

## **EPA CENTER AT HSPH FOR PARTICLE HEALTH EFFECTS**

### **6<sup>TH</sup> YEAR PROGRESS REPORT**

The EPA Particle Center at HSPH includes three research themes: The first focuses on the assessment of human exposures to ambient particles and gaseous co-pollutants. The second focuses on the investigation of susceptible sub-populations. And, the third examines the biological mechanisms which are responsible for the observed particle health effects. Each of the three research themes encompasses several research projects whose progress is briefly presented below.

#### **THEME 1: ASSESSING PARTICLE EXPOSURES**

During the sixth year of our EPA Center, we continued our examination of factors affecting exposures to PM<sub>2.5</sub> and its components using data collected in our panel studies of multi-pollutant exposures conducted in Baltimore, MD, Boston, MA, Steubenville, OH, and Atlanta, GA. In addition, we used exposure data from our Steubenville and St. Louis panel studies to examine the ability of ambient pollutant concentrations to reflect personal pollutant exposures and assessed the impact of measurement error on the relationship between pollutant exposures and intermediate cardiovascular health markers.

##### **Project 1a: Factors Affecting Personal PM Exposures**

Factors affecting personal PM exposures were examined by continuing our efforts to (1) identify housing and activities that modify the relationship between ambient particle concentrations and corresponding personal exposures and (2) examine infiltration of particles into indoor environments and how this infiltration varies with particle composition.

##### **A. Relationship between ambient particle and gas exposures and personal particulate exposures**

The relationship between ambient concentrations and personal exposures to fine particles and gases was examined using data from Boston, MA and Steubenville, OH. These studies used similar methods to measure indoor, outdoor, and personal fine particulate and gaseous levels. In both studies, personal multi-pollutant (PM<sub>2.5</sub>, SO<sub>4</sub><sup>2-</sup>, EC, O<sub>3</sub>, NO<sub>2</sub>, and SO<sub>2</sub>) exposures and corresponding ambient air pollution concentrations were measured simultaneously over 24-h periods for a cohort of individuals. In Boston, study participants included 20 healthy senior citizens and 23 schoolchildren, while in Steubenville; study participants included 10 senior citizens. In both studies, we analyzed personal exposure and ambient concentration data using correlation and mixed model regression analyses to examine relationships between: 1) ambient PM<sub>2.5</sub> concentrations and corresponding ambient gas concentrations; 2) ambient PM<sub>2.5</sub> and gas concentrations and their respective personal exposures; 3) ambient gas concentrations and corresponding

personal PM<sub>2.5</sub> exposures; and 4) personal PM<sub>2.5</sub> exposures and corresponding personal gas exposures.

We found similar results in Boston and Steubenville. In Boston, we found strong correlations between ambient PM<sub>2.5</sub> concentrations and corresponding personal exposures over time. Additionally, our results support the earlier finding that summertime gaseous pollutant concentrations may be better surrogates of personal PM<sub>2.5</sub> exposures, especially personal exposures to PM<sub>2.5</sub> of ambient origin, than surrogates of personal exposures to the gases themselves. Particle health effects studies that include both ambient PM<sub>2.5</sub> and gaseous concentrations as independent variables must be analyzed carefully and interpreted cautiously, since both parameters may be serving as surrogates for PM<sub>2.5</sub> exposures (Sarnat et al, 2005).

Similarly in Steubenville, we found strong associations between ambient particle concentrations and corresponding personal exposures as well as between ambient O<sub>3</sub> and NO<sub>2</sub> and their corresponding exposures. These associations, in particular for O<sub>3</sub>, were highest for individuals spending the majority of their time in high as compared to low ventilated environments. In cross-pollutant models, we found significant associations between ambient particle concentrations and personal gas exposures, with particularly strong associations between ambient SO<sub>4</sub><sup>2-</sup> and personal O<sub>3</sub> and between ambient EC and personal NO<sub>2</sub>. Findings that ambient gas concentrations reflect corresponding personal exposures have implications for air pollution epidemiology, suggesting that confounding of PM-associated health effects by gaseous pollutants may occur given the often strong correlations among the ambient pollutants. Furthermore, findings that ambient PM may represent exposures to both PM<sub>2.5</sub> and gases, suggest that time-series health studies based on 24-hour ambient concentrations may not be able to separate the independent effects of particles and gases. Findings from this study have been submitted to *Environmental Health Perspectives* as a research article.

## **B. Identification of factors affecting personal sulfate and EC exposures**

In the sixth year, we also analyzed data from Boston to characterize the relationships between personal, home indoor, home outdoor and ambient levels of SO<sub>4</sub><sup>2-</sup>, EC, and PM<sub>2.5</sub> for a panel of sensitive individuals with either chronic heart disease or COPD. We investigated four main factors likely to affect personal exposures: time spent in key microenvironments, such as the home; infiltration into the home; spatial variability in home outdoor concentrations, and; measurement error.

This investigation was based on simultaneous 24-hour integrated personal, home indoor, and home outdoor PM<sub>2.5</sub>, SO<sub>4</sub><sup>2-</sup>, EC, O<sub>3</sub>, SO<sub>2</sub> and NO<sub>2</sub> concentrations that were measured in 25 single-family homes in the Boston, MA area. Fifteen homes were measured in each of two seasons, winter and summer with five homes measured during both seasons. In total, there were 25 study participants including 18 individuals with self-reported chronic heart disease and seven individuals with physician-diagnosed chronic obstructive pulmonary disease (COPD). For eight participants during winter and six during summer,

a partner living in the home also participated in the personal exposure portion of the study.

