lung and cardiac vessels. **Project 4** (Toxicological Evaluation of Realistic Emission Source Aerosol-TERESA study) investigates the effects of primary and secondary vehicular emissions from a traffic tunnel in Boston, using the same animal models and biological measurements as in Project 4. The **Biostatistics Core provides design and analysis support for all four Projects, as well as** supports population studies in US cites examining PM effects on mortality and morbidity. Finally, the **Particle Core** supports the development of particle exposure systems for human and animal inhalation studies and sampling techniques for exposure assessment investigations. Below, we highlight some of our Center accomplishments and discuss our plans for the remaining two years. This overview focuses on biological responses as they relate to mechanisms, to susceptibility and PM components/sources.

Oxidative Stress: It has been hypothesized that a primary mechanism by which particles impact human health is the induction of pulmonary inflammatory responses mediated through the generation of reactive oxygen species (ROS). The NAS project examines the importance of oxidative stress pathways to the relationship between PM exposures and cardiac outcomes. Park et al 2006 postulated that two hemochromatosis (HFE) polymorphisms (C282Y and H63D), associated with increased iron uptake, may modify the effect of metal-rich particles on the cardiovascular system. [The protein product of the HFE gene modulates uptake of iron and divalent cations from pulmonary sources and reduces their toxicity.] Among men in the NAS cohort, $PM_{2.5}$ exposures decreased the high-frequency component of HRV in persons with the wild-type genotype, whereas no relationship was observed in persons with either HFE variant. The effect of PM on cardiac autonomic function was shielded in subjects with at least 1 copy of an HFE variant compared with wild-type subjects. Using the NAS cohort we have shown that reduced defenses against oxidative stress due to glutathione S-transferase M1 (GSTM1) deletion modify the effects of $PM_{2.5}$ on HRV [1]. Furthermore, polymorphisms in hemeoxygenase (HMOX-1) have been shown

to affect the ability to respond to oxidative stress. A high number of (GT)n dinucleotide repeats in the 5'-flanking region of the gene have found to increase risk of coronary artery disease. Our recent NAS paper by Chahine et al. [2] showed that individuals with a high number of (GT)n repeats are more susceptible to the PM effects. $PM_{2.5}$ was not associated with decreased HRV in subjects with the short repeat variant of *HMOX*-1, but was significantly associated in subjects with any long repeat. We also found that HMOX-1 interacts with GSTM1. In subjects with the *GSTM1* deletion and the *HMOX-1* long repeat, high frequency power of HRV decreased by 28% per 10 $\mu g/m^3$ increase in $PM_{2.5}$. Together these findings suggest that oxidative stress is an important pathway for the particle autonomic effects.

We have pioneered animal CAPs toxicological studies which have produced a large spectrum of biological responses. These investigations provide a unique opportunity to address important mechanistic questions raised by epidemiologic studies. For instance, how could particles inhaled by individuals with lessened defenses against ROS result in HRV changes? We have shown that measures of cardiac ROS are directly related to autonomic nervous system activity [3]. More recently animals with or without blocked afferent neural fibers in the lung were exposed to CAPs [4]. During exposures, ROS levels in the heart were measured using *in situ* chemiluminescence. Cardiac conduction parameters were also monitored. This study showed that blocking afferent neural response fibers in the lung, which mediate autonomic nervous system responses, blocked both increases in ROS levels in the heart and changes in cardiac conduction times. Consistent with our human studies these findings link respiratory and cardiac autonomic mechanisms with ROS and serve as a prime example of the benefits of integrated research within our Center.

Inflammation: We investigated PM-mediated impacts on several markers of inflammation and thrombotic activity, which may play key role in the physio-pathological processes leading to cardiovascular disease and mortality [5]. We examined the impact of particles on inflammation and thrombotic activity using white blood cell counts (WBC), C-reactive protein (CRP), sediment rate, and fibrinogen, and investigated whether these impacts were modified by age, health status, polymorphisms in GSTM1, and statin medications. We found both Black Carbon (BC) and Particle Number (PN) to be increase inflammation and thrombotic activity. Effects were strongest for subjects older than 78 years, individuals with chronic pro-inflammatory states (as shown for obese individuals), and individuals with reduced defenses to ROS species (as shown for people with the GSTM1-null genetic polymorphism or in non-statin users).

Vascular Dysfunction: Our previous epidemiological studies have shown that PM increases BP in diabetic populations [6,7]. Three of the Center projects (NAS, and human and animal CAPs) investigate the relationship between PM exposures and BP. We have exposed humans to particle free air, fine CAPs and coarse CAPs. Both particle size fractions induced vascular responses: the diastolic BP increased by 2.73±1.18 and, 2.86±0.86 mmHg for coarse and fine CAPs, respectively; while the systolic BP increased by 3.03±1.28 and 2.29±1.12 mmHg, for coarse and fine CAPs, respectively. It is also worth mentioning that our animal studies have shown that exposure to surrogate particles or CAPs enhances ischemia and increases blood pressure in both canine and rat models [8,9] .

Autonomic Dysfunction: Many investigators have identified short term outcomes related to PM exposures, such as HRV, an indicator of autonomic response, and arrhythmias that could lead to cardiovascular deaths. In the NAS study exposures to $PM_{2.5}$ and O_3 were associated with decreased HRV, High Frequency (HF) and, Low Frequency (LF) and increased LF/HF ratio [10]. In a later analysis we found that $PM_{2.5,}$ SO_4 and BC exposures were associated with increased LF/HF ratio on days where most of the pollution was related to local sources [11]. In a panel study of 32 elderly subjects in Steubenville, Ohio, we found that $PM_{2.5}$ and SO_4 exposures decrease HRV [12]. Furthermore, irregularities in cardiac repolarization may play an important role as they can lead to the development of an arrhythmia. Using data from the NAS, we investigated the association between PM exposures and the QT interval (QTc), which is a

measure of repolarization. Preliminary results show that the traffic-related pollutants, BC, NO_2 and CO are associated with increased QTc.

Susceptibility: Individuals with cardiac and vascular disease are more susceptible to PM effects. One potential pathway of diabetic vulnerability is via inflammation and endothelial dysfunction, processes in which cell adhesion molecules and inflammatory markers play important roles. O'Neill et al. [13] measured plasma levels of soluble ICAM-1; VCAM-1; and vWF and particle exposure in 92 Boston patients with type-2 diabetes. Among participants not taking statins, associations between $PM_{2.5}$, BC and VCAM-1 were particularly strong. Furthermore, PM exposures were associated with changes in reactive hyperemia and increased arrhythmias (Zanobetti et al. 2002; Sarnat et al. 2006) [6,14]. Finally, in post-MI patients, $PM_{2.5}$ exposures were associated with decreased HRV [15]. These effects were modified by beta-blocker, bronchodilator intake [15], and genetics [2].

Effect of Particle Characteristics and Sources on Particle Toxicity: While great progress has been made in elucidating the biological mechanisms which are responsible for the PM effects, understanding the role of specific PM sources and properties remains a significant challenge. One of the main objectives of the Center is to examine PM toxicity in relation to both PM components and to different sources. These issues are addressed by three different approaches: population and cohort studies; CAPs inhalation studies; and source specific inhalation studies.

*1) Population and Cohort Studies***.** In Franklin et al. [16] we found that Al, Si, S, Ni, and As significantly modified the association between daily $PM_{2.5}$ mass and mortality in 25 US communities cities. Zanobetti and Schwartz [17] analyzed hospital admissions data from Boston. This study found associations between MI emergency hospital admissions and BC, and emergency pneumonia admissions and BC, NO_2 and CO. The pattern of associations seen for MI and pneumonia underlines the importance of traffic emissions. Maynard et al. [18] used GIS to assess address-specific exposures to traffic PM for each decedent in Boston. Traffic exposure was associated with all-cause mortality as well as stroke and diabetes-related deaths. Sulfate, an indicator of long-range transported particles, was also associated with all-cause mortality risk, but its effect was smaller than that of traffic PM. Finally, in our original NAS study we found ambient $PM_{2.5}$ and BC concentrations to be associated with decrements in HRV and increases in CRP and fibrinogen levels.

*2) CAPs Inhalation Studies***.** The fine, coarse and ultrafine particle concentrator technologies developed by our group have been extensively used in Center studies and by researchers in US and around the world [19-21] . These technologies provide a unique opportunity to expose subjects to real ambient particles at concentrations higher than typical atmospheric levels. To date most of the CAPs studies have produced biological responses. In both the human and animal CAPs studies we examine PM toxicity in relation to PM composition and size. Of particular interest are the results of the Toronto human CAPs study which showed that both fine and coarse PM can produce similar increases in BP. Unlike previous inhalation studies which have used artificial test particles, CAPs composition depends on atmospheric conditions and source contributions and thus varies by experiment. This inherent variability of exposures can be very advantageous in the efforts to examine the effects of PM specific components and/or sources. For instance, animal CAPs studies in Boston have assoicated health outcomes to particulate species, such as: hematologic and bronchoalveolar lavage changes linked to Pb, Br and S [22], vascular changes linked to Si, S, EC and OC [23] and lung chemiluminescence linked to metals and heart chemiluminescence linked to traffic-related components [24].

3) Source Specific Inhalation Studies. Because there is still ambiguity in associating source tracers with actual sources, we believe that it is important to conduct exposure studies that examine the effects of both primary and secondary particles from specific sources. Our recent and current TERESA studies, co-funded by the Center and EPRI, aim to investigate the effects of specific sources such as electric power

plants and vehicular traffic, respectively. We have developed technologies to sample combustion emissions as well as to effectively simulate the aging of primary source emissions through a series of photochemical reactions. We have exposed rats to coal combustion primary and secondary particles at three US power plants equipped with different emission control technologies. As mentioned above, these toxicological assessments included measurement of respiratory parameters, inflammatory markers and cardiac function. Although we are still analyzing the data, our preliminary findings suggest that both primary and secondary coal combustion particles are less toxic than Boston CAPs. Using the developed technologies, we will investigate the toxicity of primary and secondary pollutants from mobile source emissions sampled inside the ventilation stack of a traffic tunnel in Boston. Together, the toxicological investigation of CAPs, coal and traffic particles will enhance our understanding about the effects of two very important particle sources impacting the US population.

