Final Technical Report

Date of Final Report: August 1, 2006
EPA Grant Number: R827351
Center Name: NYU-EPA PM Center: Health Risks of PM Components
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Title: NYU-EPA PM Center: Health Risks of PM Components
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Project Period: June 1, 1999–May 31, 2005 (no-cost extension to May 31, 2006)
Period Covered by the Report: June 1, 1999–May 31, 2006
RFA: Airborne Particulate Matter (PM) Centers (1999)
Research Category: Particulate Matter

Objective(s) of the Research Project:

Introduction

The Particulate Matter Health Effects Research Center (PM Center) at New York University (NYU), supported by Center Grant R827351 from the U.S. Environmental Protection Agency (EPA) from June 1999 through May of 2006, pursued a broad range of research to identify the health effects produced by exposure to particulate matter (PM) in ambient community air and the specific PM components most responsible for producing these effects. It also was engaged in post-doctoral training for future research leaders in air pollution studies, in organizing research Workshops for the PM Centers program, in organizing an *in vitro* collaborative PM Center effort to compare the toxicities of thoracic coarse, fine, and ultra-fine PM, and played a lead role in the preparation of papers for *Environmental Health Perspectives* on the overall PM Centers program. The most notable accomplishments of the NYU PM Center were to demonstrate, in Project #11 (R827351C013), that: 1) six months of 5 days/week exposures of a mouse model of atherosclerosis to a northeastern U.S. regional ambient air fine PM, that had an average concentration of 110 ug/m^3 for the 6-hour/day exposures, produced acute and chronic changes in cardiac function, an increase in aortic plaque and its invasiveness, genetic marker changes, and a reduction of cells in the substantia nigra region of the brain; and 2) in a follow-up six-month study, exposure to fine particles, at an average concentration of only 85 ug/m^3, altered vasomotor tone, induced vascular inflammation, and potentiated atherosclerosis. During this second 6-month study, we were also able to identify a remote point source of nickel that was responsible for significant acute changes in cardiac function. These subchronic studies in mice have thereby enabled us to establish a new level of biological plausibility for the human cohort studies of fine particle associated excess annual mortality, and that it is possible to identify singe causal PM components.

Summary of Findings:

The progress made in each of our 12 research projects is summarized below:

Exposure Characterization Error, R827351C001, K. Ito

Objectives. The main objective of this project was to quantitatively characterize spatio-temporal measurement error of ambient air PM and gaseous co-pollutants measured at routine regulatory-based air monitors as a function of site characteristics using the entire U.S. air-monitoring network. The rationale was that, while PM_{10} was often reported to be associated with health outcomes most significantly among the criteria air pollutants, this may have been in part due to differential exposure characterization error—PM_{10} may have less measurement error than other pollutants. Furthermore, we expected that PM_{10} might have varying exposure characterization error across U.S. due to varying source types, which may result in heterogeneity of estimated PM_{10} risk estimates across cities. The prevailing hypothesis was that the PM_{10} and gaseous co-pollutants data from a single air monitoring station could adequately reflect the population exposure for the entire city, and that resulting risk estimates and their significance are not biased. Also, during the course of this project, the new $PM_{2.5}$ chemical speciation network's data became available (from ~ 2001). Therefore, we also examined exposure characterization error across components of $PM_{2.5}$.

Technical Aspects. Monitor-to-monitor correlation was computed for PM_{10} and gaseous criteria pollutants in seven North-Central States: Illinois, Indiana, Michigan, Ohio, Pennsylvania, Wisconsin, and West Virginia (Ito, et al., 2001). These states cover 312,968 square miles, and contain over 56 million people. The study area also included major cities, such as Chicago, Cincinnati, Cleveland, Columbus, Detroit, Indianapolis, Milwaukee, Philadelphia, and Pittsburgh. Air pollution data for PM_{10}, SO_2, O_3, NO_2, and CO were retrieved from U.S. EPA's Aerometric Information Retrieval System (AIRS, now called Air Quality System, or AQS) for study period 1988-1990 for these states. The AIRS working file format AMP355 was used. The number of monitoring stations for these states was 287, 295, 241, 80, and 108 for PM_{10}, SO_2, O_3, NO_2, and CO, respectively. Since most of the PM_{10} data were collected on an every-6th-day sampling schedule (gaseous pollutants and weather variables were collected every day) at most sites, the data analyses were to be conducted for the PM_{10} sampling days only (total of 183 possible days during the 3 year period), in order to use comparable sample sizes. After removing seasonal trends, the monitor-to-monitor temporal correlation among the air pollution/weather variables within 100 mile separation distance in these areas could generally be ranked into three groups: 1) temperature, dew point, relative humidity ($r>0.9$); 2) O_3, PM_{10}, NO_2 (r: 0.8-0.6); and 3) CO, SO_2 ($r<0.5$). Using the subsets for separation distance less than 100 miles, regression analyses of these monitor-to-monitor correlation coefficients were also conducted with explanatory variables including separation distance, qualitative (land use, location setting, and monitoring objectives), and quantitative (large and small variance) site characteristics, and region indicators for Air Quality Control Regions (AQCR). The AQCRs are EPA-designated regional boundaries that were established based on jurisdictional boundaries, urban-industrial concentrations, and other factors such as air sheds, for the purpose of providing adequate implementation of National Ambient Air Quality Standards (NAAQS). The separation distance was a significant predictor of monitor-to-monitor correlation decline especially for PM_{10} and NO_2 (~0.2 drop over 30 miles). Site characteristics were, in some cases, significant predictors of