We found ambient  $\text{SO}_4^{2-}$  to be strongly correlated with personal and home indoor  $\text{SO}_4^{2-}$  for all individuals without an indoor source of  $\text{SO}_4^{2-}$  in the home. Associations were not as strong for EC and  $\text{PM}_{2.5}$ , likely due to the outdoor spatial variability and indoor sources of these pollutants. While the strength of the associations for  $\text{SO}_4^{2-}$  varied between subjects and by season, outdoor or ambient  $\text{SO}_4^{2-}$  accounted for approximately 80% or more of the variability in personal and indoor  $\text{SO}_4^{2-}$  concentrations. [It is important to note that two homes with humidifiers were excluded from this analysis due to the large contribution to personal and indoor  $\text{SO}_4^{2-}$  and  $\text{PM}_{2.5}$  in those two homes.] We found that with the exception of humidifier use, housing conditions, as indicated by the high indoor-outdoor  $\text{SO}_4^{2-}$  correlations, tended to be quite similar day-to-day, indicating that home indoor and home outdoor levels correspond consistently regardless of the differences in the absolute levels in the two microenvironments. While ambient levels and indoor source contributions of  $\text{PM}_{2.5}$  can vary by day, the infiltration into homes appears to be relatively constant, at least during a one-week monitoring period.

Contrary to the results for  $\text{SO}_4^{2-}$ , EC showed relatively weak associations between personal/indoor EC levels and outdoor/ambient levels. This is likely due to indoor and local source generation of EC. Indoor EC concentrations explained only 50% of the variation in corresponding personal exposures, likely the result of exposures to EC that occurred outside the home or of greater imprecision in the EC measurement method as compared to those for  $\text{SO}_4^{2-}$  and  $\text{PM}_{2.5}$ . Additionally, indoor-outdoor ratios were higher and more variable for EC than  $\text{SO}_4^{2-}$  (excluding the two humidified homes). This suggests that different factors affected indoor-outdoor relationships for EC than for  $\text{SO}_4^{2-}$ . These factors could include greater infiltration or a greater contribution of indoor sources of EC as compared to  $\text{SO}_4^{2-}$ . Since relatively few homes had indoor-outdoor EC ratios greater than 1, indicating few indoor EC sources, results suggest that differences in  $\text{SO}_4^{2-}$  and EC infiltration was the more important factor. Differences in their infiltration may be related to corresponding differences in their size

### **C. Estimation of infiltration factors for fine particulates**

Particle infiltration is a key determinant of the indoor concentrations of ambient particles. Few studies have examined the influence of particle composition on infiltration, particularly in areas with high concentrations of unstable particles, such as ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ). To address this issue, we conducted a comprehensive indoor air monitoring study in 17 Los Angeles area homes with joint funding from the California Air Resources Board and the Environmental Protection Agency. Additional data analyses for this study were funded by our EPA Particle Health Effects Center. Findings from our analyses were submitted in a research paper to the *Journal of Air and Waste Management Association* (JAWMA).

In this study, we used indoor/outdoor concentration ratios during overnight (non-indoor source) periods to estimate the fraction of ambient particles remaining airborne indoors,

or the particle infiltration factor ( $F_{INF}$ ), for fine particles ( $PM_{2.5}$ ), its non-volatile (i.e., black carbon, BC) and volatile (i.e., nitrate,  $NO_3^-$ ) components, and particle sizes ranging between 0.02 and 10  $\mu m$ . We found  $F_{INF}$  to be highest for BC (median = 0.84) and lowest for  $NO_3^-$  (median = 0.18). The low  $F_{INF}$  for  $NO_3^-$  was likely due to volatilization of  $NO_3^-$  particles once indoors, in addition to depositional losses upon building entry. In addition, we found that the  $F_{INF}$  for  $PM_{2.5}$  (median = 0.48) fell between those for BC and  $NO_3^-$ , reflecting the contributions of both particle components to  $PM_{2.5}$ .  $F_{INF}$  varied with particle size, air exchange rate and outdoor  $NO_3^-$  concentrations. The  $F_{INF}$  for particles between 0.7-2.0  $\mu m$  in size was significantly lower during periods of high as compared to low outdoor  $NO_3^-$  concentrations, suggesting that outdoor  $NO_3^-$  particles fall in this size range and its volatilization likely influenced the size distribution of indoor particles. This study demonstrated that infiltration of  $PM_{2.5}$  varies by component and is lowest for volatile species such as  $NH_4NO_3$ . We concluded that indoor  $PM_{2.5}$  of ambient origin may differ from that outdoors with respect to composition and size distribution, especially when the outdoor concentration of volatile particle components is high. In addition, based on these results, we believe that sulfate particles may not be suitable proxies of particles of outdoor origin in areas with high concentrations of volatile particles. Particle composition, therefore, may influence the ability for outdoor PM concentrations to represent indoor and thus personal PM exposures and can ultimately influence observed epidemiologic relationships based on ambient monitoring data.

### **Project 1b: Analysis of the St. Louis Bus Study exposure and health data**

In the 6th Year of our Center, we analyzed data collected in our St. Louis Bus study to examine the effects of ambient and traffic related pollution on intermediate cardiovascular and inflammatory health markers, including heart rate variability, systemic inflammation, and pulmonary inflammation. Results from these analyses are presented briefly below.

#### *Heart Rate Variability*

Exposure to airborne particles may increase cardiac risk by altering autonomic balance. As these risks may be particularly great for traffic-related particles, we examined associations between particles and heart rate variability for 44 subjects who participated in 4 repeated trips aboard a diesel bus. Twenty-four hour electrocardiograms were correlated with continuous particle concentrations using generalized additive models controlled for subject, weekday, time, apparent temperature, trip type, activity, medications, and autoregressive terms. Associations were assessed for short and medium-term mean concentrations.