Other Population Studies: In addition to the four Projects, our Center supports the extension of the analyses of population surveillance data to additional cities in the US. These efforts continue to provide new insights into the effects of particle exposures on cardiovascular and respiratory mortality [25], as well as on hospital admissions for MI infarctions Zanobetti, 2005 #104}, for heart failure [26], respiratory disease [27], and heart disease [28]. In an analysis of over 1.3 million deaths in 27 US communities, we reported increases in all-cause respiratory and stroke-related mortality associated with $PM_{2.5}$ and PM_{10} exposures. Finally, case-crossover methods have shown that individual characteristics modify the effect of particles on mortality [29], and that city characteristics modify the effect of PM on hospital admissions [27].

Future Plans: The NAS cohort has been an invaluable resource in our efforts to investigate biological mechanisms in human subjects. Future work will include assessment of participant-specific exposures which will be used in conjunction with the central site measurements for the health effects analyses. Future analysis will also include additional biological parameters, including BP and plasma levels of soluble ICAM-1, VCAM-1, and vWF. Despite unanticipated delays to installation of the three concentrators in Toronto, we are very pleased with the overall progress of this Project. We have recruited additional subjects for coarse CAPs exposures and analysis of the biological data is underway. Depending on the results of this analysis we will decide whether to perform additional coarse and fine PM exposures or switch to ultrafine CAPs during the last year. All Boston animal CAPs exposures have been completed and we are currently analyzing the data. Construction of the photochemical chamber for the traffic TERESA study will be completed soon. The field study will commence in the spring of 2009 and will last approximately six months. We anticipate finishing the study in approximately two years. Finally, we will continue the analysis of morbidity and speciation data from US cities to investigate species-specific associations using similar statistical methods used by the Franklin et al. [16] mortality study.

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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 $PM_{2.5}$ 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 current 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 include ECG, blood inflammatory markers, 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 $PM_{2.5}$, SO_4^{2-}, and BC exposures that are being measured for one week prior to the clinic visit and to ambient air pollution ($PM_{2.5}$, PM_{10}, $PM_{2.5-10}$, SO_4^{2-}, NO_3^-, 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-trasferase (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.

<u>Analysis of Previously Collected Data</u>

In Year Three, we continued our analysis and interpretation of data collected on the NAS participants as part of our original Harvard-EPA Particle Center. These analyses focused on the relation between ambient pollution and intermediate pulmonary (pulmonary function) and cardiac (HRV) outcomes and on how these relationships vary with individual-specific factors, such as genetic polymorphisms, weight, and airway hyper-reactivity (AHR). Results from these analyses have helped to identify important PM-mediated biological pathways and 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.

- *Relation between PM and Cardiac Function: Importance of Oxidative Stress Pathways*

  We published two papers this year that identified factors that modify the impacts of PM on cardiac function. Our paper by Chahine et al. (2007) extends our work on the oxidative stress pathway to examine polymorphisms in hemeoxygenase (HMOX-1). Polymorphisms

in HMOX-1 have been shown to vary its ability to respond to oxidative stress, with a high number of (GT)n dinucleotide repeats in the 5'-flanking region of the gene related to reduced response and to increased risk of coronary artery disease in high-risk groups with hyperlipidemia, diabetes or current smoking. Consistent with these findings, our paper shows that individuals with a high number of (GT)n repeats are also more susceptible to the effects of airborne particles. We found, for example, that $PM_{2.5}$ was not associated with decreased heart rate variability (HRV) in subjects with the short repeat variant of *HMOX*-1, but was significantly associated in subjects with any long repeat. We also found that HMOX-1 interacts with GSTM1 and $PM_{2.5}$ in predicting HRV. In subjects with the *GSTM1* deletion and the *HMOX-1* long repeat, high frequency power of HRV decreased by 28% (95% CI -8, -43) per 10 $\mu g/m^3$ increase in $PM_{2.5}$.

In a related paper by Park et al. (2008), we show that our elderly NAS individuals with long-term exposure to higher levels of lead may be more sensitive to cardiac autonomic dysfunction on high air pollution days. We found graded, significant reductions in both HF and low-frequency (LF) powers of HRV in relation to ozone and sulfate across the quartiles of tibia lead. Also interquartile range increases in ozone were associated with a 38% decrease (95% CI: -54.6% to -14.9%) in HF and a 38% decrease (-51.9% to -20.4%) in LF in the highest quartile of tibia lead after controlling for potential confounders.

- *Relation between Lung Function and Air Pollution: Oxidative Stress, Genes and Other Modifying Factors*

  In three papers, we investigated the impact of air pollution on lung function in our NAS participants. Together, these papers provide important evidence that oxidative stress is a key pathway by which ozone causes damage. The first of these papers investigated the effect of statins, an anti-inflammatory and antioxidant medication, on decline in lung function in the elderly and whether smoking modified this effect (Alexeeff et al., 2007a). We found that statin use attenuates decline in lung function in the elderly, with the size of the beneficial effect modified by smoking status. For those not using statins, the estimated decline in lung function (as assessed using FEV1) was 23.9 ml/year (95% CI: 227.8, 220.1 ml/yr), whereas those taking statins had an estimated 10.9-ml/year decline (95% CI: 216.9, 25.0 ml/yr). This paper suggests that statin use may lessen the impact of exposures to pollutants that generate oxidative stress and inflammation, such as PM and ozone.

  This suggestion was examined in part in a second paper (Alexeeff et al., 2007b), in which we found that acute ozone exposures are associated with decrements in lung function in our elderly NAS participants. This finding is contrary to the previous belief that the elderly are not responsive to ozone exposures. This paper further found that ozone has the greatest effect on lung function in elderly who are obese or have AHR. For example, a 15 ppb increase in O_3 during the previous 48 hours was associated with a greater decline in FEV_1 in the obese (-2.07%, 95% CI: -3.25%, -0.89%) than in the non-obese (-0.96%, 95% CI: -1.70%, -0.20%).

  We explored the relation between ozone and pulmonary function further in a third paper by Alexeeff et al. (2008). In this paper, we showed that the effects of ozone on lung function in the elderly are modified by the presence of specific polymorphisms in antioxidant genes (*HMOX1* and glutathione S-transferase pi [*GSTP1*]). Specifically, we found that a 15 ppb increase in O_3 during the previous 48 hours was associated with a 1.25% decrease in FEV1

(95% CI: -1.96%, -0.54%). This estimated effect was worsened with either the presence of a long (GT)n repeat in *HMOX1* (-1.38%, 95% CI: -2.11%, -0.65) or the presence of an allele coding for Val105 in *GSTP1* (-1.69%, 95% CI: -2.63%, -0.75). A stronger estimated effect of O_3 on FEV1 was found in subjects carrying both the *GSTP1* 105Val variant and the *HMOX1* long (GT)n repeat (-1.94%, 95% CI: -2.89%, -0.98%).

- *PM-Mediated Toxicity: Metabolic Pathways*

  In two papers, we investigated the role of the methionine cycle, which is involved in the control of biological processes ─ such as methyl-group transfers, homocysteine synthesis, and redox states ─ and that may be involved in PM-mediated toxicity. The activity of the methionine cycle is dependent on availability of dietary methyl nutrients and is modified by genetic variations in metabolic genes. In particular, the CT and TT genotypes of the C677T methylenetetrahydrofolate reductase (*MTHFR*) polymorphism have been associated with reduced enzyme activity, and linked, though not consistently, with increased risk of cardiovascular diseases. Conversely, the CT and TT genotypes of the C1420T cytoplasmic serine hydroxymethyltransferase (*cSHMT*) polymorphism have been associated with lower homocysteine levels, a methionine metabolism product that may be a risk factor of atherosclerosis, myocardial infarction, stroke, and thrombosis.

  Baccarelli et al. (2008) specifically examined whether PM-mediated effects on autonomic function are modified by dietary methyl nutrients, such as folate, the B-vitamins pyridoxine (B_6), cyanocobalamin (B_{12}), and methionine, and genes related to processing these nutrients. We found the effects of particles on HRV were modified by MTHFR and dietary methionine. In carriers of [CT/TT] *MTHFR* genotypes, for example, HRV (as assessed by SDNN) was 16.3% (95% CI, 25.8-5.6; p=0.004) lower than in CC *MTHFR* subjects. In the same [CT/TT] *MTHFR* subjects, each 10 $\mu g/m^3$ increase in $PM_{2.5}$ in the 48 hours before the examination was associated with a further 9.9% (95%CI: 17.6, 1.3; p=0.02) decrease in SDNN. The negative effects of $PM_{2.5}$ were abrogated in subjects with higher intakes (>median) of B6, B12, or methionine.

  In Park et al. (2008), we examined whether PM exposures impact a specific part of the methyl group pathway, homocysteine. We found exposures to particles from traffic (black carbon BC, organic carbon OC) to be associated with elevated plasma total homocysteine. No association was observed with sulfate, an indicator of coal and oil combustion particles or $PM_{2.5}$. The effects of BC and OC were more pronounced in persons with low concentrations of plasma folate and vitamin B12.

Data Collection

In Year Three, we continued to collect information for NAS participants as they came in for their every three-year health exam. At each exam, we specifically collected ECG, blood inflammatory marker, and daily diet information. We supplemented these measurements with those of 8-OHdG and creatinine in urine, which were added in Year 2 to obtain direct measures of oxidative stress in our participants. In total, we obtained new data (between July 1, 2007 and June 30, 2008) for 147 NAS participants.