monitor-to-monitor correlation, but the magnitudes of their impacts were not substantial. Regional differences, as examined with AQCR indicators were, in some cases (e.g., Metropolitan Philadelphia) substantial, to the extent that the pollutants that have generally poor monitor-to-monitor correlation in the overall 7 states data (i.e., SO_2 and CO) showed monitor-to-monitor correlation comparable with PM_{10} and O_3.

We extended the analysis to the nationwide data for years 1988-1997 (Ito, et al., 2005). We retrieved data from the EPA's AIRS database. The data analyses were to be conducted for the PM_{10} sampling days only for all the air pollutants, in order to use comparable sample sizes. The total number of monitors analyzed were 1892, 1133, 969, 352, and 632, for PM_{10}, O_3, SO_2, NO_2, and CO, respectively. Monitor-to-monitor correlations were computed for all the monitors within each of the AQCRs, limiting the scale of separation distance within the region. The medians of the within-AQCR median separation distance for PM_{10}, SO_2, O_3, NO_2, and CO were 26, 19, 26, 18, and 11 miles, respectively. The AIRS database contains monitor characteristic data elements associated with each air pollution monitor. These include: *Land Use* (Residential, Commercial, Industrial, Agricultural, Forest, Desert, and Mobile); *Location Setting* (Urban, Suburban, and Rural); and *Monitoring Objective* (Maximum Concentration, Population Exposure, Background, Source, and Objective Changed). The overall rankings in monitor-to-monitor correlation on the average were, in descending order, O_3, NO_2, and PM_{10}, ($r \sim 0.6$ to 0.8) > CO ($r < 0.6$) > SO_2 ($r < 0.5$). The resulting median monitor-to-monitor correlation for each monitor was modeled as a function of qualitative site characteristics (i.e., land-use, location-setting, and monitoring-objective), and quantitative information (median separation distance, longitude/latitude or regional indicators) for each pollutant using Generalized Additive Models (GAM). To assure convergence of the GAM's iterative estimation procedure, the convergence criteria parameters, as suggested by Dominici, et al. (2002), were as follows: epsilon = 10^{-14}; back-fitting epsilon = 10^{-14}; maximum iteration = 1000; and back-fitting maximum iteration = 1000. A smoothing function of median separation distance was included in the regression with locally estimated smoothing. The correlation was assumed to decline uniformly as a function of separation distance. Therefore, a relatively wide span of 0.4 was chosen for all the air pollutants. Based on our previous analysis using a smaller geographic coverage of North-central states (Ito, et al., 2001), having a very large or small variance of temporal fluctuations was a significant predictor of a low correlation. Therefore, indicator variables for the monitors with the largest and smallest variance in the five percentiles were also included in the regression model. Regional variation, or heterogeneity, of the monitor-to-monitor correlation was modeled using two alternative approaches: 1) using the seven regional categories; and 2) applying a smooth function of longitude/latitude. As with the qualitative monitor characteristic variables, the seven regional categories were arbitrarily numbered and modeled as a group, using the smoothing spline function with six degrees of freedom. Since the regional differences in the distribution of median separation distance may influence the estimate of regional variation, even with the simultaneous inclusion of median distance in the model, the analysis was also repeated using the data stratified by the separation distance. The data were split in half at the median, and also in fourths at quartile values. In the model using smoothing function of longitude/latitude ('loess' in S-plus), a series of spans (0.4, 0.3, 0.2, 0.10, 0.05, 0.02, and 0.01) were used because we did not have strong assumptions regarding the pattern and smoothness of the regional variation pattern. The span for the final model was chosen for each air pollutant based on: 1) a visual inspection of the spatial pattern of the predicted values; 2) distribution of the predicted values; and 3) the

generalized cross-validation values computed for each of the spans. Both the separation distance and regional variation were important predictors of the correlation. For PM_{10}, for example, the correlation for the monitors along the East Coast was higher by ~0.2 than for western regions. The qualitative monitor characteristics were often significant predictors of the variation in correlation, but their impacts were not substantial in magnitude for most categories.

