Johns Hopkins Particulate Matter Research Center

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Project 1: Estimation of the Risks to Human Health of PM and PM components

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Overall Center Report

Johns Hopkins Center for Particulate Matter Research

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Overall Center Goal

The Johns Hopkins Center for Particulate Matter (PM) Research has an agenda targeted towards identifying those characteristics of particles that determine the risk of PM to health. This topic was highlighted as a key area of research need by the National Research Council's Committee on Research Priorities for Airborne Particulate Matter. More complete understanding of this topic is needed, if there is to be a shift away from mass-based standards for PM towards approaches that targets the most toxic particles and the sources of these particles.

The Center's approach is inherently multidisciplinary, interweaving epidemiology, exposure assessment and atmospheric monitoring, and toxicology. We use the epidemiological evidence on risks to health as the focal point for the Center's work. Underlying our approach is the hypothesis that differences in particle characteristics contribute to the variation of the health risks of PM across the United States. In the Center's work, we first characterized this heterogeneity of the risks to health of PM across the country and then used it as the basis for establishing a sampling frame for selecting locations for both detailed PM characterization and for collection of bulk samples of PM, both $PM_{2.5}$ and $PM_{10\text{-}2.5}$. The PM samples will be evaluated for toxicity in murine models of two human diseases, asthma and congestive heart failure, associated with susceptibility to PM. In these models, we anticipate finding similar indications of toxicity across the samples, as found in the epidemiological analyses. Together, the detailed PM characterization and the bioassay results should lead to more focused hypotheses with regard to particular components that can be tested in further studies.

To accomplish this first phase of its research agenda, the Johns Hopkins Center includes three component projects. The first (Project 1) involves analyses of data bases of national scope on PM exposure and risk for hospitalization and mortality and on geographic and seasonal variation in the components of PM, as monitored by the EPA's Speciation Trends Network (STN). Project 2 investigators have developed the suite of monitoring equipment to be brought to locations identified in Project 1 and a cyclone device for collecting a substantial mass of particles for analysis and use in bioassays. Project 3 involves the development of bioassays for assessing the particles collected in Project 2. In a later phase of its research, the Center investigators will test more focused hypotheses using epidemiological and toxicological approaches, as well as carrying out exposure assessment studies directed at specific PM components.

Progress

Together, the three project teams have set the foundation for meeting the overall Center goal. Project 1 has characterized the variation in the PM-associated risk for hospitalization and mortality across the country and also analyzed the available data from the STN for the years 2000-2005. These analyses were critical to the selection of locations for PM collection and monitoring Project 2. The variability of PM mass across the 203 counties covered was largely

accounted for by seven components, suggesting that the 52 components assessed by the STN, some tightly co-varying spatially and temporally, share sources. The seven were sulfate, nitrate, silicon, elemental carbon (EC), organic carbon matter (OCM), sodium ion, and ammonium ion. These seven components, in aggregate, constituted 83% of the total $PM_{2.5}$ mass, whereas all other components individually contributed less than one percent. .There was substantial variation in the makeup of the PM, both spatially and temporally. Using the Medicare data base for cardiovascular and respiratory admissions, the Project 1 investigators also found geographic variation in risks for hospitalization for these diseases; distributions of risk were defined within five broad geographical regions of the country and from the locations, counties were selected that were at the high and low ends. This analysis confirmed overall significant variation in risks across the country and provided the sampling frame for selecting the nine monitoring sites. The nine monitoring sites were: 1) King WA, 2) Sacramento CA, 3) Maricopa AZ, 4) Hennepin MN, 5) Harris TX, 6) Allegheny PA, 6) Jefferson KY, 8) Kings KY, 9) Pinellas FL.

The Environmental Protection Agency has considered regulation for one size-specific component of PM, $PM_{10-2.5}$—so-called "coarse" PM. Evidence on the risks of $PM_{10-2.5}$ has been limited and consequently we used the Medicare data set to explore risk for hospitalization associated with $PM_{10-2.5}$, finding weak evidence as to whether $PM_{10-2.5}$ has an independent association with risk, after taking account of $PM_{2.5}$ concentration. We have also initiated analyses to explore associations of PM components, as assessed by the STN, with risk for hospitalization in the Medicare population. These analyses, which use all locations available through the STN, complement the Center's overall approach by generating hypotheses with regard to toxicity-determining components of PM.

To refine understanding of how PM characteristics determine risk, we need to be able to have a substantial mass of particles for chemical and physical characterization and for testing in biological assays that give insights into comparative toxicity of different samples and opportunities to explore the mechanistic basis of the effects of PM. The collection of PM is complicated by the potential to alter the PM as a consequence of the action of the collecting device, by the difficulty of collecting the small particles, termed "ultrafine" or $PM_{0.1}$ and by the unavoidable loss of volatile and semi-volatile components. Our approach, which utilizes a custom-made sequential cyclone operating at a high collection volume, recognizes these difficulties and provides the compromise of collection of several grams of PM over a one-month period; we have a known loss of $PM_{0.1}$ and cannot avoid the volatilization of components over the month-long collection period. On the other hand, the samples are collected in a standardized fashion, along with detailed monitoring, in all locations. The protocol for field monitoring and collection has been tested and sampling and collection have not been carried out at four locations.

For control of the health effects of PM, regulators need to know if all particles, regardless of source have similar or differing profiles of toxicity. In Project 3, the investigators have developed a set of bioassays for this purpose. Initially, they carried out in vitro (cell system)

assays with human lung epithelial and endothelial cells and already collected samples of PM from Baltimore. These experiments pointed to the diverse mechanisms by which PM may trigger biological responses. Two in vivo (mouse) models of human disease will be used to compare the toxicity of PM samples collected in the various locations identified in Project 1: one is a long-used mouse model of asthma that uses the asthma-susceptible AJ mouse strain and involves the induction of asthma by ovalbumin; and the second is a mouse model of cardiomyopathy that leads to congestive heart failure, a prevalent human disease that conveys susceptibility to PM. In the initial phase of development of the bioassays, the existing Baltimore PM samples were used. The models were characterized as to their responsiveness to PM and the nature of dose-response relationships with the instilled PM. Work in progress is pursuing molecular signatures of the effects of PM using genomic approaches.

Expectations and Future Work

The Johns Hopkins PM Center has a unifying goal and draws on a multidisciplinary group of investigators to address this goal. In its first three years, it has completed a comprehensive set of analyses of variation in the components of PM across the STN and in variation in the risks of hospitalization associated with PM—$PM_{2.5}$, $PM_{10-2.5}$, and components of PM. Overall, the results support the hypothesis that PM risks vary with characteristics, thereby supporting the rationale for our overall approach. We have also developed the PM monitoring and collection methods and are in the process of monitoring the eight selected sites across the country.

Within the next 18 months, we anticipate completing the PM monitoring and collection and having much of the bioassay work done as well. At that point, we will have an understanding of whether there are differing particle characteristics at places where differing risks to health have been observed epidemiologically. We will also know if findings in animal models parallel those used in the community; if they do, the animal models could prove to be quite useful for assessing mechanisms of toxicity.

In the last phase of the Center's work, we anticipate the development and testing of more refined hypotheses. We will continue to use multidisciplinary approaches.

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Center Director: Dr. Jonathan M. Samet
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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:
In this project we will develop and apply statistical methods to national data sources to: 1) carry out multi-site time series studies for estimating short-term effects of PM and PM components on mortality and morbidity **(Phase I**); 2) carry out cohort-studies for estimating long-term effects of PM and PM components in susceptible populations (**Phase II**); and 3) assess coherence of evidence from bioassays and epidemiological studies on PM toxicity and susceptibility; and explore linkages of sources of harmful PM components to human health risks **(Phase III**).

By providing individual-level health data for the entire US population of elderly, the National Medicare Cohort will allow us to take full advantage of all existing and future air quality databases on PM and its characteristics. This project will address the following objectives of the Center on a national scale: 1) mapping risks of PM and PM constituents to human health across the United States; 2) using the maps to identify a sampling frame of locations with contrasting higher and lower risks; 3) carrying out more refined epidemiological studies to estimate further the risks of the more toxic particles to susceptible individuals.

Progress Summary/Accomplishments:

Progress is described in relation to the original specific aims for this project.