Figure 1. Indoor PM Monitoring Locations

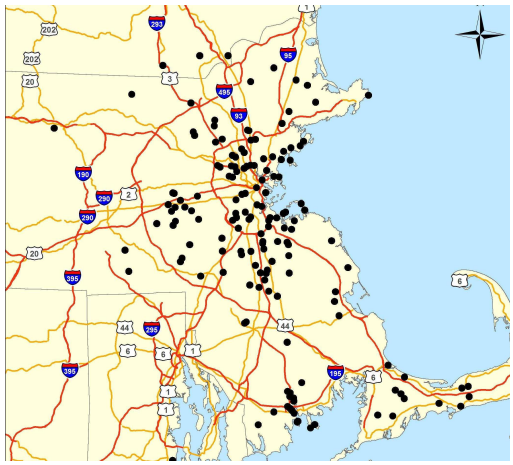


Figure shows locations through March 25, 2008; total of 160 samples

In addition to the health measurements, we also collected one-week long indoor $PM_{2.5}$ 104 NAS participants (July 2007 through June 2008). In total, indoor concentrations were measured for 202 participants. In Year Three, 107 individuals completed indoor sampling, with three of these individuals completing indoor monitoring but not having in a clinic visit (one was hospitalized, two did not come in for their visit). Forty-three participants with a clinic visit did not participate in indoor monitoring, The reasons for non-participation were varied, including lived out of state (7 participants), declined to participate (14), clinic visit scheduled too late to send monitor (13), lived in smoking household (4), received but forgot to operate indoor monitor (2), excessive monitor noise (1), technician error (1), and unable to contact (1). For both clinic visits and indoor monitoring, our participation rate was high and was greater than we had anticipated from our last year's visit number. Of those that participated, subjects generally lived within the Route 495 beltway, although several participants also lived in southern Massachusetts, southern New Hampshire and Rhode Island (Figure 1).

Laboratory Analysis

We continued to analyze collected health data, with laboratory analysis of HRV, blood and urine samples conducted in the appropriate laboratories. ECG tapes were analyzed using software from Forest Medical for ST segments, arrhythmias, and QT length. Indoor $PM_{2.5}$ samples have been analyzed for mass and reflectance.

Figure 1. Indoor $PM_{2.5}$ Concentrations

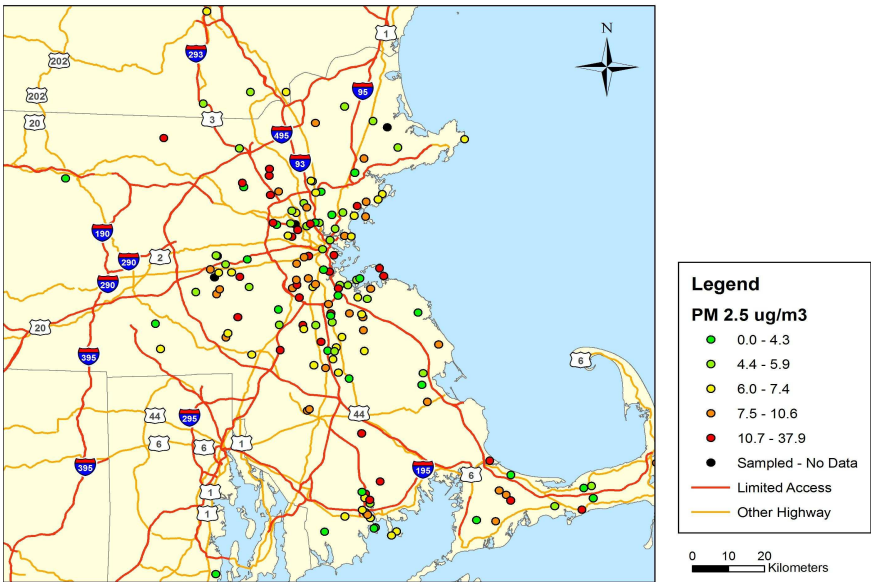


Figure shows locations through March 25, 2008; total of 160 samples.

Figure 2. Week-Long Indoor BC Concentrations

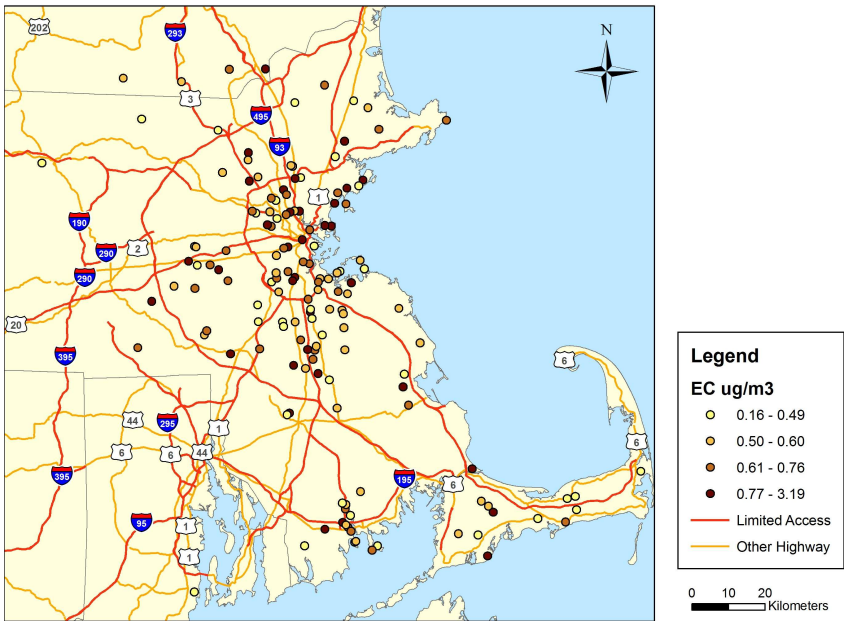
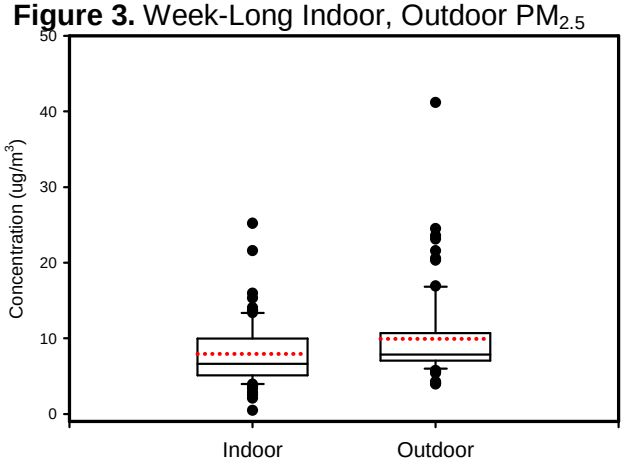


Figure shows locations through March 25, 2008; total of 155 samples.

Indoor Concentrations

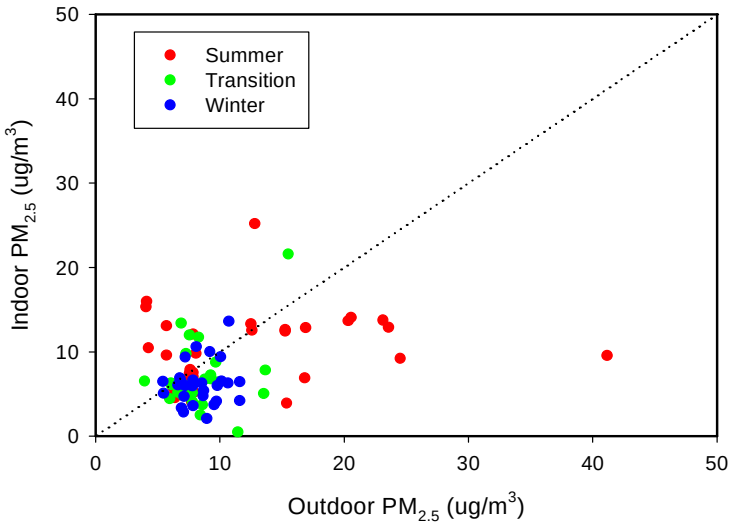
One-week integrated indoor $PM_{2.5}$ concentrations varied substantially, with values ranging between zero and 37.9 ug/m^3 (Figure 2). Week-long indoor BC concentrations (as estimated using reflectance) also ranged widely (Figure 3). The minimum and maximum concentrations equaled 0.16 and 3.19 ug/m3, respectively. Indoor $PM_{2.5}$ concentrations were generally lower than outdoor levels measured at our stationary ambient monitoring (SAM) site located in downtown Boston, on the roof of Countway Library, with lower mean and median concentrations (Figure 4). Lower indoor levels suggest the presence of few $PM_{2.5}$ sources inside our NAS participant homes. The association between indoor and outdoor $PM_{2.5}$ concentrations was weak, with an R^2 of only 0.09 (Figure 5). Observed variation in indoor $PM_{2.5}$ and BC concentrations and its relation to corresponding outdoor concentrations likely reflects temporal variation, as samples were collected over different time periods. It also likely reflects variation in home-specific factors, such as distance to road and home ventilation. The impact of these factors is currently being examined in our analysis of indoor concentrations.

Figure 3. Week-Long Indoor, Outdoor $PM_{2.5}$



Data collected July 2006-Sept. 2007

Figure 4. Week-Long Indoor vs. Outdoor $PM_{2.5}$



Includes data collected between July 2006-Sept. 2007

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Alexeeff SE, Litonjua AA, Sparrow D, Vokonas PS, Schwartz J. Statin use reduces decline in lung function: VA Normative Aging Study. Am J Resp Crit Care Med, 2007a; 176: 742-747.