Heart rate variability was significantly and negatively associated with fine particulate matter. Significant positive associations were demonstrated with heart rate and the low to high frequency power ratio. Associations were strongest with 24-hour mean concentrations although strong and significant short-term associations also were found during bus periods, independent of daily exposures. Overall, associations were largest for high frequency power with 16 (95% CI: -17, -15), 19 (95% CI: -22, -17), and 14

(95% CI: -16, -13) percent decreases per inter-quartile changes in the 24-hour PM<sub>2.5</sub> (4.6 µg/m<sup>3</sup>), black carbon (458 ng/m<sup>3</sup>), and fine particle count (39 pt/cm<sup>3</sup>) concentrations, respectively. Eleven percent (95% CI: -13.6, -7.8) decreases in high frequency power were predicted per inter-quartile change in the 5-minute PM<sub>2.5</sub> (10 µg/m<sup>3</sup>) aboard the bus. This investigation indicates that fine particles are negatively associated with heart rate variability, with an overall trend towards reduced parasympathetic tone. While daily associations were evident for all particles, short-term associations were predominantly limited to bus periods and possibly fresh traffic-related particles. A manuscript of these findings has been prepared and will be submitted to *Environmental Health Perspectives* in October 2005.

### *Systemic Inflammation*

Inflammation may represent a pathway through which airborne particles lead to increased cardiac risk. Therefore, we investigated associations between ambient particles and acute systemic inflammation among repeated measures of 44 seniors and examined susceptibility by conditions linked to chronic inflammation. Mixed models were used to identify associations between fine particle concentrations (PM<sub>2.5</sub>) averaged over 1 to 7 days and measures of C-reactive protein (CRP), interleukin-6 (IL-6), and white blood cell counts (WBC). Effect modification was investigated for diabetes, obesity, hypertension, and elevated mean inflammatory markers.

Positive associations were consistently found between ambient PM<sub>2.5</sub> and WBC across all participants, with an 11% (95% CI: 0.19 to 22%) increase per 10 µg/m<sup>3</sup> increase in PM<sub>2.5</sub> averaged over the previous week. PM<sub>2.5</sub> and CRP also exhibited positive associations among all individuals for averaging periods longer than 1 day with the strongest associations for persons with diabetes, obesity, and hypertension. For example, a 10 µg/m<sup>3</sup> increase in the 5 day mean PM<sub>2.5</sub> was associated with a 24% increase in CRP (95% CI: -8.8 to 67%) for all individuals and a 170% (95% CI: 36 to 420%) increase for persons with diabetes, obesity, and hypertension. Persons with diabetes, obesity, and hypertension also exhibited positive associations between PM<sub>2.5</sub> and IL-6. Individuals with elevated mean inflammatory markers exhibited enhanced responsiveness for CRP, IL-6, and WBC. This investigation demonstrates that air pollution is positively associated with acute systemic inflammation and indicates enhanced sensitivity for individuals with diabetes, obesity, hypertension, and elevated mean inflammatory markers. A manuscript of these findings has been submitted to *Environmental Health Perspectives*.

### *Pulmonary Inflammation*

Airborne particles have been linked to pulmonary oxidative stress and inflammation. As these effects may be particularly great for traffic-related particles, we examined associations between particle exposures and exhaled nitric oxide (FE<sub>NO</sub>) in a study of 44 seniors, which involved repeated trips aboard a diesel bus. Samples of FE<sub>NO</sub> collected before and after the trips were correlated with micro-environmental and ambient particle concentrations using mixed models controlled for subject, day, trip, vitamins, collection

device, mold, pollen, room air nitric oxide, apparent temperature, and time to analysis. While ambient concentrations were collected at a fixed location, continuous group-level personal samples characterized micro-environmental exposures throughout facility and trip periods.

In pre-trip samples, both micro-environmental and ambient exposures to fine particles were positively associated with  $FE_{NO}$ . For example, an inter-quartile increase of  $5.8 \mu\text{g}/\text{m}^3$  in the daily micro-environmental  $PM_{2.5}$  concentration was associated with a 10% (95% CI: 2 to 20) increase in  $FE_{NO}$ . After the trips, however,  $FE_{NO}$  concentrations were associated only with micro-environmental exposures, with significant associations for concentrations measured throughout the whole trip day and only during bus periods. Associations with bus exposures were strong and statistically significant with an 18% (95% CI: 11 to 26) increase in  $FE_{NO}$  predicted per inter-quartile increase of  $8.4 \mu\text{g}/\text{m}^3$  in  $PM_{2.5}$ . Although pre-trip findings were generally robust, our post-trip findings were sensitive to several influential days. Fine particle exposures may result in airway inflammation in senior adults, with associations best assessed by micro-environmental exposure measurements during periods of high particle exposures. A manuscript describing these findings is currently in the final stages of preparation.

### **Project 1c: Modeling the Relationship between Mobile Source Particle Emissions and Population Exposures**

For year 6 of project 1c, we had proposed extending our intake fraction (iF) methodology to address motor vehicle emissions, as a way of informing PM control decisions and future analyses. Our specific objectives were to:

- Evaluate geographic patterns in primary and secondary particulate matter iFs for mobile sources, using a national-scale source-receptor (S-R) matrix
- Determine the relative contributions of near-source and long-range populations to particulate matter iFs for mobile sources in different geographic locations
- Develop predictive regression equations for iFs to explain geographic patterns as a function of population density and meteorological covariates

We have completed this analysis, with the following key findings:

- For primary fine particulate matter emitted from mobile sources, the intake fractions varied across source counties from 0.14 to 23 per million (median of 1.2 per million). These values were highly correlated with near-source population density; the population in the source county explained 43% of the variability in the above estimates, and a multivariate regression model with population at various radii from the source explained 86% of the variability. Spatial analyses of residuals indicated generally strong model performance, with greater errors along the coasts, where wind fields are more difficult to characterize and downwind populations may be less significant.
- For secondary ammonium sulfate formed from  $SO_2$  emissions, the median intake fraction (0.43 per million) was somewhat lower than for primary PM. The