A. Multi-site time series studies for estimating short-term effects of PM and PM components on mortality and hospitalization (Phase I)

A1. **Characterize spatial and temporal variability of $PM_{2.5}$, $PM_{2.5}$ components, and gaseous pollutants across the US to identify locations for a more in-depth PM characterization for biological studies;**

Progress to date:

We have characterized the spatial and temporal variability of $PM_{2.5}$ components in the U.S. with the objective of identifying components for assessment in epidemiological studies (Bell et al. 2007). We constructed a database of 52 $PM_{2.5}$ component concentrations for 203 U.S. counties for 2000 to 2005 from the EPA's Speciation Trends Network (STN). First, we described the challenges inherent to analysis of a national $PM_{2.5}$ chemical composition database. Second, we identified components that substantially contribute to and/or co-vary with $PM_{2.5}$ total mass. Third, we characterized the seasonal and regional variability of targeted components. We identified substantial seasonal and geographical variation in $PM_{2.5}$ chemical composition. Only seven of the 52 components contributed 1% or more to total mass for yearly or seasonal averages (NH_4^+, elemental carbon, organic carbon, NO_3^-, Si, Na^+, and $SO_4^=$). The strongest correlations with $PM_{2.5}$ total mass were with NH_4^+ (yearly and each season), organic carbon (winter and autumn), NO_3^- (winter), and $SO_4^=$ (yearly, spring, and summer), with particularly high correlations for NH_4^+ and $SO_4^=$ in summer. Components that co-varied with $PM_{2.5}$ total mass, based on daily detrended data, were NH_4^+, organic carbon, elemental carbon, NO_3^-, $SO_4^=$, and bromine. As a basis for further investigation, we identified a subset of $PM_{2.5}$ components that should be further investigated to determine whether: 1) their daily variation is associated with daily variation of health indicators; and 2) their seasonal and regional patterns can explain the regional and seasonal heterogeneity in PM_{10} and $PM_{2.5}$ health risks.

A2. **Develop and apply statistical methods for multi-site time series studies for estimating short-term effects of PM_{10}, $PM_{2.5}$, and coarse particles (PM_{10}- $PM_{2.5}$) on hospitalization and mortality from the National Medicare Cohort, over the entire year and by season for the largest 300 counties in the USA with PM data available. Identify locations with the largest and smallest short-term effects of PM on the health indicators ("cold and hot spots") to identify locations for a more in-depth PM characterization for biological studies;**

Progress to date:

Multi-site time series studies of PM2.5 and hospital admissions (1999-2005)

As reported in Dominici et al. (2006) we have conducted a multi-site time series study to estimate risks of cardiovascular and respiratory hospital admissions associated with short-term exposure to $PM_{2.5}$ for Medicare enrollees and to explore variation of risks across regions. We assembled a national database comprising daily time-series data for the period 1999-2005 on hospital admission rates for cardiovascular and respiratory diseases and injuries (as a control), ambient $PM_{2.5}$ levels, and temperature and dew-point for 204 US urban counties. Daily hospital admission rates were constructed from the Medicare National Claims History Files (NCHF). Our study population included 11.5 million Medicare enrollees living on average 5.9 miles from a $PM_{2.5}$ monitor. We found that short-term exposure to $PM_{2.5}$ increases hospital admission risks for cardiovascular and respiratory diseases. By linking Medicare, pollutant, and weather data, we

created a national database for continued research that can be updated and analyzed repeatedly to track the health risks of air pollution.

Multi-site time series studies of PM2.5 and hospital admissions, stratified by season and geographical regions (1999-2005)

In Bell et al 2008, we investigated whether short-term effects of fine particulate matter ($PM_{2.5}$) on risk for cardiovascular and respiratory hospitalizations among the elderly vary by region and season in 202 U.S. counties for 1999-2005. We fit three types of time-series models to provide evidence for: (1) consistent PM effects across the year; (2) different PM effects by season; and (3) smoothly varying PM effects throughout the year. We found statistically significant evidence of seasonal and regional variation in PM effect estimates. Respiratory disease effect estimates were highest in winter with a 1.05% (95% posterior interval 0.29, 1.82%) increase in hospitalizations per 10 □g/m^3 increase in same day $PM_{2.5}$. Cardiovascular diseases estimates were also highest in winter with a 1.49% (1.09, 1.89%) increase in hospitalizations per 10 □g/m^3 increase in same day $PM_{2.5}$, with associations also observed in other seasons. The strongest evidence of a relationship between $PM_{2.5}$ and hospitalizations was in the Northeast for both respiratory and cardiovascular diseases. Heterogeneity of $PM_{2.5}$ effects on hospitalizations may reflect seasonal and regional differences in emissions and in particles' chemical constituents. Our results can help guide the development of hypotheses and further epidemiological studies on potential heterogeneity in the toxicity of constituents of the PM mixture.

Multi-site time series studies of Coarse PM (PM10-2.5) and hospital admissions

As reported in Peng et al. (2008) we have estimated the risk of hospital admissions for cardiovascular (CVD) and respiratory diseases (RESP) associated with exposure to $PM_{10-2.5}$, controlling for $PM_{2.5}$. We assembled a database for 108 US counties for the period 1999 to 2005 with a study population of approximately 12 million Medicare enrollees (≥ 65) living on average 9 miles from collocated pairs of PM_{10} and $PM_{2.5}$ monitors. We found a positive and statistically significant association between same-day concentrations of $PM_{10-2.5}$ and CVD admissions. When adjusted by same-day concentrations of $PM_{2.5}$, this association was positive but not statistically significant. The associations between $PM_{10-2.5}$, and RESP admissions, adjusted and unadjusted by $PM_{2.5}$, were positive but not statistically significant. The effect of $PM_{10-2.5}$ on CVD admissions was statistically significantly higher in more urban counties compared to less urban counties. We also found continuing evidence of a positive and statistically significant association between same day concentrations of $PM_{2.5}$ and CVD admissions in the most recent Medicare data.

Rationale for Sampling Strategy for Selecting US Locations for In-depth $PM_{2.5}$ Characterization and PM collection

Underlying the general approach of the Johns Hopkins PM Center was the proposition that results of epidemiological analyses could be used to identify locations having greater and lesser risk to human health associated with PM exposure. The analyses carried out for the National Morbidity, Mortality and Air Pollution Study (NMMAPS) had shown geographic heterogeneity in the effect of PM on mortality and PM characteristics are known to vary across the country. In the PM Center application we proposed to analyze national mortality and Medicare morbidity

data to create a sampling frame for selection of locations for Phase II monitoring and PM collection. We recognize that alternative sampling frames could be used, e.g., based on location or source mix; however, for identification of PM characteristics that could contribute to risk, the health risk-based approach provides the most directly relevant sampling frame. We have completed analyses to identify locations for which we have the greatest confidence that they lie at the higher or lower end of the risk distribution.

Analytic Approach

We use time-series data for the period 1999-2005 and for the same 203 US counties included in the study by Dominici et al. (2006) on $PM_{2.5}$ and hospital admissions in Medicare enrollees. We focus our analyses on lag 0 $PM_{2.5}$ concentrations because relative risk estimates are generally larger at this lag. As health outcomes, we use hospital admissions for all cardiovascular disease combined and hospital admissions for all respiratory diseases combined. Specifically, hospital admissions for cardiovascular diseases includes five cardiovascular outcomes: heart failure (ICD 9, 428), heart rhythm disturbances (426-427); cerebrovascular events (430-438); ischemic heart disease (410-414, 429) and peripheral vascular disease (440-448), Hospital admissions for respiratory diseases includes two respiratory outcomes: chronic obstructive pulmonary disease (COPD) (490-492) and respiratory infections (464-466 and 480-487).

We then

1. fit Bayesian two-stage normal-normal models to estimate short-term effects of $PM_{2.5}$ on hospital admissions for cardiovascular and respiratory hospital admissions outcomes;
2. map the Bayesian estimates of the county-specific relative rates and quantify evidence of spatial heterogeneity
3. use the maps to identify geographical locations where there are larger and smaller estimates of short-term effects of $PM_{2.5}$ on hospital admissions. Specifically:
    a. we group the 203 US counties in five large geographical regions North East (NE), Mid West (MW) ,South East (SE), North West (NW), South West (SW). We anticipate that these five large geographical regions have very different mixtures of $PM_{2.5}$ chemical composition;
    b. within each region, we fit a two-stage Bayesian model to the 203 US counties and we calculate the posterior t-statistics. A posterior t-statistic is defined as the ratio of the county-specific posterior mean divided by the county-specific posterior standard deviations of the relative risk;
    c. within each region and for each outcome, we rank the posterior t-statistics. A location having a posterior t-statistic above the 75-th percentile, consistently across the health outcomes, is identified as a location with a high risk. A location having a posterior t-statistic below the 25-th percentile, consistently across the outcomes, is identified as a location with a low risk.
    d. these locations must have at least one STN site (ESAC recommendation on July 9 2007)

The selected locations are:

- NW: King WA
- NE: Bronx, NY and Allegheny PA
- SW: Sacramento, CA and Maricopa AZ
- SE: Harris, TX and Pinellas, FL
- MW: Jefferson KY and Hennepin, MN

4. Characterize the $PM_{2.5}$ chemical composition of the selected locations

For each location, we calculated the t statistics for the $PM_{2.5}$-associated risk for the outcome, along with a categorization of the relative ranking of the estimate. We identified locations that have generally lower estimates across all outcomes while others have generally higher estimates. Within each of the regions, we identify locations in the strata of lower and higher risk. We plan to use these locations for particle collection and characterization in Project #2 so as to assure representativeness across the country.