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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 4, we anticipate that 120 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 previous years, 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 health and exposure data 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-OHgD, and ICAM and VCAM data. We will characterize our indoor $PM_{2.5}$ and BC concentrations and will predict indoor $PM_{2.5}$ levels from outdoor levels, location-specific data, housing characteristics, and GIS-based spatial models. Once complete, indoor predictions will be incorporated into our health analyses.

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

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

Date of Report: August 1, 2008
EPA Grant Number: R-832416-010
Center: EPA Center at HSPH for Ambient Particle Health Effects
Center Director: 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, 2007 – July 31, 2008

Objective(s) of the Research Project:

Our original design included controlled human exposures of 50 subjects each with 4 exposures including filtered air (FA), fine CAPs, coarse CAPs and ultrafine CAPs. Based on recommendations from our Science Advisory Committee (SAC) (July 2006, meeting) a number of changes were made to the study design. According to the revised design, each of the 25 subjects will receive four 2-hr exposures including: FA, fine CAPs (target 250 $\mu g/m^3$; max 500 $\mu g/m^3$), coarse CAPs (target 200 $\mu g/m^3$; max 400 $\mu g/m^3$), and a 2nd coarse CAPs exposure at the same levels. Exposures are randomized and at least 2-weeks apart to allow for washout. We will examine and compare the acute cardiovascular and respiratory effects of coarse and fine CAPs in healthy adults, using our newly constructed controlled particle exposure facility, in Toronto, ON, Canada. The revised design does not include ultrafine CAPs, at least for the first series of studies (25 subjects). When completed, the 25 fine CAPs results @ 250 $\mu g/m^3$ will be added to our EPA-STAR fine CAPs+O_3 cardiovascular study (33 subjects, completed November 2007) with a mean CAPs mass of 140 $\mu g/m^3$. This will allow us to look at dose-response relationships using common vascular and inflammatory endpoints. Cardiovascular outcomes will be measured pre-, post- and 24 hrs post-exposure, and will include measurements 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 for 10-min periods every 30 min using a Finometer finger cuff (pulse pressure) that includes calculated determinations of cardiac output, stroke volume and systemic vascular resistance. Simultaneously, we will measure end-tidal CO_2 using nasal prongs, so as to directly compare the two outcome measures. 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 (HRV analyses). Filter samples will be collected during exposures for both mass and chemical composition (inorganic ions, trace elements, OC/EC) 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 $PM_{2.5}$, as well as pollen characterization (GRIPST-2000 pollen sampler) and fungal spores/pollen (Burkard sampler). 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 and fine size fractions result in cardiovascular responses consistent with vascular narrowing, vascular/autonomic dysfunction, inflammation, and/or endothelial activation compared to FA (control) exposures
- Respiratory inflammatory responses (induced sputum and nasal scraping tests), pulmonary function (flow-volume curves) and nasal/respiratory symptom responses will be greater with coarse CAPs than fine CAPs, compared to FA
- Associations between CAPs and cardiovascular responses will differ by particle size fraction and PM composition

Progress Summary/Accomplishments:

Human Ethics Approval: Since the last report, we submitted amendments to the protocol including: 1) addition of sputum induction – to examine acute lung inflammation; 2) addition of nasal scrapings and a nasal symptoms questionnaire – to examine nasal irritation/inflammation; and 3) addition of blood tests to measure DNA Methylation (based on the work of Andrea Baccarelli). Amendments 1 & 2 were in response to the SAC comments that course CAPs may cause more respiratory changes and thus we should include tests of respiratory function. The three amendments were approved by the St. Michael's Hospital (SMH) Human Research Ethics Board (REB) in March, 2008 and the tests subsequently added in all subjects. Of note, the SMH REB is the primary Board, as SMH handles our grant money, but ethics approval has also been obtained from the University of Toronto, Health Canada and the US EPA.

New CAPs Human Exposure Facility: Construction of our CAPs (coarse, fine, ultrafine) exposure facility, funded through a Canadian infrastructure grant (CFI) and the Harvard/EPA Center, began the end of July, 2006 and the coarse and fine CAPs were ready for initial testing and calibrations in October 2007. The first human exposures began late in November 2007. The ultrafine concentrator testing (by ProFlow) was delayed due to electrical installation delays, but is scheduled for final testing July 10-11, 2008. The new CAPs exposure facility, including newly designed plexiglass subject enclosure with facemask delivery of the CAPs has been running well with no problems in attaining target CAPs levels. As of July 2, 2008 we have recruited 15 subjects and carried out 33 human exposures: 17 coarse and 7 fine CAPs and 9 FA.

Preliminary Results: To date, four subjects have completed all four exposures (FA, fine CAP and 2 coarse CAPs). Findings are summarized as follows: **Blood pressure (BP)** was measured during the exposure at 30-min intervals, using an automated BP cuff.

Three BP measures were taken at each time point. As we have observed in our previous CAP studies, more consistent findings were observed when the average of the 2nd and 3rd BP measures was used. Linear regression analysis was then used to determine the slope of the line fitted over the 5 times points (0, 0.5, 1, 1.5 & 2-hrs). The mean ± SE change in ***diastolic BP*** over 2-hrs for 16 coarse CAPs exposures increased by 2.93±1.12 mmHg (9 subjects). In our previous EPA-STAR fine CAPs±O_3 study, the observed mean change for the 2-hr fine CAPs alone (no O_3) exposure was similar, 2.86±0.86 mmHg (29 subjects). This compared to a smaller increase in the EPA-STAR CAPs±O3 study (1.28±1.27 mmHg, n=27). An even larger change in diastolic BP was observed when BP measured just before and immediately after coarse CAPs exposures were compared (a mean increase of 4.38±1.21 mmHg or 7.0%). This is an interesting finding because we have previously reported that diastolic BP measured just before and after fine CAPs+O_3 was not significantly different. ***Systolic BP*** for the 16 coarse CAPs exposures also increased, 3.46±1.27 mmHg compared to 2.29±1.12 mmHg (n=29) for the EPA-STAR fine CAPs. However, to date, we only have data for six fine CAPs exposures and seven for FA exposures, too few to report given the variability of this measure. **DNA Methylation.** Blood samples of two subjects were analyzed as a pilot study. Results showed decreased global methylation with exposures to coarse and fine CAPs and a suggestion of resolution with *i*NOS 24-hrs after CAPs exposures. **Pulmonary function.** Flow-volume curves were performed before, just after and 24-hrs after exposures. As we have reported in previous controlled exposure studies, flow and volume parameters increased slightly from pre to post exposure with FA. For example, in the seven subjects who have completed filtered air tests, FVC and FEV1 increased 1.14±0.68 and 3.03±1.39%, respectively. Changes were small for both coarse and fine CAPs exposures. For example, after coarse CAPs (n=14), FVC decreased 0.07±0.71% and FEV1 increased 1.53±0.79%. After fine CAPs (n=6), FVC increased 0.27±0.57% and FEV1 increased 2.70±1.49%. **Traffic Density.** In May 2008, we set up a video camera, to record traffic flow adjacent to the CAP inlet on College Street. Detailed data was obtained for traffic during the exposures, including minute-by-minute eastbound and westbound counts, separately for cars and trucks. Data to date includes eight exposures, with counts ranging from 5-42 vehicles per min, of which 5-9% were trucks (diesel). Total vehicle counts over the exposure period, now extended to 2 hours and 10 minutes, ranged from 2,341 to 2,550 vehicles. We do expect to see more variation in traffic counts as we obtain more data over the entire year. The traffic counts will be used as predictors of health outcomes in regression analyses and to examine potential relationships between traffic flow and changes in PM_{10} mass concentrations at the concentrator inlet.

<u>Health outcomes with no results to report at this time:</u> **Flow-mediated dilation (FMD).** In our recently completed EPA-STAR study, we observed a significant decrease in FMD 24-hrs following fine CAPs exposure, compared to an increase with FA. We anticipate that we will see a larger decrease with fine CAP exposures at the higher target mass concentration level of 250 $\mu g/m^3$ (mean level of 140 $\mu g/m^3$ for the EPA-STAR fine CAPs study). **Finometer.** In discussions with Dr. Rob Brook, and after some trial testing, we decided to use 10-min continuous recordings every 30-min for the Finometer, as it was very uncomfortable to keep the finger cuff on longer. We have thus extended the exposure length by 10 min, to accommodate the Finometer reading at the end of the

exposure. Finometer data has recently been sent to an expert in this area (Gianfranco Parati) with customized software analyses, and we are awaiting analyses. **Echocardiography.** We have added echocardiography to this study as a non-invasive determination of cardiac output (CO) – a primary determinant of systemic BP. Briefly, cardiac images of the aortic annulus (AA) are obtained for determination of the (diameter) cross sectional area. Next, the aortic velocity waveform at the AA is obtained by Doppler, and the velocity time integral measured (Terason software). The product of the two measures, stroke volume, is multiplied by heart rate to obtain CO. **Nasal scrapings.** Nasal scrapings and RNA gene expression (micro-array) will shortly be included as an outcome measure for all newly recruited subjects. This test is a component of another study (AllerGen NCE, Dr. Jeremy Scott) in subjects with allergic rhinitis, recruited from the PM Center study subjects. **CBCs, cytokines and endothelins/NO.** Data are batch analyzed for cytokines and endothelins/NO. We have previously observed increased white cell counts and IL-6 following exposure to fine CAPs. **Heart Rate Variability.** Subjects wear a Mortara holter during the entire exposure day and keep it on overnight, returning the next day, thus giving a 24-hr recording. ECG digital data are stored on flash cards and sent to Harvard every 2-3 exposures for data recovery and analyses. **Exposure characterization.** We have been working closely with Jeff Brook at Environment Canada (EC) and Harvard to coordinate our filter sample collections. Teflon filters (47 mm) have been sent to EC for pre- and post-exposure conditioning and weighing. Mean ± SD mass concentrations for 12 coarse CAPs exposures were 199.3±26.5 $\mu g/m^3$ (target 200 $\mu g/m^3$) and four fine CAPs 276.5±16.8 $\mu g/m^3$ (target 250 $\mu g/m^3$). After gravimetric measurements are completed, filters are batch analyzed at EC for inorganic ions by IC. Quartz filters (25 mm) are pre-fired to remove organics and after exposure sent back for OC/EC analyses by TOT using a 1.45 cm^2 punch. A punch will also be taken for selected organics, including a motor vehicle tracer (engine lubricant), by GC-MS. Teflon filters (37 mm) are collected for trace element analyses and will be sent to the DRI lab for XRF as a batch analyses (with Harvard samples). Biologic components are collected on 25 mm polycarbonate filters for endotoxin and β-glucans, both exposure and outdoor samples. Continuous data are also collected for PM mass (TEOM, DustTrak), black carbon (PSAP), temperature, RH% and criteria gases.