These results suggest that the apparent regional heterogeneity in PM_{10} effect estimates, as well as the differences in the significance of health outcome associations across pollutants may, in part, be explained by the differences in monitor-to-monitor correlations by region and across pollutants. To examine this issue, we also conducted a regression analysis to see if the heterogeneity of PM_{10} risk estimates across the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) 90 cities (publicly available) could in part be explained by the difference in the extent of PM_{10} monitor-to-monitor correlation. Of the 90 cities, 83 cities could be matched with the median PM_{10} monitor-to-monitor correlation discussed above. The inverse-variance weighted regression of PM_{10} mortality risk estimates on the median PM_{10} correlation showed a positive prediction of PM_{10} mortality risk estimates with a slope of 0.14 (95% CI: [-0.02, 0.30]) per 0.1 increment of PM_{10} correlation.

Possible exposure characterization errors across $PM_{2.5}$ components were examined using $PM_{2.5}$ chemical speciation data collected at three locations in New York City during 2001–2002 (Ito, et al., 2004). The species that are associated with secondary aerosols (e.g., SO_4, NH_4, NO_3, organic carbon [OC], etc.) tended to show high monitor-to-monitor correlations, whereas the species that are likely associated with more local sources (e.g., elemental carbon [EC] as a traffic source marker) showed lower monitor-to-monitor correlations. Source-apportionment using these data was also conducted for each monitor's data. The estimated source-apportioned $PM_{2.5}$ mass generally showed the highest monitor-to-monitor correlation for the secondary aerosol factor (r range: 0.72–0.93). The correlation for the more localized traffic-related factor was more variable (r range: 0.26–0.95). The estimated mean $PM_{2.5}$ mass contributions by source/pollution type across the monitors varied least for the secondary aerosol factor. We also extended the analysis to 28 metropolitan statistical areas (MSA's) where multiple monitors generated $PM_{2.5}$ chemical speciation data for the years 2001-2003. We analyzed a set of key $PM_{2.5}$ components that were of interest in terms of toxicological effects, source signature, and generally large signal-to-noise ratios: i.e., Ni, V, Pb, Cr, Mn, Fe, Si, As, Se, SO_4, NH_4, NO_3, EC, and OC. Again, the species associated with secondary aerosols (e.g., SO_4, NO_3) showed high monitor-to-monitor correlation. However, the monitor-to-monitor correlation for other species varied widely across the MSA's, likely reflecting the variation in the levels and major source types across the MSAs.

The monitor-to-monitor correlations discussed above are pertinent to the interpretation of results from short-term effects (i.e., time-series and longitudinal) studies. However, we also examined potential exposure characterization errors that are pertinent to long-term effects (i.e., cohort and cross-sectional) studies, using the same 28 MSA's. We found that, for the key $PM_{2.5}$ components, the coefficient of variation (CV) for across-MSA variation was generally far larger than those for within-MSA variations, with only a few exceptions. This result suggests that the quality of spatial resolution of the key $PM_{2.5}$ components is sufficient and adequate for the analysis of cross-sectional cohort data.

The results from the source apportionment workshop (Thurston, et al., 2005; Hopke, et al., 2006; Ito, et al., 2006; Mar, et al., 2006) also provided information regarding the exposure characterization error associated with source-apportioned $PM_{2.5}$. The comparison of source-apportioned $PM_{2.5}$ across investigators using the $PM_{2.5}$ chemical speciation data sets from Phoenix, AZ and Washington, DC found that soil-, secondary sulfate-, residual oil combustion-, and salt-associated mass were most unambiguously identified by various methods, whereas vegetative burning and traffic were less consistently identified. Combined with the result suggestive of varying exposure characterization error across $PM_{2.5}$ species, and across U.S. regions mentioned above, a systematic examination of multi-city time-series health effects analysis will be needed. We compared the mean levels of key $PM_{2.5}$ chemical species and the published PM_{10} mortality risk estimates in the 60 MSA's in the NMMAPS study for which speciation data were available, and found that the city-to-city variation of PM_{10} risk estimates could be better explained by some $PM_{2.5}$ chemical species (Ni and V) than others (Lippmann et al., 2006). Thus, the city-to-city variation in PM health risk estimates may be modified by components of $PM_{2.5}$. Our future research will directly examine this issue.