At the 2006 SAC meeting we were advised to consider seasonal differences in regional particle composition. We are currently evaluating the logistical aspects of visiting some sites more than once to determine if seasonal factors are an important determinant in terms or risk for health effects. The additional visits would involve a subset of sampling equipment and would include the bulk particle sampling system developed as part of Center work

A3. **Develop and apply statistical methods for multi-site time series studies that take into account exposure measurement error for: 1) estimating short-term effects of $PM_{2.5}$ components on hospitalization and mortality from the National Medicare Cohort; and investigating whether spatial and seasonal variability of the $PM_{2.5}$ components explain spatio-temporal variability of short-term effects of PM_{10}, (PM_{10}- $PM_{2.5}$) and $PM_{2.5}$ on hospitalization and mortality estimated in A.2**

Progress to date:

New methods for adjustment uncertainty

We are developing new methods to account for "adjustment uncertainty" in time series studies of air pollution and health. Specifically in the paper by Crainicenau et al. (2007) we propose a general statistical framework for handling adjustment uncertainty in exposure effect estimation for a large number of confounders, a specific implementation, and associated visualization tools. We also show that when the goal is to estimate an exposure effect accounting for adjustment uncertainty, Bayesian Model Averaging (BMA) can fail to estimate the true exposure effect and over- or under-estimate its variance.

Bayesian hierarchical distributed lag model for estimating the time course of hospitalization risk associated with particulate matter air pollution

In a paper by Peng et al. 2007, we developed new methods for estimating the distributed lag function in time series studies of air pollution and health. We have proposed a Bayesian hierarchical distributed lag model that integrates information from national databases with prior knowledge of the time course of hospitalization risk after an air pollution episode. We have applied the model to a database of particulate matter air pollution monitoring and health information on 6.3 million enrollees of the US Medicare system living in 94 counties covering the years 1999–2002. We have obtained estimates of the distributed lag functions relating fine particulate matter pollution to hospitalizations for both ischemic heart disease and acute exacerbation of chronic obstructive pulmonary disease (COPDAE). We found that the effect of an increase in fine particulate matter on ischemic heart disease is immediate, with the bulk of hospitalizations occurring within 2 days of an air pollution increase, while for COPDAE the

effect of fine particulate matter appears to be distributed over a week or more.

Estimating trends in the short-term effects of PM_{10} on mortality

In the paper by Dominici et al. 2007**,** we have evaluated change in the short-term effect of airborne particles over a period of increasingly stringent regulation that might have changed the chemical composition and toxicity of the airborne particles. We use updated data and methods of the National Mortality Morbidity Air Pollution Study (NMMAPS) to estimate national average relative rates of the effects of PM_{10} on all-cause, cardiovascular and respiratory mortality, and other-cause mortality for the period 1987-2000. We have estimated national average relative rates of the effects of $PM_{2.5}$ (<2.5 μm) on all-cause mortality for the period 1999-2000. We found strong evidence that lag 1 exposures to PM_{10} and $PM_{2.5}$ continue to be associated with increased mortality. We also found a weak indication that the lag 1 effects of PM_{10} on mortality declined over the period 1987-2000 and that this decline mostly occurred in the eastern U.S. The methodology presented here can be used to track the health effects of air pollution routinely on regional and national scales.

Multi-site time series studies of PM2.5 and hospital admissions, stratified by season and geographical regions (1999-2005)

In Bell et al 2008, we investigated whether short-term effects of fine particulate matter ($PM_{2.5}$) on risk for cardiovascular and respiratory hospitalizations among the elderly vary by region and season in 202 U.S. counties for 1999-2005. We fit three types of time-series models to provide evidence for: (1) consistent PM effects across the year; (2) different PM effects by season; and (3) smoothly varying PM effects throughout the year. We found statistically significant evidence of seasonal and regional variation in PM effect estimates. Respiratory disease effect estimates were highest in winter with a 1.05% (95% posterior interval 0.29, 1.82%) increase in hospitalizations per 10 □g/m^3 increase in same day $PM_{2.5}$. Cardiovascular diseases estimates were also highest in winter with a 1.49% (1.09, 1.89%) increase in hospitalizations per 10 □g/m^3 increase in same day $PM_{2.5}$, with associations also observed in other seasons. The strongest evidence of a relationship between $PM_{2.5}$ and hospitalizations was in the Northeast for both respiratory and cardiovascular diseases. Heterogeneity of $PM_{2.5}$ effects on hospitalizations may reflect seasonal and regional differences in emissions and in particles' chemical constituents. Our results can help guide the development of hypotheses and further epidemiological studies on potential heterogeneity in the toxicity of constituents of the PM mixture.

Multi-site time series studies of PM2.5 chemical components and hospital admissions (1999-2005)

In Peng et al 2008, we estimated the associations between daily levels of $PM_{2.5}$ components and risk of hospital admissions in 119 US urban communities for 12 million Medicare enrollees (aged 65 years or older) using Bayesian hierarchical statistical models. We used a national database comprising daily data for 2000—2006 on hospital admissions rates for cardiovascular and respiratory outcomes, ambient levels of major $PM_{2.5}$ chemical components (sulfate, nitrate, silicon, elemental carbon, organic carbon matter, sodium and ammonium ions) and weather. In multiple-pollutant models, an interquartile range (IQR) increase in elemental carbon was associated with a 0.80 (95% posterior interval [PI], 0.34, 1.27) percent increase in risk of same day cardiovascular admissions, and an IQR increase in organic carbon matter was associated

with a 1.01 (95% PI: 0.04, 1.98) percent increase in risk of respiratory admissions on the same day. Ambient levels of elemental carbon and organic carbon matter, which are generated primarily from vehicle emissions, diesel, and wood burning, were associated with the largest risks of emergency hospitalization across the major chemical constituents of fine particles.

B. Cohort studies based on the National Medicare Cohort for estimating longer-term effects of PM and PM composition in susceptible populations and for cause-specific health outcomes (Phase II)

B1 **Develop statistical methods for cohort studies for estimating associations between longer-term exposure to PM_{10}, $PM_{2.5}$, PM_{10}, $PM_{2.5}$, and $PM_{2.5}$ components and hospitalization and mortality adjusted by individual-level and area-level confounders;**

Progress to date

Diagnosing confounding bias in studies of chronic effects of air pollution on health
In the paper by Janes H et al. 2007 we have proposed a new method for diagnosing confounding bias under a model that estimates associations between a spatially and temporally varying exposure and health outcome. Specifically, we have decomposed the association into orthogonal components, corresponding to distinct spatial and temporal scales of variation. If the model fully controls for confounding, the exposure effect estimates should be equal at the different temporal and spatial scales. We have shown that the overall exposure effect estimate is a weighted average of the scale-specific exposure effect estimates.

Using approach, we have estimated the association between monthly averages of fine particles ($PM_{2.5}$) over the preceding 12 months and monthly mortality rates in 113 US counties from 2000 to 2002. We have decomposed the association between $PM_{2.5}$ and mortality into 2 components: (1) the association between "national trends" in $PM_{2.5}$ and mortality; and (2) the association between "local trends," defined as county-specific deviations from national trends. This second component provides evidence as to whether counties having steeper declines in $PM_{2.5}$ also have steeper declines in mortality relative to their national trends.

We have found that the exposure effect estimates are different at these 2 spatiotemporal scales, which raises concerns about confounding bias. We conclude that the association between trends in $PM_{2.5}$ and mortality at the national scale is more likely to be confounded than is the association between trends in $PM_{2.5}$ and mortality at the local scale. If the association at the national scale is set aside, there is little evidence of an association between 12-month exposure to $PM_{2.5}$ and mortality.