- variability was similar to that for primary PM, but with more regional variability rather than small-scale spatial variability. In spite of the regional influence on atmospheric chemistry, multivariate regressions with only population terms had an  $R^2$  of 0.78, indicating the significance of population patterns even in this context. However, there was relatively greater statistical significance for population beyond 200 km from the source, relative to primary PM, and relatively lower statistical significance for population within 200 km, reflecting expected concentration patterns.
- Secondary ammonium nitrate formed from NO<sub>x</sub> emissions had an even lower median intake fraction (0.072 per million), with spatial variability driven somewhat by population patterns ( $R^2$  of 0.63 in multivariate regression model) but also by relative ambient concentrations of sulfate, nitrate, and ammonium. Higher values tended to be found in the Midwest, where there is adequate ammonia to neutralize nitrate (and lower ambient sulfate), versus higher levels in the Ohio River Valley and Northeast for secondary sulfate and primary PM.
  - We also quantified the extent to which SO<sub>2</sub> controls might free up ammonia to react with nitrate, thereby increasing ammonium nitrate concentrations. We determined that the public health benefits of SO<sub>2</sub> emission controls (due to sulfate reductions) would be offset by ammonium nitrate increases by an average of 9%, ranging from 1% to 29% across U.S. counties.
  - As mentioned above, one of our primary objectives was to determine the relative importance of near-source and long-range populations. The median distances within which half of the total intake fraction was realized was about 150 km for primary PM, 450 km for secondary sulfate, and 390 km for secondary nitrate. However, these values varied substantially by setting (i.e., range for primary PM from 0 km, indicating that more than 50% of the iF was realized in the source county, to 1800 km). In dense urban areas, often a majority of the intake fraction was realized within the source county, indicating that more geographically resolved dispersion modeling may be warranted.

Comparing our results with the published literature to date, the magnitude of our estimates appear reasonable, and this analysis remains the first attempt to characterize spatial variability in mobile source intake fractions and to derive conclusions about the model scope and resolution needed to accurately estimate public health benefits of pollution control from mobile sources. Specifically, we concluded that a national-scale county-resolution dispersion model is likely sufficient for secondary particulate matter or primary particulate matter in rural areas with substantial downwind populations, but that more resolved models should be explored in dense urban areas or less-populated areas without significant downwind populations.

The manuscript based on this work is undergoing final revisions and will be submitted to the journal *Atmospheric Environment* in September 2005. It will be submitted jointly with a power plant intake fraction analysis derived from Wilson (2003), a manuscript also supported by the EPA Particle Center at HSPH.

Based on the findings from this analysis, we have proceeded with follow-up work addressing potential within-county heterogeneity in primary PM mobile source intake fractions, as well as the questions of the spatial extent of the iF for sources within urban areas and the potential biases in estimates based on county-level resolution. We are using the CALINE dispersion model to simulate the influence of line-source emissions on concentrations under a variety of meteorological conditions, population patterns, and building/road configurations (i.e., presence/absence of a street canyon). This simulation will allow us to determine heterogeneity in primary PM intake fractions as well as the circumstances under which the near-source populations may dominate the intake fraction. Model development is ongoing and initial findings will be presented at the International Society for Exposure Analysis conference in November 2005.

## **THEME 2: SUSCEPTIBILITY AND EPIDEMIOLOGICAL METHODS**

During year six, we continued to work on our major themes of addressing questions of confounding, making methodological advances, and addressing susceptibility through three projects. The results are summarized below.

### **Project 2a: Investigation of factors influencing PM Mortality/Morbidity Relationships**

We have investigated factors influencing PM mortality and morbidity relationships in several ways. First, we have examined the association of air pollution with more specific responses than have generally been examined. For example, we have multi-city papers examining the association of PM<sub>10</sub> with hospital admissions for congestive heart failure (Wellenius, Schwartz, and Mittleman 2005A,B), stroke (Wellenius, Schwartz, and Mittleman 2005C), and myocardial infarction (Zanobetti and Schwartz, 2005). Further, since the studies were case crossover analyses of individual data, rather than count data, we were able to examine the role of other pre-existing medical conditions (e.g. diabetes, COPD) as modifiers of those associations in all of these studies, providing further evidence of who is susceptible to the effects of particle exposures. For example, persons with COPD or pneumonia were more susceptible to the effects of PM<sub>10</sub> triggering an MI. We have also examined the association of daily mortality for more specific causes of death, and examined factors that modify that association (Zeka, Zanobetti, and Schwartz 2005A).

In another study, we examined the effects of particulate air pollution on subjects with type II diabetes, and found an effect of both traffic particles and sulfate particles on flow mediated dilation, a measure of the function of the endothelial layer of the arteries, and a risk factor for atherosclerosis (O'Neill et al, 2005A).

### **Project 2b: Effects of Exposure and Confounding on Air Pollution Epidemiology**

The major potential confounders for the short term effects of air pollution are temperature and other gaseous air pollutants. To examine their role as confounders, we first need to understand better what their real association with the health outcomes of concern are. In addition, we have made methodological advancements that allow us to control for confounding without fully understanding the nature of the association between the potential confounders and health outcomes.