Conclusions. There are differential exposure characterization errors across PM and gaseous pollutants at ambient levels. PM_{10}, $PM_{2.5}$, O_3, and NO_2 tend to have moderate to high temporal correlations across monitors within cities, compared to CO and SO_2. These errors also vary by region and site-specific characteristics. Some of the differences in the observed health effects across pollutants in the past health effects studies may be explained by our findings. However, the estimated ecologic level exposure characterization errors did not explain the city-to-city variation in the PM_{10} mortality risk estimates substantively. Components of PM may play roles in the city-to-city variation of PM health risks. A more comprehensive assessment of the overall exposure characterization error will need to consider personal level exposure error, but such information is currently available from only a few cities.

The project was technically feasible to conduct.

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X-ray CT-based Assessment of Variations in Human Airway Geometry: Implications for Evaluation of Particle Deposition and Dose to Different Populations, R827351C002, B.S. Cohen, E.A. Hoffman

Objectives. To address the paucity of data regarding PM deposition in the lungs of people with pre-existing pulmonary disease and the normal elderly; subpopulations which may be at special risk, this project investigated the potential for retrieval of morphometric data from three-dimensional images of tracheobronchial airways obtained *in vivo* by x-ray Computerized Tomography (CT). The study also explored the potential for the use of stereolithography (STL) to produce hollow airway casts of normal and abnormal lung airways for the experimental determination of site-specific deposition and for experimental verification of particle deposition models. The ultimate goal was to quantify the impact of the airway variability on PM deposition and dose. The project was a collaboration between the extensive imaging expertise at the University of Iowa and NYU PM Center particle deposition expertise.

Progress Years 1–2. A volumetric rendering of the interior surface of a hollow airway cast (used in previous studies at NYU) was generated, producing a surface representation of the airway tree. These three-dimensional images were then converted to a STL file format required for the rapid prototyping of airway casts. This was accomplished by shape-based interpolation to create isotropic voxels and to smooth the surface, after which a volumetric rendering of the resultant

segmented luminal space of the airway tree phantom was generated. The stereolithography unit uses a computer-controlled arm connected to a plastic extrusion device to build volumetric structures layer-by-layer. Two heads are present on the machine, one to lay down the plastic compound for the structure of interest and a second head to lay down needed support material for the structure as it is being built, and which can later be separated from the structure. Close concordance was seen between the original hollow airway cast and the STL produced replicate. The casting process was subsequently converted to utilize a water soluble material to build supporting structures.

Thin multi-slice helical CT scanning allows the acquisition of high-resolution volumetric image data sets of the lung in a breath-hold or at multiple phases within a respiratory cycle. From these scans, hollow airway casts that include 5 or 6 bronchial generations can be created. The process was utilized to obtain an image, and then produce a cast, from a living person. The casts can be accurately replicated for use in studies of inhaled particle deposition in replicate casts of both healthy and diseased airways using realistic air flow rates.

Progress Years 3 and 4. Work was performed preparatory to planned *in vivo* studies to compare inhaled particle deposition pattern and efficiency in sheep with the deposition measured in a hollow airway cast prepared from the same animal's three-dimensional image. Our Iowa collaborators continued to work on the development of sheep models for the testing of various measures of pulmonary perfusion, regional ventilation, airway structure and distensibility, diaphragm and rib cage mechanics, etc. We fine-tuned our methods of respiratory gating and succeeded in developing methodology that allows us to gate image acquisition very accurately to an inductance plethysmographic (Respitrace) signal, and acquire volumetric images of the lung at multiple points within the respiratory cycle over a period of 30 cycles. While it is common to monitor airflow at the mouth and lung volumes, the accuracy required by the above described respiratory-gated image acquisition requires much tighter tolerances than most pulmonary function testing equipment.

Significant advancements in computerized analysis have been made in the areas of lung, lobe and airway segmentation, airway tree matching, and lung feature matching. A set of reproducible feature points are first identified, including airway branching points, for each CT image to establish correspondences across subjects.

The binary airway tree is skeletonized to identify the three-dimensional centerlines of individual branches and to determine the branchpoint locations. Graph algorithms can then be applied to match corresponding branchpoints. A program was developed that visualizes two airway trees side by side and allows a human observer to navigate through the trees in the three-dimensional space and to define matching branchpoints by hand. This is an invaluable tool that provides independent standards. An evaluation using phantom data as well as *in vivo* scans showed excellent agreement (between 85% and 97%) between the automatically obtained matches and matches provided by human experts. These methods will assist development of concordant measures across bronchial branches in different individuals. We also began to establish methodology to perform computational fluid dynamics measures on specific airway geometries imaged by CT so as to predict deposition patterns and to then compare them with direct CT-based measures of deposition.