Fine Particulate Matter and Mortality: A Comparison of the Six Cities and American Cancer Society Cohorts with a Medicare Cohort
In the paper by Eftim et al. 2008, using Medicare data, we have assessed the association of $PM_{2.5}$ with mortality for the same locations included in these studies. We have estimated the

chronic effects of $PM_{2.5}$ on mortality for the period 2000-2002 using mortality data for cohorts of Medicare participants and average $PM_{2.5}$ levels from monitors in the same counties included in the SCS and the ACS. Using Medicare data, which lack information on some potential confounding factors, we estimated risks similar to those in the SCS and ACS, which incorporated more extensive information on individual-level confounders. We propose that the Medicare files can be used to construct cohorts for tracking the longer-term risk of air pollution over time.

Methods development for estimation of the long-term effects of $PM_{2.5}$ on mortality and morbidity outcomes is in progress. The challenge of adequately adjusting for individual-level and area-level confounders needs to be addressed.

Mortality in the Medicare Population and Chronic Exposure to Fine Particulate Air Pollution in Urban Centers (2000-2005)

In Zeger et al (2008) we estimated the relative risk of death in a U.S. population of elderly associated with long-term exposure to $PM_{2.5}$ by region and age-groups, for the period 2000-2005. By linking fine particulate matter ($PM_{2.5}$) monitoring data to the Medicare billing claims by zip code of residence of the enrollees, we have developed a new retrospective cohort study, the Medicare Cohort Air Pollution Cohort Study. The study population comprises 13.2 million participants living in 4,568 zip codes having centroids within 6 miles of a $PM_{2.5}$ monitor. Relative risks adjusted by socioeconomic status and smoking were estimated by fitting log-linear regression models. In the East and Central regions, a 10 $\mu g/m^3$ increase in six year average of $PM_{2.5}$ is associated with a 6.8% (95% CI: 4.9 to 8.7%) and 13.2% (95% CI: 9.5 to 16.9) increases in mortality, respectively. We did not find evidence of an association in the West and for persons above 85 years of age. Averaged over all the regions and age groups, the relative risk estimate is 2.5% (95% CI: 1.2 to 3.7%). Caution is warranted in relying upon an overall estimate because we found geographic heterogeneity in the effect of $PM_{2.5}$ across the United States.

Publications/Presentations:

Bell M, Peng R, Dominici F (2006) The Exposure-Response Curve for Ozone and Risk of Mortality and the Adequacy of Current Ozone Regulations, *Environmental Health Perspectives,*114: 532-536.

Bell M, Dominici F, Ebisu K, Zeger SL, Samet JM (2007) Spatial and Temporal Variation in $PM_{2.5}$ Chemical Composition in the United States for Health Effects Studies (2000-2005). *Environmental Health Perspectives,*115,989–995.

Bell M, Kim JY, Dominici F. (2007) Potential confounding of particulate matter on the short-term association between ozone and mortality in multi-site time series studies. *Environmental Health Perspectives,*115,1591-5.

Bell M. Ebisu K. Peng R.D. Walker J. Samet J.M. Zeger .S.L. Dominici F**.** Seasonal and regional short-term effects of fine particles on hospital admissions in 202 U.S. counties, 1999-2005, *American Journal of Epidemiology* (in press)

Dominici F**,** Peng D, Bell M, Pham M, McDermott A, Zeger SL, Samet JM (2006) Fine Particulates Air Pollution and Hospital Admission for Cardiovascular and Respiratory Diseases, J*ournal of the American Medical Association*, March 8, 295:1127-1135.

Dominici F, Peng RD, Zeger SL, White RH, Samet JM (2007) Particulate Air Pollution and Mortality in the United States: Have the Risks Changed from 1987 to 2000? *American Journal of Epidemiology,* 166,880-888, *(*with discussion).

Dominici F**,** Peng RD, Zeger SL, White RH, Samet JM (2007). Respond to ''Heterogeneity of Particulate Matter Health Risks'' *American Journal of Epidemiology,* 166,892-893.

Bell M, Dominici F (2008) Effects Modification by Community Characteristics on the Short-Term Effects of Ozone on Mortality in 98 U.S. Communities, *American Journal of Epidemiology,* 167, 986 – 997

Crainiceanu CM**,** Dominici F, Parmigiani G. Adjustment Uncertainty in Effect Estimation, *Biometrika (*in press*).*

Dominici F, Peng R, Ebisu K, Zeger SL, Samet JM, Bell M. (2007) Does the effect of PM_{10} on mortality depend on PM nickel and vanadium content? A re-analysis of the NMMAPS data, *Environmental Health Perspectives,*115,1701-3.

Dominici F. Wang C. Crainiceanu CM, Parmigiani G. (2008) Model Selection and Health Effect Estimation in Environmental Epidemiology, *Epidemiology,* 19, 558-560.

Eftim S, Janes H, McDermott A, Samet JM, Dominici F**.** (2008**)** A Comparative Analysis of the Chronic Effects of Fine Particulate Matter, *Epidemiology,*19, 209-216.

Janes H, Dominici F**,** Zeger SL (2007) Trends in Particulate Matter and Mortality in 113 U.S. Counties, 2000-2002: Evidence on the Long Term Effects of Air Pollution. *Epidemiology,* 18, 416-423 (with discussion).

Janes H, Dominici F**,** Zeger SL (2007) Partitioning Evidence of Association Between Air Pollution and Mortality. *Epidemiology*. 18, 427-428.

Peng R, Dominici F**,** Zeger SL (2006) Reproducible Epidemiological Research, *American Journal of Epidemiology,* 163: 783-789.

Peng R, Chang H, Bell M, McDermott A, Zeger SL, Samet JM, Dominici F. Coarse Particulate Matter Air Pollution and Hospital Admissions for Cardiovascular and Respiratory Diseases Among Medicare Patients, *Journal of American Medical Association* 299, 2172-2179.

Peng R, Dominici F**.**,Welty L. A Bayesian Hierarchical Distributed Lag Model for Constrained Distributed Lag Functions: Estimating the Time Course of Hospitalization Associated with Air Pollution Exposure, *Journal of the Royal Statistical Society, Series C* (in press).

Thomas D, Jerrett M, Kuenzli N, Louis T, Dominici F**,** Zeger SL, Schwartz J, Burnett R, Krewski D, Bates D (2007) Bayesian Model Averaging in Time-Series Studies of Air Pollution and Mortality, *Journal of Toxicology and Environmental Health*, Part A, 70:1-5.

Welty L, Zeger SL, Dominici F. Bayesian Distributed Lag Models: Estimating Effects of Particulate Matter Air Pollution on Daily Mortality, *Biometrics* (in press).

Zeger SL**,** Dominici F, McDermott A, Samet JM Mortality in the Medicare Population and Chronic Exposure to Fine Particulate Matter, *Environmental Health Perspectives* (in press).

Presentations:

The American Thoracic Society International Annual Meeting, May 2006, San Diego CA, " Fine Particulate Air Pollution and Hospital Admissions for Cardiovascular and Respiratory Diseases."

The Air and Waste Management Association Annual Meeting, June 2006, New Orleans TN, " The Shape of the Exposure Response Curve in PM and Mortality"

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Relevant Web Sites

www.jhsph.edu/particulate_matter

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Center Director: Jonathan M. Samet
Project Title: PM Characterization and Exposure Assessment
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EPA Project Officers: Stacey Katz and Gail Robarge
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Period Covered by the Report: August 1, 2007 – July 31, 2008

Objective(s) of the Research Project:
The focus of Project 2 is the measurement of specific chemical components and physical characteristics of PM from different areas of the country in support of the Center focus, which is assessing characteristics of PM that determine toxicity. The goals of Project 2 are to collect bulk PM samples for use in biological assays and for detailed characterization including mass, inorganic ions, elemental carbon and organic compounds, specifically polycyclic aromatic hydrocarbons , elemental metals and their oxides, and sulfur isotope ratios. We will also collect information on the distribution of particle size. The objectives of Project 2 include: 1) the development of methods for collecting bulk ambient PM, and a system for characterizing the chemical and physical properties of ambient PM; 2) the identification of specific regional differences in PM characteristics that may contribute to differential biological responses demonstrated by *in vitro* and *in vivo* bioassay systems; 3) the assessment of the relationship between human exposure to $PM_{2.5}$ and biological response during high $PM_{2.5}$ exposure period and low $PM_{2.5}$ exposure periods. The goals and objectives have remained unchanged over this time period.