Collaborations/meetings: 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. In April, 2008, Dr. Petros Koutrakis came to Toronto to give a presentation and to see our new CAP facility and discuss our project. During the American Thoracic Society (ATS) Conference in Toronto May 18-21, 2008, we had an open house at our new CAPs facility and investigators involved with PM research were present to view our facility and discuss our recent findings. At the ATS conference we set up several meetings with our Harvard collaborators (Drs. Joel Schwartz, Brent Coull and, Diane Gold) to discuss our project progress/results, new collaborations, statistical issues and new manuscripts.

Health Canada: Oxidative Stress Collaboration: We have established collaboration with Health Canada on oxidative stress measures in response to coarse CAPs exposure, as part of our EPA PM Center project. Blood and urine samples are provided to Health Canada, initially for 9 subjects (study completed) with ongoing plans to add 16 more subjects in 2008-2010. Specifically, measures in urine 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-α for inflammation, oxidative potential, Isoprostane-8, TBARS and conjugated diene for oxidative stress.

AllerGen NCE Collaborations: We have collected filter samples during all FA (control) coarse and fine CAP exposures for determination of biological material including airborne endotoxin and markers of fungi. Samples will be batch analyzed at a later date. In addition, we have recently started to collect filter samples from pre-exposure calibration runs of coarse and fine CAPs for the specific objective of adapting DGGE and DNA macro-array technologies for the characterization of airborne and dust-borne fungal contaminants and to investigate the value of these data in the context of studying indoor/outdoor exposures and health outcome measures. The latter information will inform the Canadian Healthy Infant Longitudinal Development study (a multi-disciplinary, longitudinal, birth-cohort study).

EPA-STAR Coarse CAP Study Collaboration: University of Michigan collaborator Dr. Rob Brook has been funded to: 1) examine vascular dysfunction following 2-hr coarse CAPs exposures; 2) compare responses to CAPs from both urban and rural sources; 3) compare racial differences in responses; 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. Through funding from the grant an additional vascular measure will be added to the Toronto study; a SphygmoCor, large arterial compliance, central aortic BP and hemodynamics device. Common measures will be compared between the two sites, including: ultrasound baseline diameter and reactivity measures; Finometer continuous BP measures; BP measures during exposure; SphygmoCor measures; and biological constituents collected on filters, with the characterization coordinated by Dr. Diane Gold at Harvard for measures of airborne endotoxin and markers of fungi.

Evaluation of Autonomic Responses to CAPs in Controlled Exposure Studies: Holter recordings from our previous Toronto exposure studies have been sent to Harvard and analyzed for HRV. Statistical analyses are ongoing and initial results show an association between increasing diastolic BP and decreasing HRV during exposures. The results will inform the current study by enabling the investigators to assess the interrelation between pollution exposures, HRV autonomic responses, vascular, and inflammatory responses.

Evaluation of Ambient Exposures on Baseline Systemic Inflammation: Dr. Aaron Thompson from the Toronto group just completed a work term at Harvard as part of his training in Occupational Medicine. In collaboration with Harvard and the Toronto group,

he evaluated the contribution of prior ambient pollution exposure on pre-exposure (baseline) systemic inflammatory measures.

Acute Blood Pressure Changes in Outside Workers: As a direct result of the blood pressure responses observed in our CAPs studies, an MSc student is carrying out a pilot project in outside workers (e.g., University of Toronto grounds keepers). Starting July 11, 2008, over the summer and fall he will measure blood pressure at the start and end of a work day and also obtain both personal and area PM measures in 20-30 outside workers to test for associations between BP changes and PM levels.

Publications/Presentations:

At present, there are no direct publications from the study.
A number of manuscripts are planned once sufficient data is collected, including:

1. Design of a human exposure facility for coarse, fine and ultrafine concentrated ambient particles;
2. Comparison of cardiovascular responses to coarse and fine concentrated ambient particles;
3. Acute nasal and respiratory responses for coarse compared to fine concentrated ambient particles and;
4. Heart rate variability during acute exposures to coarse and fine concentrated ambient particles.

In addition, manuscripts in preparation listed below will inform the current project. These were made possible through our collaborations with Harvard, Environment Canada (Dr. Jeff Brook), Health Canada (Dr. Ling Liu) and the University of Michigan (Dr. Rob Brook):

1. Fine CAPs induce an IL-6 inflammatory response in asthmatics and non-asthmatics;
2. Air pollution and systemic inflammation: Interleukin-6, fibrinogen & the criteria pollutants;
3. Exposure to fine CAPs ± O_3: endothelial function, BP and systemic inflammatory responses.
4. HRV and BP changes during exposure to fine CAPs ± O_3;
5. Blood endothelins and NO metabolites in response to controlled fine CAPs ± O_3 in young healthy asthmatics and non-asthmatics and in children and;
6. End-tidal CO_2 (capnography), a sensitive marker of ventilation/perfusion changes in response to fine CAPs ± O_3 in young healthy individuals.

Future Activities:

Human Exposures. Exposure testing will continue until 25 subjects are completed. Interim analyses will be carried out to guide future 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: July 1, 2008
EPA Grant Number: R-832416-010
Center: EPA Center at HSPH for Ambient Particle Health Effects
Center Director: 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, 2007 – July 31, 2008

Objective(s) of the Research Project:

The objective of this project 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) were 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, captures particles predominantly from local sources, while, exposures starting about 10:30 am are relatively more enriched in transported particles. Specific biologic outcomes included: breathing patterns, indicators of pulmonary and systemic inflammation, blood pressure, *in vivo* oxidant responses in the heart and lung, and quantitative morphology of lung and cardiac vessels. To control for circadian variations all outcomes were assessed during both time periods, in relation to those of filtered air (sham) exposures. Animal exposures were 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. Studies of cardiopulmonary mechanisms in relationship to *in vivo* oxidant responses in the heart and lung were also carried out using CAPs.

Progress Summary/Accomplishments:

Early-late experiments. Exposure data from all the experiments are shown in Table 1. CAPs mass concentrations (Early - 505.8 ± 75.8 $\mu g/m^3$ and Late 407.2 ± 45.7 $\mu g/m^3$) were slightly higher than previous published studies from our laboratory, and not significantly different from each other using a paired two-tailed t-test. 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 (when diurnal variation in sulfate production from oxidation of SO_2 is greater than in other seasons), 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. Even though statistically significant differences were found between AM and PM exposures, the differences were modest.

Table 1. CAPs mass and component concentrations during the early and late exposure periods (concentrations are expressed in $\mu g/m^3$ and fractions in %)

Measures	Early mean±SE	Late mean±SE	p =
CAPs Mass	505.8 ± 75.8	407.2 ± 45.7	0.083
***Black Carbon Mass**	10.5 ± 0.9	7.3 ± 1.1	**0.002**
***Elemental Carbon**	22.5 ± 2.6	16.5 ± 2.8	**0.032**
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
***Iron**	13.3 ± 1.9	9.7 ± 1.0	**0.035**
Zinc	1.0 ± 0.1	1.1 ± 0.1	0.857
***Nickel**	0.07 ± 0.015	0.04 ± .007	**0.033**
Vanadium	0.03 ± 0.01	0.01 ± .009	0.144
Magnesium	1.2 ± 0.3	1.4 ± 0.3	0.293
***Copper**	0.4 ± 0.06	0.2 ± 0.03	**0.019**
Manganese	0.2 ± 0.03	0.3 ± 0.04	0.349
***EC Percent of Mass**	5.3 ± 0.5	3.9 ± 0.4	**0.007**
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
***Sodium Percent of Mass**	2.3 ± 0.7	2.7 ± 0.7	**0.026**
Chlorine Percent of Mass	2.3 ± 0.9	3.1 ± 1.3	0.180
***Silicon Percent of Mass**	2.5 ± 0.4	2.9 ± 0.5	**0.040**
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
***Potassium Percent of Mass**	0.68 ± 0.07	0.77 ±0.08	**0.017**
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
***Nickel Percent of Mass**	0.019 ± 0.004	0.009 ± 0.001	**0.025**
Vanadium Percent of Mass	0.007 ± 0.002	0.003 ± 0.002	0.074
***Magnesium Percent of Mass**	0.29 ± 0.07	0.39 ± 0.07	**0.048**

***Copper Percent of Mass**	0.09 ± 0.01	0.07 ± 0.01	**0.043**
***Manganese Percent of Mass**	0.06 ± 0.01	0.08 ± 0.01	**0.040**

These data can also be assessed by strain for each study carried out. These analyses are shown for selected elements in Table 2. In this analysis, it can be seen that for specific experiments there are greater differences between early and late exposures than are apparent in the overall averages. Different levels of exposures received by the different strains and seasonal groups were due to the random variations in composition of the ambient pollution in Boston for different exposure days.