The Iowa team also examined how spatial resolution varies as a function of position in the field of view. A phantom was made containing 37 copper spheres 1/32 inch (0.8 mm) in diameter that were placed in 3 concentric rings at 50mm, 100mm, and 150mm. The phantom was scanned and reconstructed and a computer simulation was performed to construct a volume similar to that generated by the scanners. Issues of falloff of resolution away from the isocenter remain to be resolved. At NYU we have tried to develop a suitable monodisperse radio-opaque test aerosol. We have x-ray tested common contrast media to determine the smallest layer that can be distinguished from a unit density background, but results to date are not satisfactory.

Technical Aspects. The positive aspects of this project were the development of a computational capacity to create a 3-D computer model of lung airway dimensions from CT scans of an original cast model on which measurements had been made of airway branch diameter, length, and branching angle. We then demonstrated excellent agreement on measurements made from a silastic reproduction as compared with the original to assure accurate reproduction of these metrics.

We then successfully demonstrated the concordance of lung airway sizes in humans *in vivo*, as measured by CT, with those measured in a hollow lung cast that was produced by stereo-lithography using the dimensions provided by the CT scans of the same human lung.

The negative aspects were that the sheep studies were not initiated because we have not yet been able to identify appropriate contrast media. Also, our collaborators at the University of Iowa were not yet able to completely correct for the variation of spatial resolution as a function of position in the field of view. Additionally, within the confines of our resources we were not able to extend the study to the production of hollow airway casts of patients with well characterized chronic lung diseases or to model the influence of abnormal airway structures on lung function.

Technical Effectiveness, Economic Feasibility, and Benefits. Successful techniques were developed for smoothing the data acquired from individual slices to enable the production of three dimensional models of the lung airways from a set of CT scans. Production of such hollow casts is economically viable. The benefits are that reproductions of the airways of patients who receive CT scans for medical purposes, and whose medical history and pulmonary function test are available, can be produced for study of airflow and deposition of particles in accurate, economical and reproducible models. This can provide improved knowledge of how airway particle deposition or airflow varies with morphometry in particular diseases with the possible determination of improved intervention

Asthma Susceptibility to $PM_{2.5}$, R827351C003, G.D. Thurston, J. Reibman

Objectives. The objectives of this research project were to investigate which ambient air $PM_{2.5}$ component(s) and PM mechanisms affect asthmatics most strongly, and to prospectively follow a cohort of non-smoker asthmatics and evaluate PM effects on their health status. The ultimate goals were: to establish technical and operational feasibility for a combined epidemiological/clinical research study; demonstrate associations between specific ambient air $PM_{2.5}$ components and commonly occurring asthma biomarkers attributable to air pollution; and

develop hypotheses regarding the mechanisms of the $PM_{2.5}$-health effects association that could potentially be tested via toxicological studies by other researchers in the NYU-EPA PM Research Center (e.g., via controlled exposure studies).

Technical Aspects. We originally recruited patients during 1999-2000 for our cohort of adult non-smoking asthmatic subjects willing to be followed by prospective monitoring, on days following low vs. high $PM_{2.5}$ concentrations. Because of difficulties in the first summer (of 1999) in inducing sputum in asthma patients, we felt we needed to improve our induced sputum technique. Approval was obtained to induce sputum from normal volunteers. Ten subjects were recruited; and duplicate procedures were performed on these subjects. Subjects with asthma were recruited from the previous summer cohort, clinics and local advertisements. Participants were asked to be "on call" for 1 day notice to come for 4 visits, 2 "High" and 2 "Low" $PM_{2.5}$ visits. These correspond to 2-day lag visits from the defined day. Subjects then underwent pulmonary function testing (PFT), blood draw, pre-medication with bronchodilator, followed by sputum induction. "High" and "Low" PM days were defined based on analysis of previous data: "High"=$PM_{10} \geq 40\ \mu g/m^3$, while "Low"=$PM_{10} \leq 20\ \mu g/m^3$.

Sputum induction was performed by use of increasing concentrations of hypertonic saline (3%, 4%, 5%) via an ultrasonic nebulizer that were inhaled for 7 minutes. Subjects underwent spirometry for measurement of FEV_1 at the start of the procedure, and after each period of saline inhalation. If the FEV_1 dropped 20%, the procedure was terminated. After each saline inhalation, subjects coughed into a sterile container. Sputum plugs were separated from saliva and examined within 2 hours. After weighing, sputum plugs were dissolved in dithiothreitol (0.1%) and phosphate buffered saline. The suspension was then filtered and a total nonsquamous cell count performed. Cell viability was determined by trypan blue exclusion. Cytospins were prepared, stained with Wright's stain, and a differential cell count of nonsquamous cells types performed. Metachromatic cells were detected in preparations stained with toluidine blue. Cell pellets were also prepared for RNA analysis.