Progress Summary/Accomplishments:

I. Introduction

The rationale for this project is based on the conclusion that "[t]*he diversity of PM characteristics and the array of possible health effects define a potentially large and complex matrix for investigation; in fact different features of particles might be relevant to different health outcomes" (NRC 2004)* As a result we proposed to assess the specific chemical components and physical characteristics of particulate matter (PM) from samples taken in different areas of the country. These locations have been selected based on a gradient of estimated risks to health. Specifically, we proposed to develop a new method for collecting bulk PM for use in biological assays; to develop a portable system for the characterization of chemical

and physical properties of ambient PM; and to identify specific regional differences in PM characteristics that may contribute to differential biological responses in *in vitro* and *in vivo* bioassay systems. This report will describe activities carried out by Project 2 (PI.Patrick Breysse) and the PM Characterization, Sampling and Analysis Core (PI Alison Geyh) since funding, giving emphasis to progress during the past year. The overall goal of developing a PM monitoring and collection approach and then deploying it at the sites identified through the Project 1 analyses has been accomplished.

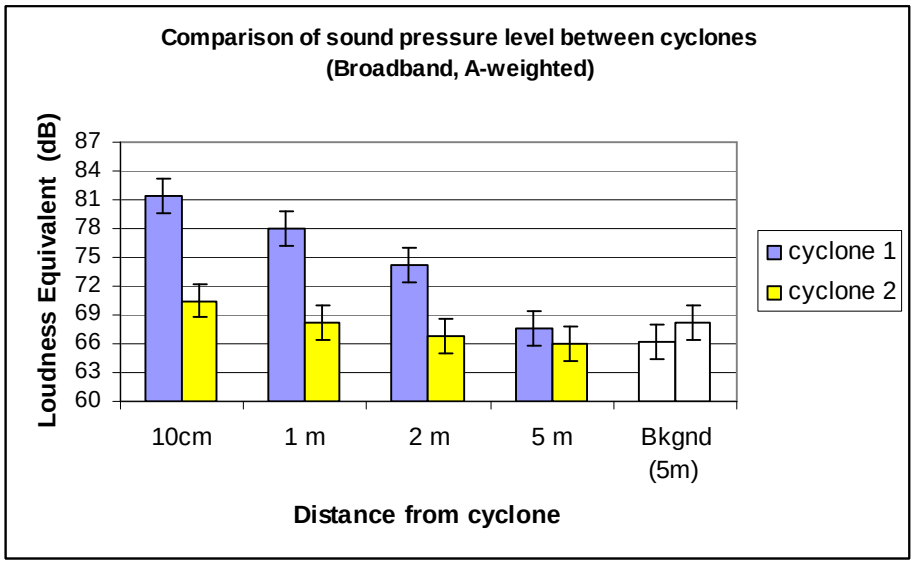
II. Protocol development and testing of the Hopkins Sequential Cyclone Sampler (HSCS)

As reported previously, the cyclone system has been re-designed over the first two years to include a commercially available PM-10 inlet instead of a cyclone. The second stage cyclone was retained, and designed to collect inhalable coarse particles (<10 μm and >2.5 μm). When tested with a challenge aerosol, a D_{50} cut size of 2.33 μm was obtained at a flow rate of 1000 L/min. The third stage is a commercially available cyclone with a D_{50} cut-size of 0.3 μm tested at the same flow rate for collection of PM <2.5 μm and >0.3 μm.

During the past year, Project 2 has been working with a commercial manufacturing company, Hi-Q Environmental Products Company, to produce the systems that have now been deployed in the field. There have been significant delays in the manufacturing process of the first two complete systems, which resulted in delays in the testing the systems in Baltimore and their ultimate deployment to the field.

Protocols and procedures for leak testing the system and flow calibration were developed. After deployment in Seattle and Sacramento, temperature and noise reduction strategies were required before sampling in Phoenix due to the unique characteristics of the Phoenix site, which was located in a residential area – less than 15 feet away from neighboring houses – and where temperatures can reach 120 ºF during summer. After completing the field effort in Sacramento both HSCS systems were shipped to Baltimore to perform these tests. To achieve noise reduction, the inside of the HSCS cabinet was lined with a sound dampening material; sound pressure level testing showed a significant reduction when standing 10 cm in front of the HSCS with the door closed (Figure 1)

Figure 1



To reduce cabinet temperature, two strategies were tested: an insulating material was installed inside of the cabinet; and a reflective material was wrapped around the outside of the cabinet. Neither resulted in a significant temperature decrease inside the cabinet, and the insulating material was suspected of retaining heat generated by the pump. Therefore, none of the temperature control strategies was implemented. Continuous monitoring inside the cabinets, both in Baltimore and Phoenix, showed temperatures of 130 F or lower, well below the 200 F maximum recommended by the manufacturer.

While noise and temperature reduction were being tested in Baltimore, the system ran for 3 weeks in order to collect Baltimore bulk PM.

A manuscript describing the characterization and testing of the sequential cyclone system has been prepared, as well as a Report of Invention (ROI) entitled "Hopkins Sequential Cyclone System for the Collection of Bulk Particulate Matter" to Johns Hopkins Technology Transfer (JHTT). A provisional application for a patent was filed on March 6, 2008.

III. PM Monitoring

PM Monitoring and locations: Monitoring locations identified by Project 1 are found in Table 1. We plan to monitor the nine sampling sites. Given the timeline and the budgeted resources. we anticipate that we will be able to complete monitoring for at least 7 out of the nine sampling locations with Allegheny PA included. Beginning April, 2007 the Project 2 team began the process of contacting state and county environmental agencies to discuss potential monitoring locations and to beginning scheduling monitoring dates. To date, agencies in the following five counties/states have been contacted: King County WA, Sacramento CA. Maricopa AZ, Hennepin MN, and Harris TX. Contact was facilitated through a letter sent by EPA to the state and local air quality agencies introducing the project and stating EPA support. To date monitoring has been conducted in three counties with a fourth scheduled for September 2009.

King County, WA: Monitoring in King County, WA was carried out from October 25 to December 1, 2007. The location within King County was an air quality monitoring site maintained by the Department of Ecology Air Quality Program (John Williamson and Doug Knowlton). It is located on Beacon Hill in Seattle, WA, a centrally located hilltop surrounded by urban development that was identified by the local agencies as representative of urban PM in the county. In addition, the monitoring station at this location was large enough to house and had adequate power to run the equipment listed in Table 2.

Sacramento County, CA: Monitoring in Sacramento County was carried out January 12 – March 13, 2008. Sacramento Metropolitan Air Quality Management District (SMAQMD -John Ching and Ken Lashbrook) identified one site in the City of Folsom that was adequately powered and had enough space to house Project 2 equipment. The City of Folsom is approximately 20 miles east of Sacramento and is impacted by air quality from the City of Sacramento. The monitoring site was located behind City Hall away from busy roads and freeways. SMAQMD required proof against legal liability in the form of a Hold Harmless agreement before monitoring could

be conducted. Negotiation between the University and SMAQMD required several months and were finalized January 2008.

Maricopa County, AZ: Monitoring in Maricopa County was carried out June 1 – July 20, 2008. Maricopa County Air Quality Department (MCAQD –Ben Davis) and the Arizona Department of Environmental Quality (AZDEQ – Raymond Redman) were both responsible for helping us to identify an appropriate monitoring site. On December 21, 2008 Drs. Ana Rule and Alison Geyh traveled to Phoenix to explore potential monitoring locations. The site selected was located in a residential neighborhood not directly impacted by busy road, freeways or other specific sources. The site was a residential housing lot, which contained one small trailer housing MCAQD equipment. The MCAQD provided a second trailer with power specifically to house Project 2 equipment.

Hennepin County, MN: Contacts with the MN Pollution Control Agency (MNPCA) (Rick Strassman) began April 2008. At the end of May 2008, Dr. Patrick Breysse traveled to Minneapolis to explore potential monitoring locations. Dr. Breysse could not identify any possible monitoring locations within Hennepin County, as all sites in this county are roof top and support only one or two instruments. Two options were offered. The first option was a trailer that could be provided by MNPCA in a park in Hennepin County. Project 2 would be responsible for providing power. The cost to provide power was estimated at between $10,000 and $15,000, and to take several weeks or months to establish. The second option was a monitoring site located at a small airport catering to private aircraft in a county adjacent to Hennepin, Anoka County. The airport has an average of 2-3 small aircraft per day. The site is 4 miles north of the Hennepin border, has adequate power and space to house Project 2 equipment and is available immediately. An analysis of the correlation between $PM_{2.5}$ concentrations measured at all locations within and surrounding Hennepin County showed the correlation to be 0.92. After discussions with Project 1 and the Center PI, Project 2 has accepted the offer of the site at the Anoka airport. We anticipate monitoring will begin at this location the middle of September 2008.

Harris County TX: Preliminary discussions have begun with the Texas Commission of Environmental Quality (Kristin Bourdon). We have been offered space at the Deer Park monitoring station which is the STN site in Houston and anticipate the beginning of the field effort for February 2009.

Table 1 summarizes the status of the field monitoring effort.