Specifically, we have spent a good deal of time examining the association of temperature with mortality and hospital admissions, as temperature seems the most reasonable potential confounder. We have examined, for the first time, the association of temperature with cardiovascular hospital admissions in a large multi-city study (Schwartz, Samet, and Patz, 2004). This association differed greatly from that seen for mortality, with admissions increasing monotonically with increasing temperature. We have also demonstrated that there are social and racial gradients in the temperature related mortality risks that are much larger than those seen for air pollution, suggesting that the air pollution effects are not due to confounding, since they show a different pattern (O'Neill et al, 2005B; O'Neill et al, 2005C). Finally, we have begun to examine the role of medical condition as a susceptibility factor in temperature related deaths (Schwartz, 2005B).

Moving on to the issue of confounding, we have used the case crossover approach and matched control days to have the same temperature as the day for each death (Schwartz 2004A). Since the case and control days are identical on temperature, it cannot be a confounder. We found that this approach yielded the same risk estimates as when control days were not matched on temperature, and temperature was controlled with regression splines. This provides considerable reassurance that the results for PM are not due to confounding by temperature. In addition, the same approach was applied to examining the ozone-mortality association in a large multi-city study, with similar results.

With regard to confounding by gaseous air pollutants, we used the same approach of choosing matched control days. Control days were matched either on the level of  $O_3$ ,  $SO_2$ ,  $NO_2$ , or  $CO$ , in a case-crossover study, and the effect of  $PM_{10}$  remained in all cases (Schwartz 2004B). Because this approach, unlike the NMMAPS approach, is not sensitive to how the other pollutant was controlled (linear, nonlinear, what form, etc), it provides strong evidence of an independent effect.

In a last examination of confounding, we have used a recently developed hierarchical method to control for confounders in the presence of measurement error (Schwartz and Coull, 2003) to re-examine the NMMAPS mortality study (Zeka and Schwartz, 2004). We found that after controlling for measurement error, PM was still associated with daily deaths, independent of the other pollutants.

## **Project 2c: Effects of Chronic Exposures**

The effect of chronic particulate exposures was examined using the Normative Aging Study cohort. In Year 6, we demonstrated that the effect of PM<sub>10</sub> on heart rate variability was enhanced in hypertensive individuals (Park, 2005). A follow-up to that study (Schwartz et al, 2005) has demonstrated that the effect is also enhanced in the obese, and in persons without the gene to manufacture glutathione S transferase M1. Since obesity increases systemic oxidative stress and GSTM1 is an important part of defenses against oxidative stress, this strongly indicates that oxidative stress is an important pathway for the autonomic effects of PM.

While cohort studies have shown much larger effects of PM on mortality than time series studies, some have argued that these are the results of lifetime or very long term exposure. If so, benefits of reducing air pollution will take a long time to appear. To examine this issue we conducted a further 10 year follow-up of the Six City Study. We compared the covariate adjusted mortality rate in each of the six cities in each of the two follow-up periods to the average exposure in each follow-up period. We found that in cities where exposure fell substantially between the two follow-up periods, the covariate adjusted mortality rate fell substantially, whereas if there was little change in exposure, there was little change in the mortality rate. This indicates that the health improvements from reducing PM exposure should be seen relatively soon after reducing PM concentrations in the air (Laden et al, 2005).

### **THEME 3: MECHANISMS OF AMBIENT PARTICLE TOXICITY**

Theme III proposed three projects which aim to define the biological mechanisms that lead to adverse health outcomes, and to identify the particulate and gaseous pollutants responsible for increased cardiac vulnerability. Inhalation studies using controlled exposures to concentrated ambient particles (CAPs) have been conducted using the myocardial infarction (MI) rat model and have demonstrated the adverse effects of PM, including combustion particles and road dust, on cardiac health. The first of the Theme III projects explored mechanisms increasing *in vivo* chemiluminescence in the heart and lungs of rats exposed CAPs. Novel approaches were used to determine whether the primary effect is mediated by the autonomic nervous system, systemic inflammation, or a combination of these mechanisms. In addition, studies were initiated to assess the direct effects of particles on anti-oxidant enzymes. The second project continued our studies on the effect of ambient particles and gas mixtures (exposures to CAPs with either O<sub>3</sub> or NO<sub>2</sub>) on the rat model of myocardial infarction. The third project proposed development of methods to investigate the relative toxicity of primary emissions and secondary particles (same primary emissions aged inside a photochemical chamber) using normal rats as well as a model of MI. This was a very ambitious plan for one year, and considerable progress was made. Moreover, although not included in the original plans, it was decided to use Center resources to support a fourth project to investigate whether ambient particulate matter exhibits direct and selective inhibitory effects on oxidative stress enzymes.

#### **Project 3a: Oxidant-mediated cardio-toxicity of ambient air pollutants**

During the last year we have used an *in vivo* model of inhalation exposure to ‘real world’ particles to test the hypothesis that the lung-heart signaling after particle deposition in the lung operates through neural mechanisms. Our data show that CAPs exposure increases cardiac oxidants. PM-induced oxidative stress is mediated by autonomic signals and the resulting oxidative stress is associated with significant functional alterations in the heart (Rhoden et al, 2005).

We used pharmacological strategies to determine whether oxidants are implicated in PM-dependent cardiac dysfunction and whether PM-induced increase in autonomic stimulation on the heart mediates cardiac oxidative stress and toxicity. Adult Sprague-Dawley rats were exposed to either intra-tracheal instillation of urban air particles (UAP 750  $\mu\text{g}$ ) or to inhalation of concentrated ambient particles (CAPs mass concentration  $740 \pm 300 \mu\text{g}/\text{m}^3$ ) for 5 hours. Oxidative stress and cardiac function were evaluated immediately after exposure. Instillation of UAP led to significant increases in heart oxidants measured as organ chemiluminescence (UAP:  $38 \pm 5 \text{cps}/\text{cm}^2$ , sham:  $10 \pm 1 \text{cps}/\text{cm}^2$ ) or thiobarbituric acid reactive substances (TBARS, UAP:  $76 \pm 10$ , Sham  $30 \pm 6 \text{ nmol}/\text{mg}$  protein). Heart rate values were increased immediately after exposure (UAP:  $390 \pm 20 \text{ bpm}$ , sham:  $350 \pm 10 \text{ bpm}$ ) and returned to basal levels over the next 30 min. Heart rate variability (SDNN) was unchanged immediately after exposure, but significantly increased during the recovery phase (UAP:  $3.4 \pm 0.2$ , Sham:  $2.4 \pm 0.3$ ).