At that time, sputum samples were successfully collected on both normal subjects (n=10) and from subjects with asthma (n=11). In addition, some 44 blood serum samples were collected. While this did not provide a database sufficient for the originally envisioned high vs. low $PM_{2.5}$ day comparisons, these samples did provide a basis for evaluating which biomarkers can be successfully used to assess PM-induced effects. For example, preliminary findings from several of these samples have already demonstrated the ability to detect and measure inflammatory cells in sputum samples, as well as the presence of elevated levels of eosinophils. In addition, sputum samples were analyzed for the presence of dendritic cells (CD1a+), and the quality of mRNA was tested in sputum cell pellets.

Overall, progress was made in 2000 toward our study goals, but practical problems arose. The number of subjects that reliably participated was too limited, and only 2 days in the summer of 2000 met the "high" pollution day criteria, as opposed to an expected 18 days. Furthermore, only 50% of our previous subjects agreed to return for the study. Forty subjects were screened by PFT and clinical parameters. Twenty of these subjects failed screening on PFT criteria, even after modification of exclusion criteria; 13 patients agreed to participate in the screening. These factors combined to significantly reduce the number of sample-days that could be collected.

The limitations in our ability to collect and analyze samples forced us to re-examine and adjust our approach in order to better work towards achieving our planned goals. Based upon the above-discussed prior findings from the already collected samples, new subject blood samples were collected bi-weekly on asthma subjects during the summer of 2001. This schedule design avoided past problems experienced in trying to bring in subjects on short notice.

Our methods involved monitoring a panel of 17 subjects with asthma over a three-month period in the summer of 2001 by spirometry (every 2 weeks), AM and PM peak flow measurements (daily), symptom questionnaire (severity scale, albuterol use), and serum samples (every 2 weeks). We also collected $PM_{2.5}$ and other pollution data continuously over this 3 month period. Our goal was to determine whether there is an association between ambient air $PM_{2.5}$ levels and these defined health outcomes. In particular, we aimed to test the hypothesis that increases in plasma levels of specific chemokines related to asthma (i.e., those involved in eosinophil recruitment and Th2 responses) are associated with elevations in ambient air $PM_{2.5}$. Thus, blood samples and PFT measurements were collected during subject visits over 12 weeks during July-September 2001 (total = 6 samples/subject).

All patients were recruited from the Bellevue Hospital Primary Care Asthma Clinic (BHPCAC) in New York City, and all were using beta-2 agonists, as required. The initial screening of subjects was based on inclusion criteria, such as age range (18-70), FEV_1 ($\leq$85% and >50% of predicted), and smoking history ($\leq$ 10 pack year, and no smoking in the past one year). The exclusion criteria included: presence of concurrent lung disease (such as respiratory infections), substance abuse, and the use of oral corticosteroids within the last month. Additionally, daily diary data were obtained from each of the participating subjects, during the study period. Information was collected on peak expiratory flow readings in the morning and evening, frequency of wheeze, shortness of breath, hours spent in air-conditioned rooms, and number of puffs of albuterol used. Serum cytokine levels were determined using commercially available sandwich Enzyme-Linked Immunosorbent Assays (ELISA), for RANTES (Regulated on Activation, Normal T Expressed and Secreted cytokine) (Endogen, Rockford, IL) and for eotaxin, TARC and IP-10 (R&D, Minneapolis, MN). Serum samples were stored at -70 degrees Celsius, and were allowed to gradually equilibrate to room temperature before running the assay. Microtiter plates were read using a microplate reader, and absorbance was estimated by subtracting the 450 nm readings from 550nm readings. Cytokine levels were quantified by converting absorbance to concentration, picograms per milliliter (pg/ml), using the relationship between absorbance and concentration obtained from the standard curve. The sensitivities of the RANTES, eotaxin, TARC and IP-10 assays were 2, 5, 7, and 1.67 pg/ml, respectively.

$PM_{2.5}$ data were collected at Hunter College on First Avenue in Manhattan, near Bellevue Hospital, by the NYU School of Medicine, and daily weather data were obtained from the weather station located at Kennedy International Airport. Gaseous pollution concentrations (e.g., ozone, O_3) were obtained from the New York State Department of Environmental Conservation (NYSDEC), as measured at nearby Manhattan monitors. All analyses were conducted using linear-mixed effect models assuming random intercepts and slopes for each subject, and since repeated measurements over time were observed for each subject, serial correlation between observations was also taken into account. Potential confounders to the association between

ambient $PM_{2.5}$ and changes in serum cytokine levels, pulmonary function, and respiratory symptom data, such as number of puffs of albuterol, hours spent in air conditioning, day-of-week effects, and hot/humid days, were included in the corresponding regression models. Additionally, biomarker levels were included in the regression model in three ways: 1) as raw variables (pg/ml); 2) as z-transformed variables (in order to remove the effect of varying baseline levels of the cytokine between subjects); and 3) as a deviation from the mean biomarker level for each subject.