Table 1. Monitoring Schedule

Order	**County**	**Start Date**	**End Date**
1	King, WA*	October 25, 2007	December 1, 2007
2	Sacramento CA*	January 12, 2008*	March 13, 2008
3	Maricopa AZ*	June 1, 2008	July 20, 2008
4	Hennepin MN**	September 15, 2008	October 30, 2008
5	Harris TX	February 1, 2008	April 30, 2008
6	Allegheny PA	June 1, 2009	July 15, 2009
7	Jefferson KY	TBD	TBD
8	Kings NY	TBD	TBD
9	Pinellas FL	TBD	TBD

* Completed
**Scheduled
TBD – to be decided

Establishing a site: At each location, the equipment listed in Table 2 is deployed. This set of instruments was prepared and tested for field use during the first two years and the feasibility of using a van for deployment was assessed, leading to the present approach of moving the equipment from site to site. To establish the site, a team of three people spend between 5 – 7 10-hour days setting up equipment, building inlets and calibrating instruments. Once established, the site is managed by one member of Project 2 who visits the site each day to conduct a daily site check or a weekly data download. At the end of the monitoring period, two members of Project 2 dismantle the site and ship the equipmentto the next location.

Table 2. Summary of PM Sampling

Continuous PM Monitoring Instruments			Integrated PM Sampling Instruments			
Type	Analyte (units)	Sampling Frequency	Type	Analyte	Collection Frequency	No. samples per sampling period
TSI Aerosol Particle Sizer	<0.5 - 20 um (particle counts/cm^3)	every 15 min	Hopkins Sequential Cyclone System	coarse PM	integrated over entire site monitoring period	1
				fine PM	integrated over entire site monitoring period	1
TSI Scanning Mobility Particle Analyzer	17.9 - 881.7 nm (particle counts/cm^3)	every 15 min	Harvard Impactor (Telfon filters)	PM_{10}	7 days	2
				$PM_{2.5}$	7 days	2
Echochem PAS2000	un-differentiated particle-bound PAHs (ng/m^3)	every 5 min	PMASS (quartz fiber filters)	PM2.5	7 days	4
Magee Scientific Aethalometer	black carbon (ng/m^3)	every 5 min				
Thermo Environmental SPA 5020	particulate sulfate (ug/m^3)	every 15 min				

Yields for bulk PM collected from deployment of the HSCS are reported in Table 3.

Table 3. Bulk PM mass results

Site	Dates	Coarse PM (mg)	Fine (mg)
Seattle	Nov 2007	89	705

Sacramento	Feb / Mar 2008	111	397
Baltimore	Apr / May 2008	325	1551
Phoenix	Jun / Jul 2008	1181	1510

IV. Data Management

Data sets reporting concentration data from the continuous instruments listed in Table 2 are currently being processed by two member of Project 2. Data management activities are summarized below.

Data storage: Downloaded data are stored on a network drive at JHU-SPH server. Once the data are stored in the network drive, the files are not altered. The files are also backup onto another server, and to a DVD.

Data management procedure and documentation: Data cleaning and processing have begun on data sets from King and Sacramento Counties. Weekly raw data files for each instrument are manually reviewed for completeness. The weekly raw data files are imported into EXCEL. Any identified problems are highlighted and logged. The weekly files for each instrument are collapsed into a composite data set for the entire monitoring period. Flagging codes are being developed which will reflect common problems across all instruments and specific problems unique to each instrument. An additional level of quality assurance is provided by culling and validating the raw data based on daily operating status as recorded in the monitoring site log book and daily checklists. Raw data in the EXCEL files are uploaded into SAS for further cleaning. In this stage, observations predating the official start-up time for the specific monitoring site are removed from database. Repetitive observations, such as two or more identical observations for each time point, are flagged. Additionally, negative observations, possible outliers (> 99^{th} percentiles), and missing observations are flagged. These flags are summarized into an EXCEL file. All data management procedures are documented in a separate WORD file.

V. PM Characterization

Table 3 presents the analysis plan for the integrated PM samples collected at each location. Sample analysis has begun. All samples have been evaluated for mass and a mass concentration determined from samples collected by the Harvard Impactor and PMASS. Protocol development has begun for the analysis of soluble and insoluble metals from the same sample in the Geyh laboratory at JHSPH. Bulk coarse and fine PM from King County, Sacramento County and Baltimore, as well as $PM_{2.5}$ samples collected on quartz filters from Sacramento, have been delivered to the laboratory of Dr. Steven Chillrud for Pt-group elements and PAH characterization (see below).

Table 3. Sample Analysis Plan

Type	Mass	Inorganic Ions	Soluble Metals	Insoluble Metals	Elemental carbon	Pt-Group Elements	PAHs	Oxidation States
HI - PM10	✓#	✓#	✓#	✓#		✓		

HI - PM2.5	✓#	✓#	✓#	✓#		✓		
PMASS - PM2.5					✓		✓	
PM Bulk Coarse	✓	✓	✓	✓	✓	✓	✓	✓
PM Bulk Fine	✓	✓	✓	✓	✓	✓	✓	✓

Pt - Group Element and PAH Analysis (Work conducted at LDEO-CU: Steven Chillrud, James Ross and Beizhan Yan)
In the past year, we have purchased an isotopically enriched platinum spike from Oak Ridge National Laboratory, prepared it, and verified that it agrees with our other Pt standard (High Purity Standards, Charleston SC). We have used high resolution (M/delta M ~ 4000) to determine that the optimal isotopes for Rh and Pd (respectively 103 and 105) were not interfered with by polyatomic species. And we have carried out multiple digests of 5 mg of reference material BCR-723 (tunnel dust), using the microwave/aqua regia/cation exchange method developed previously, to verify that we can measure Rh and Pd in this low sample mass. In the past few months we have encountered a recurring problem with the Pt blank. At present we are trouble-shooting our procedure in order to identify the source of the problem. Once this problem is resolved, we will be ready to determine PGE's in bulk and filter samples collected at each location.

Dr. Beizhan Yan, an organic geochemist specializing in the analysis of trace organic compounds including PAHs and their compound-specific stable isotope ratios, has joined Dr. Chillrud's group starting September 2007. Dr. Yan is responsible for the analysis and interpretation of results for PAHs within the bulk and filter based PM samples. Dr. Yan is also interested in developing new methods for resolving specific compounds within the "unresolved complex mixture" of hydrocarbons related to fuel combustion. Dr. Yan has begun analysis for PAHs of the bulk and $PM_{2.5}$ quartz filter samples.

Oxidation States and Coordination Chemistry (Work conducted at the Brookhaven National Lab (BNL): Saugata Datta (GCSU) and Steve Chillrud (LDEO))
During this last year, the BNL has awarded Project 2 investigators three additional time slots on the National Synchrotron Light Source: November 2007 (5 days Beamline X11A), March 2008 (4 days; Beamline X23A), July 2008 (5 days; Beamline X23B). The purpose of the November visit was to establish the best media for sample support. Previous experiments had suggested the quartz filters would be ideal. In response $PM_{2.5}$ samples collected on quartz filters were included in the sample collection field protocol. Filters loaded with a PM mass estimated to be reflective of the mass loading for actual samples were evaluated. Filters were analyzed singly and in stacks to increase the total mass presented to the beam. The mass on a single filter was found to be too low for detection of the elements of interest (Fe, V, Mn, Cr, Ni). Significant interference was found from the quartz itself when the filters were stacked. A new sample support method was developed. The new method is a polyethylene frame designed to the dimensions of the beam. The new holder was developed to minimize the mass need for analysis of the bulk PM. The new sample holder was evaluated during the March 2008 visit using SRM 1648 and BCR

723. In addition, bulk PM from Baltimore as analyzed for Fe and Ni oxidation states. Baltimore samples were also analyzed to evaluate the impact of different in storage conditions on oxidations states (Air/RT vs. Argon/ 4 C). During the July 2008 visit coarse and fine bulk PM from King, Sacramento, and Maricopa Counties and Baltimore were evaluated for Ni, V, Mn, Cr, and Fe.

Delivery of bulk PM to Project 3. Bulk fine PM from King county, Sacramento county and Baltimore has been delivered to Project 3.