To determine the role of ROS in the development of cardiac malfunction, rats were treated with 50mg/Kg N-acetylcysteine (NAC) 1 hour prior to UAP instillation or CAPs inhalation. NAC prevented changes in heart rate and SDNN in UAP-exposed rats ( $340 \pm 8$  and  $2.9 \pm 0.3$  respectively).

To investigate the role of the autonomic nervous system in PM-induced oxidative stress rats were given 5mg/Kg atenolol ( $\beta$ -1 receptor antagonist), 0.30 mg/Kg glycopyrrolate (muscarinic receptor antagonist) or saline immediately before exposure to CAPs aerosols. Both atenolol and glycopyrrolate effectively prevented CAPs-induced cardiac oxidative stress ( $\text{CL}_{\text{ATEN}}$ :  $11 \pm 1 \text{cps}/\text{cm}^2$ ,  $\text{CL}_{\text{GLYCO}}$ :  $10 \pm 1 \text{cps}/\text{cm}^2$ ,  $\text{TBARS}_{\text{ATEN}}$ :  $40 \pm 6 \text{ nmol}/\text{mg}$  protein,  $\text{TBARS}_{\text{GLYCO}}$ :  $38 \pm 6 \text{ nmol}/\text{mg}$  protein)(Rhoden et al, 2005).

These data show that PM exposure increases cardiac oxidants via autonomic signals and the resulting oxidative stress is associated with significant functional alterations in the heart. The observed preventive effects of NAC suggest that treatment with low doses of this antioxidant could be used to ameliorate the toxic effects of particulate air pollution.

### **Project 3b: Effects of ambient particles and gas mixtures (exposures to CAPs with O<sub>3</sub>) on the rat model of myocardial infarction.**

We have compared the effects of the following exposures: filtered air, CAPs alone, ozone alone, and CAPs plus ozone. CAPs exposure levels will be approximately 30 times the ambient level. Ozone exposures started at 120 ppb, comparable to the current ambient 1-hour standard, and at a level where effects might be expected. Because of the extensive

efforts required for Project 3a, described above, we have not yet completed the full range of studies planned for this project. Additional CAPs-Ozone exposure tests were conducted in October 2005. Preliminary findings (not including the October 2005 tests) are very encouraging, suggesting significant increases in both ventricular premature beats and supraventricular premature beats in rats exposed to ozone alone, none with sham exposures, non-significant increases with CAPs alone, but with exposures to the combination of CAPs and Ozone a markedly significant increase in both types of arrhythmias is observed.

### **Project 3c: Toxicological Evaluation of Realistic Emissions of Source Aerosols: Development of a mobile laboratory for the assessment of sources of ambient air pollution.**

Sources such as coal-fired power plants may produce only small quantities of primary particulate matter (PM), and most of the concern over the health impacts of power plant emissions focuses on the secondary particles formed via atmospheric oxidation of emitted SO<sub>2</sub>. The effects of secondary particles formed from vehicular emissions are also of considerable concern. However, toxicological studies of these secondary particles are difficult to carry out. The TERESA (Toxicological Evaluation of Realistic Emissions of Source Aerosols) study involves the use of mobile laboratories at power plants or sources of vehicular emissions to age these emissions, followed by animal exposures. Development of methods to carry out these studies was supported by the EPA PM Center at HSPH in conjunction with the Electric Power Research Institute and the US Department of Energy. Several power plants were included in the project to allow assessment of different coals and pollution control configurations. The primary goal of TERESA is to evaluate the comparative toxicity of secondary particles derived from coal-fired power plant emissions and vehicular sources.

Primary emissions were drawn from the power plant stack into a mobile laboratory, where several atmospheric scenarios were simulated (primary particles only, secondary particles, secondary particles + secondary organic aerosol, and secondary particles + ammonia + secondary organic aerosol). Male Sprague-Dawley rats were exposed to these atmospheric mixtures for 6 hours. Control animals were exposed to filtered air. Pulmonary function and breathing pattern data were collected during exposure, and bronchoalveolar lavage cells and fluid and blood were collected post-exposure for cytological and biochemical analyses (Rohr et al, 2005; Godleski et al, 2005).

PM<sub>2.5</sub> concentrations ranged from 200-450 µg/m<sup>3</sup> in all of the secondary particle scenarios. At the first plant studied, where primary particle emissions were very low, no differences in breathing pattern or pulmonary function, in BAL, or in blood parameters was observed between control and exposed animals. At the second plant where primary particle emissions were 3 times higher, but still quite low, significant differences were noted for several parameters. Significant changes in breathing pattern toward a rapid shallow breathing pattern were observed in both the secondary particles and the secondary particles + organic aerosol scenarios.

For each exposure scenario, statistical modeling was used to assess the size and strength of association between exposure and each respiratory outcome. Additive mixed models were applied to 10-minute averaged data collected from all exposed and Sham animals exposed during that scenario. A form of repeated measures model for longitudinal data, additive mixed models represent an extension of linear regression models that allows one to (1) estimate potentially non-linear effects of independent variables, and (2) include random effects as independent variables in order to account for clustering of observations that results from repeated measurements being taken on the same animal during the exposure period. For each outcome, additive mixed models were fit using as independent variables (1) a general nonlinear mean trend for sham animals over the exposure period, (2) an exposure indicator, which implies a constant shift in the mean trend due to test exposure, and (3) random animal effects reflecting animal-to-animal heterogeneity that results in correlation among 10-minute averages taken on the same animal over time. All models were fit using the gamma function in the R software (R Development Core Team [2004]).