Table 2. CAPs mass and component concentrations during the early and late exposure periods by time of year and by strain (concentrations are reported in $\mu g/m^3$, particle count is in 1,000 particles per cm^3)

	Strain / Group	Fine Mass	Black Carbon	Particle Count	OC	EC	TC	Na	Mg	Al	Si	S
SPRING	SD-CD Early	311.1 ± 176.4	6.5 ± 4.1	17.2 ± 4.8	47.72	14.91	62.63	12.64	1.09	2.97	8.30	31.04
	SD-CD Late	207.1 ± 93.2	3.8 ± 1.8	14.7 ± 3.9	47.85	9.03	56.88	10.79	0.71	2.38	7.29	25.50
	WKY/SHR Early	381.4 ± 179.9	8.9 ± 5.7	N/A ± N/A	71.09	22.59	93.68	15.94	2.64	6.25	16.30	29.47
	WKY/SHR Late	361.0 ± 263.6	7.3 ± 8.1	N/A ± N/A	61.38	13.63	75.01	18.55	2.91	4.89	13.51	30.86
	Early	347.0 ± 181.5	7.7 ± 5.2	17.2 ± 4.8	59.41	18.75	78.15	14.29	1.86	4.61	12.30	30.25
	Late	283.7 ± 211.7	5.6 ± 6.1	14.7 ± 3.9	54.62	11.33	65.95	14.67	1.81	3.63	10.40	28.18
FALL	SD-CD Early	275.5 ± 210.0	8.5 ± 5.7	11.8 ± 4.3	72.25	18.71	90.97	1.44	0.67	4.12	12.00	14.49
	SD-CD Late	251.0 ± 262.2	7.1 ± 20.8	9.8 ± 4.3	77.51	15.57	93.07	2.62	0.89	4.62	12.90	14.30
	WKY/SHR Early	818.9 ± 735.5	15.3 ± 13.5	17.5 ± 10.9	84.93	31.20	116.13	9.50	1.34	3.06	8.76	59.22
	WKY/SHR Late	455.2 ± 362.8	9.8 ± 8.6	15.6 ± 10.9	79.95	23.43	103.39	12.53	1.50	2.67	7.88	41.08
	Early	619.6 ± 653.5	12.8 ± 11.7	15.7 ± 10.3	80.32	26.66	106.98	6.57	1.10	3.44	9.94	42.96
	Late	380.9 ± 343.9	8.8 ± 14.3	13.5 ± 9.5	79.06	20.57	99.64	8.93	1.28	3.38	9.71	31.34

Twenty one repetitions of the early-late experiments were carried out, and animals were studied for breathing pattern, BAL, and chemiluminescence outcomes, in each treatment group for each strain. The numbers of animals studied provided sufficient power to examine differences between groups, based on our previous studies. Table 3 shows the difference between all early and late sham exposed animals for respiratory parameters. Since there are significant differences between these animals, the need to control for diurnal variation in analyses of respiratory parameters is emphasized. All Data reported in this study are presented as continuous differences in parameter measurements between CAPs and Sham animal exposures to compensate for this diurnal variability.

Table 3. Early-late differences in sham animals indicating significant diurnal effects may influence these exposures.

	Δ ± SE	p value
f	20.7 ± 4.52	<0.0001
Tv	0.06 ± 0.20	NS
Ti	-0.03 ± 0.01	=0.0001
Te	-0.02 ± 0.00	<0.0001
Penh	-0.22 ± 0.05	<0.0001
EIP	0.26 ± 0.31	NS
EEP	-16.4 ± 4.37	=0.0002
EF50	0.51 ± 1.27	NS
PEF	1.88 ± 2.05	NS
PIF	4.23 ± 2.39	NS

For all rat strains, breathing pattern and *in vivo* chemiluminescence studies show significant differences between CAPs and Sham exposures. 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 to estimate overall, time -and species-specific effects of exposure. The models represent an extension of linear regression models that make it possible to estimate exposure effects while (1) controlling for potentially non-linear effects of time within exposure period, and (2) including random animal effects to account for correlation among repeated measurements taken on the same animal during the exposure period. For each outcome, four models using increasing level of detail for exposure effects were run. These models corresponded to estimating overall, time-specific, species specific effects, and time-specific effects for each species. Random forests methods were used to rank the importance of each measured CAPs component in predicting differences between CAPs and filtered air outcome means. This approach represents an extension of classification and regression trees (CART), identifying predictors that yield the largest gains in prediction accuracy of the response (in this case the CAPs-Sham mean difference in outcome for a given exposure) achieved by CART when applied to multiple bootstrap resamples of the original data. This analysis was supplemented by plotting raw CAPs - Sham mean differences for each outcome versus the components identified in the random forest analysis.

With breathing pattern, there are substantial differences between the spring and the fall. The overall pattern tends to follow the fall pattern. There was much greater variability in spring. Overall, there is an increase in respiratory frequency with concomitant shortening of the time of inspiration and expiration. An overall increase of frequency was seen in all groups/strains with CAPs exposure compared to controls, (13.081 ± 3.139 $p<0.0001$). This increase was also significant in the early morning exposure for the 3 strains (SD, SHR and WKY; $p \leq 0.01$). Inspiratory and expiratory times were decreased overall (-0.007 ± 0.003 and -0.013 ± 0.005 respectively; $p \leq 0.05$). Figure 1 shows the change in respiratory parameters for early morning and late day exposures by strain and overall. Although responses for animals exposed in the morning were often greater than those of animals exposed in the afternoon, this difference was only significant for frequency in the SHR rats ($p=0.0393$). Tidal volumes tended to increase in the overall analyses and there were no early-late differences that were significant. Inspiratory and expiratory flows had some statistically significant differences, but these tended to be increases. With increases in tidal volumes and flows in these experiments, there does not appear to be toxicologically significant changes in pulmonary function, even though there is a definite change in breathing pattern. The rapid shallow pattern suggests that the morning exposure was sensed by the animals to be sufficiently irritative to change breathing pattern more so than in the afternoon. However, change in breathing pattern was not accompanied by decrease in volume or flow suggesting that there was not either a bronchoconstrictive change or inflammatory change in the airways. This lack of change in these parameters was corroborated by BAL and histological findings which did not show any significant changes.

Changes in the pause parameters also showed little change between the early and late exposures. Penh showed a significant increase overall in the morning exposures which

appeared to be largely related to the changes in this parameter for SD rats in the fall exposure group. Univariate analyses indicated very few significant relationships. The random forest analysis for frequency and the predicted ranking of elements associated with that response are shown in figure 2. The relationship between CAPs vs. Sham differences in frequency and concentrations of the first 4 ranked components in the random forest are shown in figure 3 along with similar plots for BC and total sulfur. Zinc, lead, calcium top the random forest list and all have significant positive relationships (increasing change in frequency with increasing concentration of the component). Tin (Sn) has a negative relationship. Sulfur has a weak positive relationship, but there is no relationship with black carbon.

Figure 1: **CAPs vs Sham Differences In Respiratory Parameters**
Δ ± Standard Error By Season, Strain and Exposure Group

Figure 2: Dose-respiratory frequency response of all exposure components using the random forest as an approach to multivariate analysis

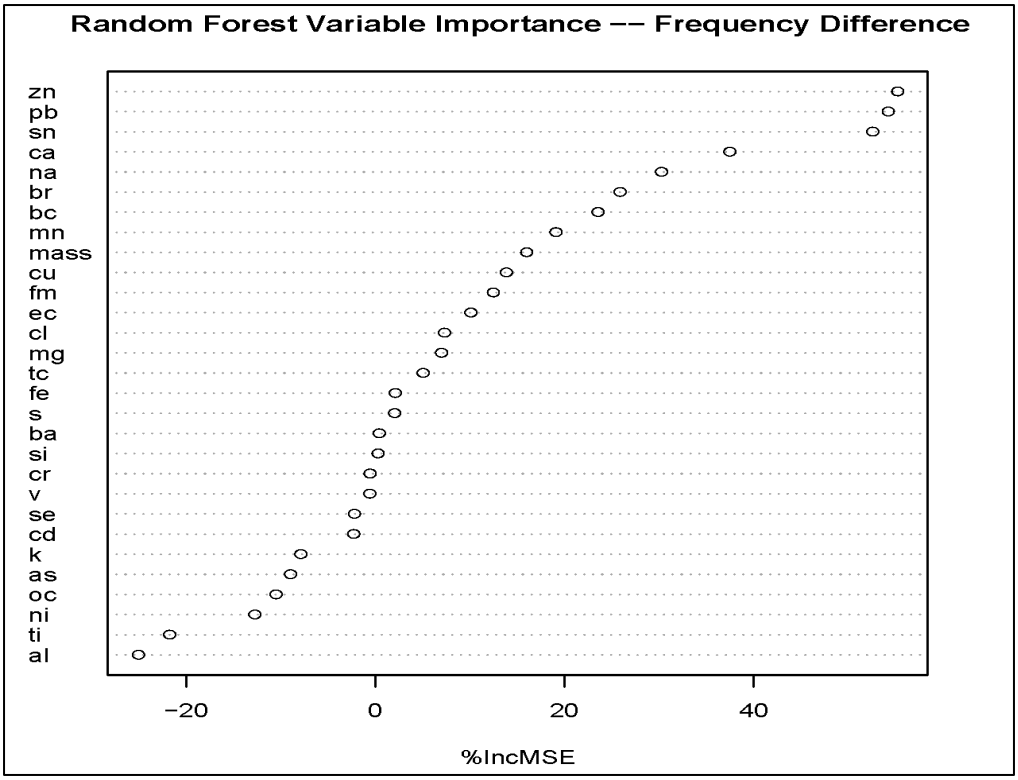
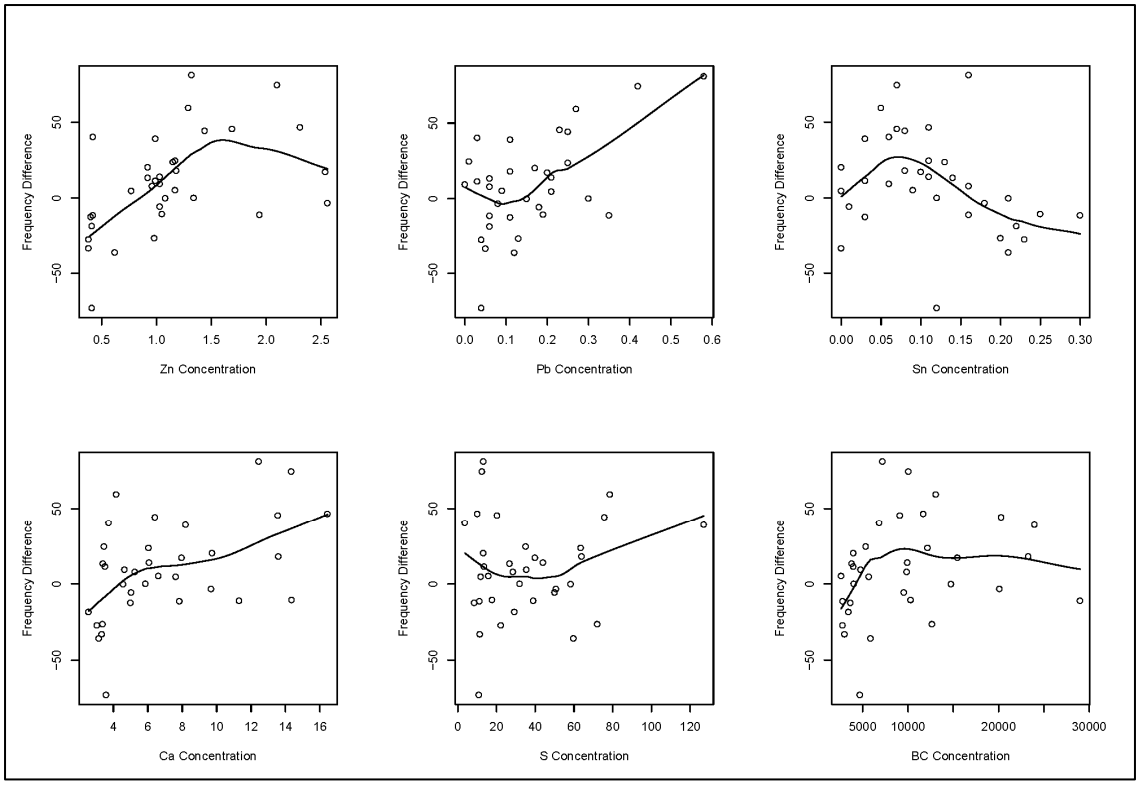