Publications/Presentations: Ana M. Rule *; Geyh AS; Ramos-Bonilla JP; Mihalic JN, Margulies JD; Kesavan; Breysse PN. "Design and Characterization of a Sequential Cyclone System for the Collection of Bulk Particulate Matter" submitted to J. Aerosol Science April 2008 (rejected). The paper was accepted for presentation AAAR Annual Conference October 20-24, 2008

Saugata Datta, Hartmann J., Protus T., Mihalic J., Rule A., Ramos-Bonilla J., Geyh A. and Chillrud SN. "New sampler holder for mass limited samples for speciation studies of Fe, Mn, Ni by XANES" submitted for presentation AAAR Annual Conference October 20-24, 2008

Report of Invention (ROI) entitled "Hopkins Sequential Cyclone System for the Collection of Bulk Particulate Matter" to Johns Hopkins Technology Transfer (JHTT)

- Patent application provisional application filed on March 6, 2008

Supplemental Keywords: Bulk particle collection, cyclone, exhaled breath condensate, markers of inflammation

Relevant Web Sites: www.jhsph.edu/particulate_matter

Date of Report: July 15, 2008, 2008
EPA Grant Number: **GAD No. 832417-010**
Center: Johns Hopkins Particulate Matter Research Center
Center Director: Jonathan M. Samet
Project Title: **B**iological Assessment of the Toxicity of PM and PM Components
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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:
Exposure to particulate matter (PM) is currently associated with development of various respiratory diseases such as lung cancer, COPD, and asthma. Hallmarks of asthma include airflow obstruction, bronchial hyper-responsiveness, and airway remodeling. Particulate matter less than 2.5 µm in diameter ($PM_{2.5}$) is derived mainly from industrial heating as well as the combustion of vehicle fuels and is considered to have clinical relevance since it deposits in the respiratory bronchioles of the lungs. $PM_{2.5}$ has been associated with premature mortality. Recent studies suggest an association between acute exposure to PM and daily mortality and morbidity, which was strongest for respiratory- and cardiovascular-related hospital admissions and cause of death in susceptible individuals. The specific objectives to be completed across the three phases of this **Project** are: 1.To characterize secretion of inflammatory cytokines/chemokines in human bronchial epithelial cells induced by PM; 2. To characterize airway inflammation in murine models of lung inflammation induced by bioavailable PMs; 3. To evaluate the role of ROS in PM-induced *in vitro* and *in vivo* airway inflammation and toxicity; 4. To link in vitro and in vivo gene expression patterns induced by PM with morbidity and mortality rates of the city where the sample was collected; 5. To link fluctuations in ambient bioavailable PM levels with relevant biomarkers (cytokines, epithelial/endothelial activation, peripheral blood mononuclear cell gene expression, exhaled breath condensates) in a panel of PM exposed human subjects; 6. To characterize signaling mechanisms of PM-induced secretion of inflammatory cytokines/chemokines and ROS burden in human bronchial epithelial cells.

Progress Summary/Accomplishments:

A. Project #3: Biological Assessment of Toxicity of PM and PM Components

Rationale. Despite numerous epidemiologic studies pointing to diverse adverse health effects of exposure to urban airborne particulate matter (PM), the physical and chemical characteristics of PM

that contribute to cardiopulmonary toxicity and dysfunction remain poorly understood. Further, relatively little is known regarding the molecular mechanism(s) of PM-induced airway inflammation and cardiovascular dysfunction, processes considered to play a critical role in cardiopulmonary morbidity and mortality. Elaboration of reactive oxygen species (ROS) and secretion of pro-inflammatory cytokines from airway epithelium exposed to urban PM may be involved not only in airway inflammation, but also in PM-mediated toxicity to cardiac tissue, distant from the lung. Project #3 studies are encompassed within 3 phases. *In vitro* and *in vivo* Phase I studies have been initiated to establish the models that will be used in carrying out bioassays with specimens collected in the various cities throughout the United States. In Phase I, in developing the models, emphasis has been placed on PM collected by cyclone-generated (single stage) extraction method for bulk PM collection from the roof of the School of Public Health (April-June 2005), yielding a PM sample in the size range of 0.1 to 10 microns (provided by Project #2 investigators: Drs. Patrick Breysse and Alison Geyh). Phase I studies have included both PM-induced changes in lung and cardiac tissue gene expression using the Baltimore PM. Several manuscripts are in preparation. Similar studies using PM derived from specific US cities will be carried out subsequently under Phase II (murine asthma) and Phase III (murine dilated cardiomyopathy). As noted in the prior review of Project #3 studies, future studies will not be emphasizing *in vitro* approaches, which were evaluated in Phase I.

B. Phase I: *In vitro* Toxicity Assessment of Baltimore PM.

Overview and summary: These studies have utilized **human bronchial epithelium** and **human lung endothelium** with evaluation of cytokine secretion (GM-CSF, IL-6, IL-8, and IL-1β), generation of ROS, such as H_2O_2 and superoxide, and signaling mechanisms regulating cytokine/ROS production, cytotoxicity, and vascular/epithelial permeability. These *in vitro* effects of Baltimore PM on lung cell function evidence a "pro-inflammatory lung cell phenotype" with increases in epithelial and endothelial permeability in PM fraction-specific pathways. PM also induces elaboration of ROS, effect which is partially reversed by the anti-oxidant N-acetyl-L-cysteine (NAC). Reflective of the prior SAC review of Project #3, future studies will focus on in vivo animal models with less emphazise on *in vitro* approaches.

Effects of Baltimore PM on human bronchial epithelium: Regulation of COX-2 Expression and IL-6 Release by Particulate Matter in Airway Epithelial Cells

Treatment of HBEpCs with Baltimore PM induced ROS production, COX-2 expression and IL-6 release. Pretreatment with N-acetylcysteine (NAC) or EUK-134, in a dose-dependent manner, attenuated PM-induced ROS production, COX-2 expression and IL-6 release. The PM-induced ROS was significantly of mitochondrial origin as evidenced by increased oxidation of the mitochondrially targeted hydroethidine to hydroxyethidium by reaction with superoxide. Exposure of HBEpCs to PM stimulated phosphorylation of NF-κB and C/EBPβ, while the NF-κB inhibitor, Bay11-7082, or C/EBPβ siRNA attenuated PM-induced COX-2 expression and IL-6 release. Furthermore, NAC or EUK-134 attenuated PM-induced activation of NF-κB; however, NAC or EUK-134 had no effect on phosphorylation of C/EBPβ. Additionally, inhibition of COX-2 partly attenuated PM-induced PGE2 and IL-6 release.

- **Effect of Baltimore PM on human lung endothelium:** We determined that PM decreases trans-endothelial electrical resistance (TER), a reflection of loss of vascular integrity in dose-dependent and time-dependent fashion. Water–soluble PM supernatants, in contrast, enhance endothelial cell barrier function whereas the water–insoluble PM pellet produces endothelial cell barrier dysfunction. The presence of NAC partially reverses the PM effect on permeability and

barrier dysfunction and induces stress fiber formation, a finding consistent with increased permeability. This particulate endothelial cell biology study of PM toxicity elucidated that PM disrupts EC barrier via an ROS-p38 MAPK-HSP27 signaling pathway. These results partially explained acute inflammatory pulmonary injury induced by PM via the induction of vascular leakage of protein into BAL.

C. Phase I: *In vivo* Effects of PM exposure in a Murine Model of Asthma

Overview and summary: We have developed and characterized an experimental model of murine asthma induced by ovalbumin (OVA) in the asthma-susceptible AJ mouse strain in order to evaluate PM effects. Briefly, 10-12 week old AJ mice received OVA (0.4 mg/kg i.p, day 0) followed by an intratracheal OVA challenge (30 mg/kg, day 14). Then PM (20 mg/kg) was administered through an intratracheal aspiration three days after OVA challenge. After 1 day, 4 days or 7 days post PM exposure, airway hyperresponsiveness (AHR) was determined via acetylcholine (1 mg/kg) intravenous injection through the inferior vena cava and animals were sacrificed for BAL extraction and tissue harvesting. As noted below, these studies highlight the interaction between PM and lung inflammatory responses in the sensitized mouse--interactions which result in enhancement of airway hyperresponsiveness. In this study, we have used a high dose of PM; however, instillation of lower doses of PM (0.01 to 1.0 mg/kg body weight) is planned in all the future studies with fine particles from different locations (Project 2). These lower doses are more comparable to exposure of humans to ambient PM. Key findings are highlighted below:

- **Baltimore PM induces AHR.** Reactivity of the airways was determined by the response to endogenous bronchoconstrictors, such as acetylcholine. Airway pressure change stimulated by exogenous infused acetylcholine was measured to represent airway responses. OVA challenge increased AHR. PM induced significant increases in AHR in both control AJ mice and asthmatic OVA challenged mice.

- **Baltimore PM induces protein leakage into airway.** BAL protein level increase indicates vascular leakage and is a key parameter of inflammatory lung injury. PM, not OVA, increased protein levels in BAL an indication of disruption of epithelial/endothelial barriers.