Finally, a more general model that relaxed the assumption of a constant shift due to test exposure was also fit to the data. This model specified distinct mean trends over the exposure period for the SHAM and test animals, again including random animal effects to account for the repeated measurements taken on each animal. The difference between these estimated trends represents the time-varying effect of the test exposure over the exposure period.

Table 1 describes the directional trends and the level of significance of the changing trend.

Table 1. Directional Trends and Levels of Significance for Different Scenarios

Scenario	Respir Rate	Tidal Volume	Time Inspiration	Time Expiration	PenH
Sec unneut organics #1	↑ ns	↓ <b>p=0.003</b>	NC.....ns	NC ns	↓ ns
Sec unneut organics #2	↑ ns	NC.....ns	NC.....ns	NC.....ns	↓ <b>p=0.001</b>
Sec unneut	↑ p=0.06	↓ <b>p=0.04</b>	↓ <b>p=0.02</b>	↓...p=0.06	↓ <b>p=0.01</b>
Sec unneut MI model	↑ <b>p=0.024</b>	NC.....ns	NC.....ns	↓ <b>p=0.005</b>	↑... <b>p=0.03</b>
Sec neut organics	↓ ns	↓ <b>p=0.002</b>	NC.....ns	NC.....ns	↓ <b>p=0.001</b>
Primary particles	↓ ns	↓ <b>p=0.001</b>	NC.....ns	NC.....ns	↓ <b>p=0.003</b>

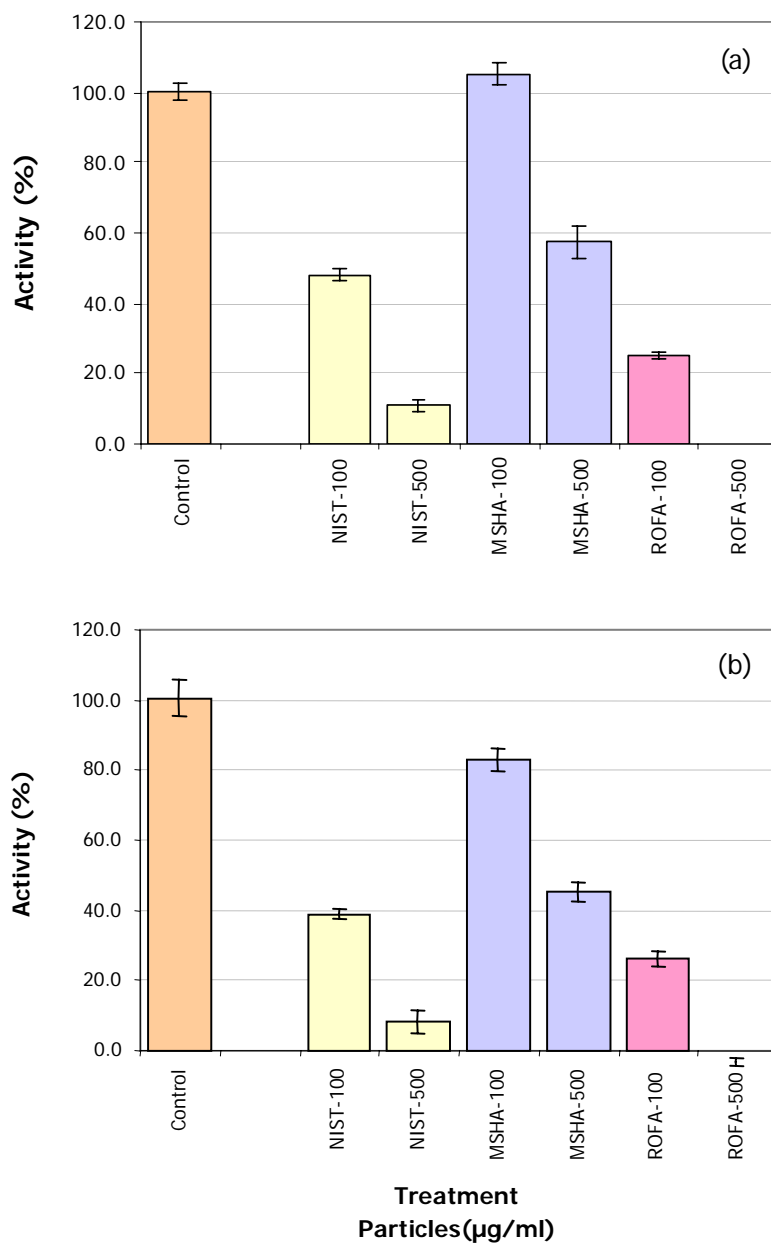
Results from the first two TERESA plants, under several different simulated atmospheric scenarios, indicate different toxicological effects with exposure to secondary particles derived from coal combustion. Definition of the basis of these differences is in progress with detailed comparative analyses of the exposure component concentrations, the type of coal burned, and other exposure variables. Studies of vehicular emissions have been planned, and arrangements are in progress for these studies to be part of our new EPA

Center. Meanwhile, analysis continues on the electrocardiographic analyses of animals exposed using the MI model.