We also analyzed the daily $PM_{2.5}$ samples collected near the NYU School of Medicine (at Hunter College) for trace elemental composition (using our PM Center Resource X-ray fluorescence [XRF] analyzer), allowing us to also examine our health effects data relative to exposures to various $PM_{2.5}$ components over time.

Results. There was a wide range of $PM_{2.5}$ levels experienced over the summer of 2001 in the New York area, with levels ranging from below 10 $\mu g/m^3$ to nearly 60 $\mu g/m^3$. This provided a range of exposures with which to look for variations in biomarkers during this period. Serum samples were collected from each of the 12 subjects, approximately every 2 weeks, over the course of the summer, for a total of 5 samples for each subject or 60 observations for each cytokine.

A preliminary analysis (without adjustment for potential confounders) of pulmonary function and respiratory symptom data revealed that only shortness of breath incidence (0 and 1-day lags) was significantly associated with ambient $PM_{2.5}$ levels. Maximum hourly O_3 levels were significantly associated with AM peak expiratory flow rates (0 and 1-day lags), and with the ratio of AM to PM peak expiratory flow rates (1-day lag). However, none of these associations remained statistically significant after adjustment for potential confounders, or in the two-pollutant models.

Of all the cytokines considered in the crude analyses, only RANTES was associated with ambient $PM_{2.5}$ levels and maximum hourly O_3 levels, although only for O_3 was the association marginally significant. Mean daily O_3 levels were also considered, but the associations were stronger (more positive and significant) for maximum hourly O_3 levels. For both pollutants, the stronger associations were observed with the same day lag, compared to the 1-day lag.

As shown in Figure 1, there was a general positive trend between the same day $PM_{2.5}$ and RANTES levels, with one regression "hinge point" (i.e., a very low value from one patient on a high $PM_{2.5}$ day) that weakened the overall positive slope. In subsequent analyses, this outlier was removed, and the effect of $PM_{2.5}$ on RANTES levels was estimated in the mixed-effects models adjusting for potential confounders to the association such as, hot and humid days, day-of-week effects, hours spent in air conditioning, number of puffs of albuterol, and serial correlation between observations for each individual.

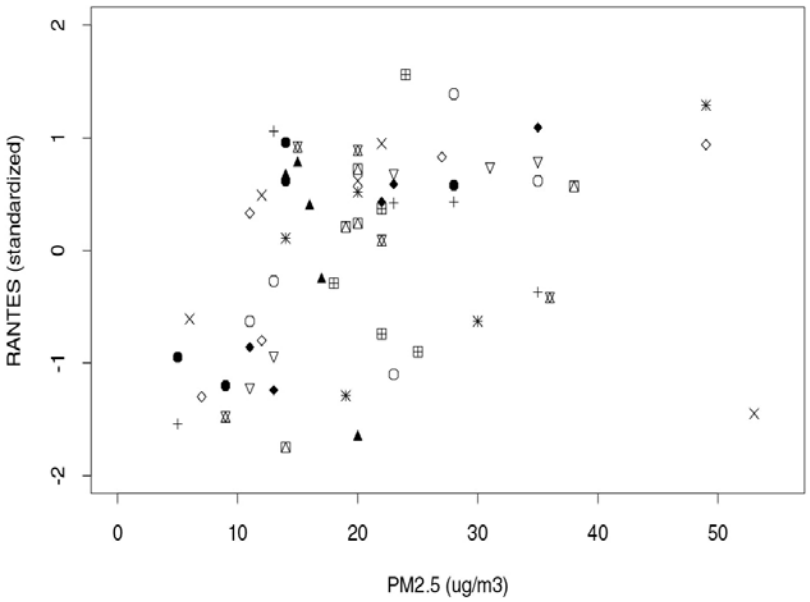

Figure 1. Plot of Serum RANTES Concentrations vs. Ambient $PM_{2.5}$ During 2001

In the fully adjusted model, after removal of the outlier, RANTES levels (raw) were found to be increased by 11,321 pg/ml (z-statistic = 2.34) per Inter-Quartile Range (IQR) of $PM_{2.5}$ (14.36 $\mu g/m^3$), which corresponds to a 17% increase based on the study average of 66640 pg/ml (see Table 1). Similar results were observed in the fully adjusted model for the z-transformed (14.8% increase per IQR of $PM_{2.5}$), and deviation from the mean (14.1% increase per IQR of $PM_{2.5}$) RANTES levels. Additionally, in the crude analyses (without adjustment for potential confounders), comparable results were observed for the untransformed or raw RANTES levels (12.03% increase per IQR of $PM_{2.5}$).