- **Baltimore PM induces inflammatory leukocyte infiltration into the airways.** PM induced inflammatory leukocyte infiltration into the alveolar and airway in both PBS control AJ mice and OVA challenged asthma mice. OVA challenge induced eosinophil and macrophage increases in BAL. Baltimore PM induced eosinophil and neutrophil infiltration in BAL.

- **Baltimore PM induces Th1 and Th2 type cytokines in BAL.** OVA challenge induced TH2 cytokine IL-4 and IL-5 secretion into BAL. PM induced not only TH2 cytokine IL-4 and IL-5 in asthma mice, and TH1 cytokine IL-6, IFN-γ and TNF-α in BAL.

- **Baltimore PM induces mucus production in murine airways.** PM and OVA challenge induced mucus-producing goblet cell generation in mice (pink PAS stained epithelial cells). PM and OVA challenge synergistically induced positive-stained goblet cells at day 4 post PM challenge.

- **Baltimore PM induces gene transcription signaling in murine asthmatic lung .** PM had a strong impact on the global expression of lung genes. 436 genes survived filtering and were

identified as significantly dysregulated by PM exposure (375 genes upregulated and 61 gene downregulated). In contrast, OVA-challenge had less impact on lung gene expression than PM at day 4 post exposure. Only 37 genes (21 genes upregulated and 16 gene downregulated) were differentially regulated by OVA sensitization even when less stringent criteria (FDR <5% and fold change >2 fold) were applied. The combination of PM and OVA treatment exhibited synergistic effects on lung gene expression, with a total of 591 genes identified as differentially regulated (492 genes upregulated and 99 genes downregulated). The PM-regulated genes were related to 22 biological processes, including innate immune response, chemotaxis, cell surface receptor linked signal transduction, inflammatory response, defense response, cell cycle, nervous system development, and DNA-dependent regulation of transcription. Similar to GO analysis, cell cycle, inflammatory response (interleukin signaling, interferon signaling) and cell surface receptor (B-cell receptor, T-cell receptor and Toll-like receptor) pathways were among the most distinctly regulated pathways. The majority of these signaling pathways are closely related to asthma development. For example, the genes in the complement system were significantly regulated by PM in both the control (PM group) and the asthma animals (PM & OVA group). These genes are implicated in the development of asthmatic phenotypes. Some asthmatic marker genes such as *It1na* (SI>1.52), *Tff2* (SI>1.60), and *Clca3* (SI>1.16) were all upregulated by PM and OVA synergistically.

D. Phase I: *In vivo* Effects of PM exposure in a Murine Model of Cardiomyopathy

Overview and summary: Transgenic mice engineered to express a cardiac-specific dominant/ negative form of transcription factor CREB-(Ser-Ala)133, essential for cardiac muscle function were used as a cardiomyopathy model for studying the cardiac effects of PM. This model induces: progressive ventricular failure, cardiac dilatation, decreased systolic & diastolic pressures, hypertrophy and interstitial fibrosis. Measurements were obtained in 10- and 20-wk-old mice exposed to 1 mg/mouse of PM/mouse with evaluation 72 hours after PM challenge. Experimental groups (CD1-PBS, CD1-PM, CREB-PBS, and CREB-PM) were exposed to PM or PBS by intratracheal instillation at 10 or 20 wks of age. Continuous electrocardiograms were recorded prior to and for 36 hours following exposure. Arrhythmia scores were based on the frequency of ventricular premature beats and episodes of ventricular tachycardia. Cardiac function was assessed by cardiac ultrasound at baseline and following PM exposure. cDNA microarray analyses were performed on the left ventricles, lung, and left atrial tissues of 20 wk groups. In conclusion, this study is the first to demonstrate that PM exposure acutely increases ventricular arrhythmias in transgenic mice with severe cardiac dysfunction. Genomic assessment revealed differential regulation of numerous genes,some of which may be involved in the pathogenesis of PM triggered ventricular arrhythmias. These results are consistent with epidemiologic studies that suggest that PM is more likely to trigger phenotypic changes in individuals with severe cardiac dysfunction. We currently are pursuing the novel molecular signatures that PM may have on lung,, left ventricle, left atrium, and carotid body tissues. In these studies, exposure of mice to 1mg/kg body weight represents ~10 times greater exposure compared to human exposures; therefore, future studies will be carried out at 0.01 to 1.0 mg/kg body weight fine particle doses. Key findings are highlighted below:

- **Baltimore PM induces reductions in baseline fractional shortening (FS)** in the 20 wk CREB groups when compared to 10 wk CREB groups (18% vs 35%; p=0.04).

- **Baltimore PM induces ventricular arrhythmias in CREB mutant mice with CHF.** CD1 mice at any age do not exhibit ventricular arrhythmias: either at baseline or 36 hours post PM exposure. CREB mice at 10 or 14 weeks of age do not demonstrate arrhythmias:

either at baseline or following PM/PBS exposure. CREB mice at 20 wks demonstrate ventricular arrhythmias at baseline (arrhythmia score 2.0 vs. 1.8; p=0.77). CREB mice at 20 wks exhibit marked increases in ventricular arrhythmias following PM in conjunction with increased expression of genes involved in cardiac arrhythmias (arrhythmia score 5.5 vs. 2.2; p=0.02). CREB mice exhibit numerous PVC's (pre-ventricular contractions) at baseline. After PM administration, the same CREB mice demonstrate increased PVC's and an idioventricular rhythm. This rhythm may be similar to that of a slow ventricular tachycardia.

- **Baltimore PM induces left ventricular differential gene expression** of several gene ontologies in CREB mice including inflammation, signal transduction, and ion channel regulation. LV RNA from control and 20 wk CREB-PM groups was utilized in Affymetrix arrays.

Baltimore PM induces carotid body dysfunction in CREB mice After undergoing Dejour's test, in which murine minute neural ventilation was assessed after a 15 second exposure to hyperoxia, significant carotid body dysfunction was found in CREB-PM mice compared to their PBS counterparts (-59.3 vs. -48.6). Further ex vivo measures of carotid body sensory responses to hypoxia confirmed these results as the function of carotid bodies from CREB/PM mice were significantly altered from the CREB-PBS animals (4.3 vs. 2.7).

E. Studies proposed for Year 3:

As noted above, we have completed all planned *in vitro* experiments. In Year 3 we will concentrate on Phase II and Phase III studies focused on screening the cardiopulmonary toxicity of the new, characterized PM samples collected by Project #2 personnel. We have received from Alison Geyh bulk fine PM samples from Baltimore, Seattle and Sacramento (200mg). We will continue to use toxicogenomic approaches in the evaluation of PM effects in the model of OVA-induced murine asthma and mice with dilated cardiomyopathy. For these experiments lower doses of particulate matter fine samples (0. 01, 0.1, and 1 mg/kg body weight) collected from various centers will be employed to assess the biological toxicity on asthma and cardiomyopathy models. Our calculations, based on published data, indicate that a dose of 1mg/kg body weight of mouse will be approximately 10 times greater than the reference human exposure. Further, we will be selecting a marker for PM induced inflammation and a marker for asthma /cardiomyopathy for characterization of these new PM fine samples (0.01 to 1.00 mg/kg body weight) as well as for further comparison among the samples based on the different geographical distribution and sample size.

Publications:

Wang T, *Moreno-Vinasco L, Huang L, Lang GD, Linares JD, Goonewardena SN, Grabavoy A, Samet JM, Geyh AS, Breysse PN, Lussier YA, Natarajan V, Garcia JGN. Murine Lung Responses to Ambient Particulate Matter: Genomic Analysis and Contribution to Airway Hyperresponsiveness. *co-first author. *Environ. Health Perspec in press* June 20 [online] 2008

Zhao Y, Usatyuk PV, Gorshkova IA, He D, Wang T, Moreno-Vinasco L, Samet JM, Geyh AS, Breysse PN, Spannhake EW, Garcia JGN, Natarajan V. Mechanisms of Particulate

Matter Induced COX-2 Expression and IL-6 Release Epithelial Cells. *AJRCMB* Jul 10. [Epub ahead of print] 2008.

Abstracts:

Moreno L, Grabavoy A, Goonewardena S, Sammani S, Natarajan V, Breysee P, Geyh A, Samet JM, Garcia JGN. Biological Effect of PM exposure in a susceptible murine strain (Abstract presented to EPA conference Oct 12th 06).

Supplemental Keywords: Differentiated and non-differentiated airway cells; ROS; particulate matter, murine models; cardiopulmonary functions; cytotoxicity; cytokines.

Relevant Web Sites

www.jhsph.edu/particulate_matter