Figure 3: Scatter plots of changes in respiratory frequency vs exposure concentrations of components identified by random forest ranking.



In summary, diurnal differences in breathing pattern were found, and these pattern differences were enhanced by CAPs exposure. Responses of animals exposed early in the day were greater than and different from the responses of animals exposed at mid day. Responses of animals exposed during the spring were very different from the responses of animals exposed during the fall. The basis of this difference is not explained by differences in exposure composition, but differences in meteorological conditions such as temperature and hours of light need to be further explored. Changes in respiratory parameters were not strongly correlated with expected differences in black carbon and sulfur concentrations. Zinc, an element usually regarded as an emission of diesel engines, had similar concentrations in the morning and mid-day, but was consistently ranked as one of the components most strongly associated with changes in respiratory parameters. Sprague Dawley and SHR rats had comparable responses to the exposures, while WKY rats consistently had a lesser response to the same exposures. These results were presented at the 2008 American Thoracic Society meeting in Toronto by Dr. Edgar Diaz.

Data from the in vivo chemiluminescence studies show that the lung had significant effects of CAPs exposures whereas chemiluminescence changes in the heart did not reach significance in these exposures. There were significant changes with both early and late exposures, and analyses showed that early and late responses were not significantly different. 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 4 illustrates many components with significant univariate relationships to lung chemiluminescence, but none of these were significant in multivariate analyses.

Table 4. Effects of specific exposure components on in vivo lung chemiluminescence. using univariate analyses

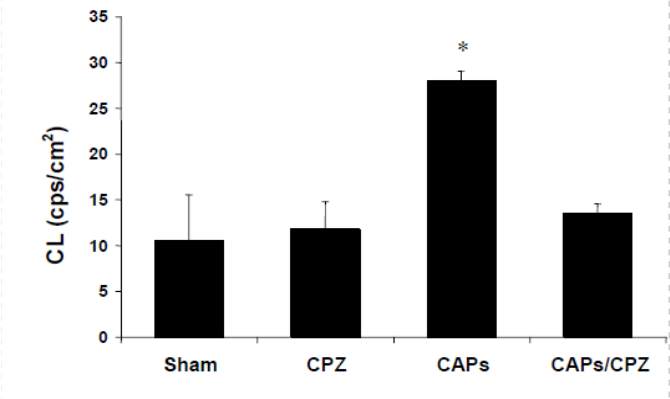
Element/ Component	Estimate ± SE	P value
CAPs Mass	0.012±0.005	**0.027**
Organic Carbon	0.106±0.038	**0.0064**
Elemental Carbon	0.298±0.121	**0.016**
Al	1.573±0.739	**0.036**
Si	0.577±0.267	**0.034**
S	0.132±0.061	**0.033**
Fe	0.509±0.218	**0.022**

Overall, the analyses 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.

Exposures of WKY and SH rats, monitoring blood pressure, electrocardiogram, and blood parameters have been completed, and data analyses are in progress.

Mechanistic studies using In vivo Chemilumenescence: A number of important mechanistic studies in this area have been completed in the past year supported within this project (Rhoden et al 2008; Ghelfi et al 2008). These animal studies are important correlates to the studies of oxidant stress and autonomic function in Project 1. Studies (Rhoden et al 2005) supported in Project 4 suggest that ambient particles modulate autonomic tone leading to cardiovascular oxidant stress and dysfunction. The relationship between inhaled CAPs, neurogenic signals from the lung, and effects on cardiac oxidative stress, has now been directly investigated (Ghelfi et al 2008). In these studies, the effect of blockade of vanilloid receptor 1 (TRPV1) was assessed in relationship to CAPs-induced cardiac oxidative stress and dysfunction in a rat model of inhalation exposure. Capsazepine (CPZ), a selective antagonist of TRPV1, was given intraperitoneally or as an aerosol immediately before exposure to CAPs. Control and CPZ-treated rats were exposed to filtered air or CAPs aerosols for 5 hours using the Harvard Ambient Particle Concentrator (mean PM2.5 mass concentration: 218 ± 23 $\mu g/m^3$). At the end of the exposure, cardiac oxidative stress was measured using in situ chemiluminescence: CL), lipid peroxidation (TBARS), and tissue edema. Cardiac function was monitored throughout the exposure, and measures of heart rate, heart rate variability, and cardiac conduction times were assessed. As shown in Figure 4, CPZ (aerosol) decreased CAPs-induced CL. Lipid TBARS and edema in the heart were similarly affected indicating that blocking TRP receptors, systemically or locally, decreases heart CL.

Figure 4. CPZ aerosolization prevents oxidative stress in the heart of rats exposed to CAPs as measured with in vivo chemiluminescence. Values represent the mean of eight independent determinations ± SEM. *p < 0.05.



CAPs exposure led to significant decreases in HR (CAPs 350±32 bpm, control: 370±29), and in the length of the RT, Pdur, QT and Tpe intervals. These changes were observable immediately upon exposure and were maintained throughout the 5 hours of CAPs inhalation. Changes in cardiac rhythm and ECG morphology were prevented by CPZ. These data are shown in Table 5 below.

Table 5: Change in ECG Parameters during Acute Exposure to either Filtered Air or CAPs with CPZ blockade of Vanilloid receptor 1 in the lung.

Group	ECG Segment				
	RT (s)	RTp (s)	Pdur (s)	QT (ms)	Tpe (s)
Control	0.048 ± 0.009	0.022 ± 0.003	0.015 ± 0.002	0.060 ± 0.003	0.025 ± 0.006
CPZ	0.044 ± 0.005	0.022 ± 0.002	0.016 ± 0.004	0.056 ± 0.003	0.022 ± 0.005
CAPs	0.038 ± 0.008 **	0.019 ± 0.005 *	0.017 ± 0.005*	0.051 ± 0.003 **	0.020 ± 0.007 **
CAPs/CPZ	0.045 ± 0.010	0.020 ± 0.003	0.016 ± 0.002	0.058 ± 0.003	0.026 ± 0.008

* p < 0.01, ** p < 0.005

The findings in Table 5 suggest that cardiac conduction current abnormalities in CAPs-exposed rats alter action potentials leading to changes in conduction velocity and

ventricular repolarization. Taken together these results suggest that inhaled CAPs stimulate TRVP1 (and possibly other pulmonary irritant receptors) and thereby activate autonomic nervous system reflexes. The end result of this reflex activation is increased cardiac oxidative stress, and functional cardiac electrophysiologic changes including increased P-wave duration and QT interval and decreased QRS and Tpe durations. Thus, CAPs exposure results in cardiac current abnormalities leading to changes in conduction velocity and ventricular repolarization, and that triggering of TRPV1-mediated autonomic reflexes in the lung is essential for the observed changes in conduction, repolarization and cardiac rhythms.

In other mechanistic studies, Rhoden et al (2008) used a specific blocker of superoxide anion to assess the role of this oxidant in induction of pulmonary inflammation with instilled ambient particles. The findings of this study indicate that superoxide anion plays a significant role in the development of inflammation. Investigators of this project have also carried out additional analyses of studies completed in the previous center and published these studies (Wellenius et al 2006). Important reviews of the cardiovascular effects of air particulate on the cardiovascular system (Godleski 2006) as well as gave several invited presentations at national meetings on this topic were given by Dr. Godleski. Finally, in collaboration with the statistical core of the Center, a number of statistical methods papers have been published by members of core and this project (Nikolov et at 2007, 2008)

Publications/Presentations:

Ghelfi, E., Rhoden, C., Wellenius, G. A., Lawrence, J., and González-Flecha, B. (2008). Cardiac oxidative stress and electrophysiological changes in rats exposed to concentrated air particles are mediated by TRP-dependent pulmonary reflexes. Toxicol Sci 102, 328-36.