### **Project 3d: Ambient Particulate Matter Exhibits Direct and Selective Inhibitory Effects on Oxidative Stress Enzymes.**

A primary mechanistic hypothesis by which ambient air particles have a significant negative impact on human health is via the induction of pulmonary inflammatory responses mediated through the generation of reactive oxygen species (ROS). The objective of this study was to evaluate whether air particulates interact directly with enzymes involved in oxidative stress responses, which are part of ROS-mediated inflammatory response pathways. We performed enzyme inhibition assays on four enzymes involved in oxidative stress responses (Cu/Zn superoxide dismutase, Mn superoxide dismutase, glutathione peroxidase, and glutathione reductase), in the presence of particles of varying toxicities, and found distinctive inhibition patterns that were both particle and enzyme specific. Results from the assays on Cu/Zn SOD and Mn SOD enzymes are shown in Figures 1a,b. In both cases, the control treatment consisted of the corresponding enzyme at 0.15 U/ml, which falls at the middle of the linear range for the assay, with no particles added. The standard curve for each enzyme was determined from a range of standards according to the assay protocol, using replicate wells in the same microplate to ensure consistency within each experiment. The effects of the particle type and concentration appear to be similar for both SODs. The NIST particles reduced the activity of both enzymes by about 50% when present at 100 µg/ml and by more than 90% at a level of 500 µg/ml. The ROFA particles were clearly more active, eliciting 75% and 100% inhibition of the enzymatic activities when present at 100 µg/ml and 500 µg/ml, respectively. Inert MSHA particles were the least inhibitory and did not reduce the activity of Cu/Zn SOD at 100 µg/ml, but they did show a small but significant inhibitory effect on the activity of Mn SOD (the 95% bootstrap confidence interval of residual Mn SOD activity was [76.3, 88.9]). On the basis of these findings, we suggest a strategy for an enzyme bioassay that could be used to assess the potential of particles to generate ROS-induced responses. This work is detailed in a manuscript submitted for publication (Hatzis et al, 2005).

**Figures 1a,b.** Effect of PM type and concentration on the activity of Cu/ZnSOD (a) and MnSOD (b). (n=5 per determination).



## APPENDIX A

### Human Subjects:

Table 2 lists the open IRB protocols for the Center Related work. The HSPH IRB requires these to be maintained open even after funding has expired as long as research work (including manuscript preparation) is on-going. We will maintain all the Center's IRB protocols active for the next two years. Attached are the approvals for all documents listed in the table.

**Table 2: EPA Particle Center at HSPH Sponsored Projects with Active Human Subjects Protocols**

Protocol No.	Study Name	IRB w/ jurisdiction	Date of Last Review
0011 PART	Particulate Air Pollution Exposure Helen Suh, PI Petros Koutrakis	HSPH	Nov 21, 2004
9905 ASSE	Assessing Human Exposures of High-Risk Sub-Populations to PM Petros Koutrakis, PI	HSPH	Feb 17, 2005
	Normative Aging Study Volkonas	VA	April 25, 2005
9905 EPAP	Chronic Effects of Exposure to PM Dockery	HSPH	April 28, 2005
0109 TRAF	Traffic-Related Particles and Cardiovascular Health in St. Louis H. Suh, P. Koutrakis	HSPH Channing	Oct 22, 2004 July 22, 2005
0005 CARD 9710 CARD	Cardiovascular Vulnerability to Particulate Pollution Diane Gold, PI (Steubenville, age 65+)	HSPH-Suh HSPH-Gold Channing	May 18, 2005 Oct 17, 2005 July 5, 2005

### Quality Assurance:

We have no new Quality Assurance issues to report since our last report. The Centers Quality Documents will be reviewed and updated in preparation for future work.

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### Theme II

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**APPENDIX B. Financial Summaries**

*YEAR 6: 6/1/2004 to 5/31/200*

Category	Year 6-2004-05 Appropriation	Adjustment	Balance from Year 5	Cumulative Appropriation	Year 6 Expense	Cumulative Balance	Comments:
Personnel	758,191		815,630	1,573,821	798,987	774,833	
Fringe Benefits	166,131		122,038	288,169	217,071	71,099	
Subtotal Personnel	924,322		937,668	1,861,990	1,016,058	845,932	
Equipment	15,000		31,630	46,630	36,181	10,449	
Travel	24,000		(35,971)	(11,971)	27,552	(39,523)	
Supplies	34,000		(84,548)	(50,548)	51,597	(102,145)	
Other	14,700		(92,071)	(77,371)	148,556	(225,927)	
Subcontracts	-		251,758	251,758	51,327	200,431	
total direct costs	1,012,022		1,008,466	2,020,488	1,331,271	689,217	
indirect costs	637,955		577,369	1,215,324	782,578	432,746	
TOTAL COSTS	1,649,977	-	1,585,835	3,235,812	2,113,849	1,121,963	
ALLOCATION					Spent to date		
Total to date	9,447,015				8,325,052		

*EXPENDITURES BY YEAR:*

Year 1	1,545,270
Year 2	1,540,462
Year 3	1,563,850
Amendment	49,998
Year 4	1,556,608
Year 5	1,540,850
Year 6	1,649,977
TOTAL	9,447,015

*CUMMULATIVE SUMMARY: as of 11/1/05*

Category	Year 1-6 Appropriation	Cumulative Expense	Cumulative Balance	Comments:
Personnel	3,464,743	2,689,909	774,833	
Fringe Benefits	675,699	604,600	71,099	
Subtotal Personnel	4,140,442	3,294,510	845,932	
Equipment	310,000	299,551	10,449	~150K fabr. Equipment
Travel	177,000	216,523	-39,523	
Supplies	231,000	333,145	-102,145	majority lab supplies
Other	471,722	697,649	-225,927	inc. animals and rel. costs
Subcontracts	807,139	606,708	200,431	
total direct costs	6,137,303	5,448,085	689,218	
indirect costs	3,309,713	2,876,967	432,746	
TOTAL COSTS	9,447,016	8,325,052	1,121,964	