Rhoden, C. R., Ghelfi, E., and González-Flecha, B. (2008). Pulmonary Inflammation by Ambient Air Particles is Mediated by Superoxide Anion. Inhal Toxicol 20, 11-5.

Rhoden, C. R., Wellenius, G., Ghelfi, E., Lawrence, J., and Gonzalez-Flecha, B. (2005). PM-Induced Cardiac Oxidative Stress Is Mediated by Autonomic Stimulation. Biochem Biophys Acta 1725, 305-313.

Wellenius, GA Coull, BA Batalha, JR Diaz, EA Lawrence, J Godleski, JJ , (2006) Effects of Ambient Particles and Carbon Monoxide on Supraventricular Arrhythmias in a Rat Model of Myocardial Infarction. Inhal Toxicol. 18: 1077-82.

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Nikolov MC, Coull BA, Catalano PJ, Godleski JJ. (2007)An informative bayesian structural equation model to assess source-specific health effects of air pollution Biostatistics 8: 609-24.

Nikolov, M. C., B. A. Coull, et al. (2008). "Statistical methods to evaluate health effects associated with major sources of air pollution: A case study of breathing patterns during exposure to concentrated Boston air particles." Journal of the Royal Statistical Society Series C (57): 357-378.

Imrich, A., Ning Y, Lawrence J, Coull B, Gitin E, Knutson M, and Kobzik L. (2007). Alveolar macrophage cytokine response to air pollution particles: oxidant mechanisms. Toxicol Appl Pharmacol 218, 256-64.

Diaz EA Lawrence J, Bonafe W, Calomeni G, Funaro G, and Godleski JJ (2008) Assessing Toxicity of Local and Transported Ambient Air Particles. Amer J Resp and Crit Care Med (Abstract)

Godleski, JJ Invited Speaker, AWMA Featured Symposium Particulate Air Pollution and Health; Session C Air Pollution and Cardiovascular Health "Effects of Ambient Particles on the Heart. June 23, 2006

Godleski, JJ Invited Speaker The Role of Air Pollutants in Cardiovascular Disease: Effects of Air Pollution on the Myocardium. October 13, 2006

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

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

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Center: EPA Center at HSPH for Ambient Particle Health Effects
Center Director: Petros Koutrakis
Project Title: Toxicological Evaluation of Realistic Emission Source Aerosol (TERESA): Investigation of Vehicular Emissions
Investigators: Petros Koutrakis, John Godleski
Institution: Harvard School of Public Health
EPA Project Officer: Stacey Katz
Project Period: April 1, 2005 – September 30, 2010
Period Covered by the Report: August 1, 2007 – July 31, 2008

Objective(s) of the Research Project:

Because particulate and gaseous source emissions undergo many transformations once released into the atmosphere, it is likely that secondary and primary pollutants exhibit different toxicities. Since most of the source-specific toxicity studies to date have focused on primary pollutants, there remains a great need to investigate the relative toxicity of source-specific primary and secondary particles. We have recently probed directly into this question with the Toxicological Evaluation of Realistic Emission Source Aerosol (TERESA) Power Plant study, a research project funded by the Electric Power Research Institute (EPRI) and the previous Harvard EPA PM Center. This study was designed to investigate the relative toxicity of primary and secondary particulate emissions from coal-fired power plants, *in situ,* and to explore the relationship between secondary particle formation processes and particle toxicity. As part of the TERESA study we have developed techniques and facilities to sample source emissions, form secondary particles inside a photochemical chamber, and expose animals to both primary and secondary particles. The TERESA approach forms an excellent foundation for future research, as it can readily be adapted to investigate other combustion sources. Using the developed technologies, this project extends this research to the toxicological investigation of primary and secondary pollutants from vehicular (mobile source) emissions released from the ventilation stack of a large roadway tunnel within the northeastern United States. We are in the preparation stages for this project that will compare the relative toxicity of primary and secondary mobile source emissions with concentrated ambient particles (CAPs) and with primary and secondary coal power plant emissions from the current TERESA study.

The specific hypotheses of this project:

- Exposures to fresh and to photochemically oxidized mobile source emissions will induce cardiovascular responses in normal animals;

- Atmospheric photochemical processes enhance the toxicity of gases and particles emitted from motor vehicles, and;

- Animal models of susceptible populations (e.g., spontaneously hypertensive rats) will have greater biological responses to particles originating from motor vehicles than the corresponding normal animal model.

Progress Summary/Accomplishments:

Permission to use an urban tunnel to provide mobile source emissions: The original plans for TERESA experiments, starting with funding from EPRI, and then expanded with funds from both DOE and the first Harvard EPA PM Center, included the goals of comparing the health outcomes from both stack gas from coal-fired power plants and from mobile sources with health outcomes from concentrated ambient particles (CAPs). After the power plant studies were initiated, we had discussions with the original TERESA scientific advisory committee about the best way to conduct the mobile source experiments. We quickly concluded that to get meaningful results it would be necessary to use actual traffic emissions rather than controlled emissions from lab tests using a dynamometer with only one or a few vehicles. To get the most representative mixtures of actual vehicle emissions, and to maximize the emission concentrations, we decided that traffic tunnels would be the optimum sources.

We made initial contacts to local agencies responsible for urban tunnels not long after we first began the TERESA project. We quickly concluded that the ideal sampling location should be at one of the ventilation shafts for the tunnels. We have had a long series of considerations to identify an appropriate site and obtain permission for use. This process has taken more than two years. However, we now have been given approval for use of an urban site which we will further characterize in terms of traffic mix and traffic emissions.

Preliminary laboratory chamber tests for simulation of secondary particle formation from mobile source emissions: Based on the experience and knowledge gained from the power plant studies, we were prepared to make the adaptations necessary to convert our mobile laboratory reaction chamber from the requirements for producing secondary particles from power plant stack gas to the requirements for producing secondary particles from mobile source emissions. The fundamental chemistry for power plant stack gas that is relevant to the TERESA studies is the oxidation of SO_2 to form acidic sulfate aerosol. Hydroxyl radical is used for the oxidation, and it is produced by high energy UV irradiation of ozone. Because ozone reacts most rapidly with NO present in the stack gas, excess ozone has to be added to have enough to use to produce hydroxyl radical. In addition, because the stack gas is diluted with dry air, and because water vapor is necessary to promote the oxidation chemistry, humidity of the reaction chamber is added using a small steam generator. For the mobile source emissions, the relevant reactions involve oxidation of volatile organic species (VOC's) by hydroxyl radicals (and ozone), in the presence of medium energy UV light (similar to natural sunlight). Unlike the power plant stack gas, excess ozone does not need to be added to titrate NO that is present in mobile vehicle exhaust gas; ozone concentrations increase rapidly in the chamber once the lights are turned on. Water vapor must also be added to promote the oxidation of VOC's. The primary difference in the reaction chamber

requirements for mobile source emission secondary particle production is the use of lower energy UV lamps.

We have started preliminary laboratory tests for mobile source emissions using the same chamber that was used for the power plant study. We use lower energy UV lamps. A ten-year old compact automobile is used as a source of emissions. The engine is run slightly fuel-rich during experiments, causing inefficient fuel combustion and resulting in CO concentrations similar to those observed in the highway tunnel. With the engine of the car idling, we expect that the CO concentration is a reasonable surrogate for VOC's. By diluting the exhaust gas to produce different known CO concentrations, we are thus able to control the VOC concentrations in the chamber and produce enough secondary aerosols to be in the range of concentrations needed for animal exposure tests. Our laboratory development tests will also involve variation in the flow rate through the chamber, to determine the effects of residence time on particle mass formation and particle size distribution. We also plan to test the effect of different concentrations of water vapor. Finally, the parallel plate membrane denuder, used in the TERESA power plant study to minimize concentrations of gaseous co-pollutants in the exposure atmospheres, will be evaluated for its efficiency in removing unreacted VOCs in the diluted and aged automobile exhaust mixture.

Publications/Presentations:

This project is the continuation of the TERESA study that was designed to compare the health effects of simulated secondary aerosol for coal-fired power plants with the effects from simulated secondary aerosol from vehicular sources. These efforts were supported by the previous Harvard/EPA PM Center, as well as by the current Center, and other support was provided by both EPRI and DOE. Analysis of the results from the three coal-fired plants has been under way for the last two years. The results of these analyses, along with descriptions of the experimental methods are included in nearly final drafts of manuscripts to be published in a special volume of *Inhalation Toxicology*. These papers will compare health outcomes for the different plants which use different types of scrubbers to clean the stack emissions, and will also include comparisons with health effects found for exposures to Concentrated Ambient Particles with those from the simulated secondary aerosol from the power plants. After the mobile source emission study health effects have been determined, we will publish a comparison paper that will include all three types of aged aerosols.

Future Activities:

Our goal is to complete laboratory tests by the end of this fall, and start field tests next winter. Keeping this schedule will depend on successful completion of the lab tests and formal approval from the Mass Turnpike Authority. The initial field tests will require optimizing the features of the reaction chamber to correspond to expected differences between the single vehicle exhaust used for lab tests and the mixed vehicle exhaust from the traffic tunnel. We expect it will take a few months to complete the optimization tests.

Toxicological exposures to primary and secondary aerosols from vehicular emissions are expected to be completed before the end of year 4 of this Center.

Supplemental Keywords: traffic related particles, acute cardiovascular effects, primary and secondary particle effects

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