The crude model results for O_3 (same day lag, no outliers removed) indicated an increase of 4,791.98 pg/ml (z-statistic = 1.91) per IQR of O_3 (25.63 ppb) which corresponds to a 7.19% increase in RANTES levels, whereas the fully adjusted model for O_3 (same day lag, no outliers removed) indicated a decrease of 543.39 pg/ml (z-statistic = -0.08) per IQR of O_3 which corresponds to a 0.82% decrease in RANTES levels. Similar results were observed for both the z-transformed and delta-transformed cytokine levels. Not surprisingly, in the fully adjusted two-pollutant models with the outlier removed, the association between $PM_{2.5}$ and RANTES remained significant, whereas the association with O_3 became non-significant (see Table 1).

Table 1. Two-Pollutant Model Slope and 95% Confidence Interval Estimates, for the Fully Adjusted Analyses After Removal of the Outlier. Slope and 95% confidence intervals were calculated per inter-quartile range of the pollutant and expressed in units of pg/ml and as a percentage of the mean cytokine level.

				% Mean	
Cytokine Format	**Pollutant (Lag)**	**Analyses**	**Z Statistic**	**Slope**	**(95% Confidence Intervals)**
RANTES (Concentration: pg/ml)					
	$PM_{2.5}$ (same day)	Fully-Adjusted*	2.13	18.94%	(1.51, 36.36)%
	O_3† (same day)	Fully-Adjusted	-0.46	-3.82%	(-20.09, 12.45)%
RANTES (Z-transformed)‡					
	$PM_{2.5}$ (same day)	Fully-Adjusted	3.41	21.06%	(8.96, 33.16)%
	O_3 (same day)	Fully-Adjusted	-1.85	-10.92%	(-22.47, 0.64)%
RANTES (Delta-transformed)§					
	$PM_{2.5}$ (same day)	Fully-Adjusted	2.23	16.72%	(2.05, 31.39)%
	O_3 (same day)	Fully-Adjusted	-0.60	-4.17%	(-17.79, 9.45)%

* Fully-Adjusted Analyses: regression of cytokine levels on ambient $PM_{2.5}$ levels adjusting for confounders such as number of hours spent in air conditioning, number of puffs of albuterol; daily maximum temperature and relative humidity (hot and humid days), day-of-week, and autocorrelation between observations for each subject.
† O_3 = daily maximum hourly Ozone levels
‡ z-transformed
§ delta-transformed

In addition to evaluating associations with the Criteria air pollutants, we also conducted source apportionment of the Hunter College $PM_{2.5}$ samples during 2001 (see Lall R, Thurston GD. Identifying and quantifying transported vs. local sources of New York City $PM_{2.5}$ fine particulate matter air pollution. *Atmospheric Environment* 2006;40(Suppl 2):S333-S346). The source components $PM_{2.5}$ contributions estimated for each day were: residual oil combustion particles, traffic particles, soil particles, transported sulfate particles, and World Trade Center (WTC) disaster particles. Different cytokines had differing associations with the various PM source component contributions. In the complete dataset, the WTC particle components were associated across all the biomarker outcomes and cytokines considered here. These initial results suggest that the very high WTC pollution had short-term inflammatory effects on the subjects in this study. We are continuing to investigate these associations.

Health Effects of Ambient Air PM in Controlled Human Exposures, R827351C004, T. Gordon, R. Reibman, L.C. Chen

Objectives. The original hypothesis of this Project was that concentrated ambient PM will produce acute adverse respiratory and cardiovascular health outcomes in volunteers under controlled exposure conditions. In Year 2, because the project was stopped (with agreement from the External Advisory Committee), we examined whether the stimulation of epithelial cells by ambient particles results in the release of cytokines which can upregulate antigen presentation by dendritic cells. In addition, in Year 5, Dr. Gordon coordinated a multi-Center collaboration which examined the *in vitro* and *in vivo* effects of size-segregated particles collected at geographically diverse sites throughout the U.S.

Technical Aspects

Human Exposure Study. The exposure of human subjects to PM was terminated largely for 2 reasons. First, the centrifugal concentrator that was used in the initial animal studies by Drs. Gordon, Nadziejko, and Chen fell into disrepair. Repeated attempts to salvage the original concentrator were unsuccessful and the concentrator achieved concentration factors of 3 to 5 instead of the original 10-fold concentration factor. A virtual impactor concentrator designed by Sioutas was recently purchased by the NYU PM Center and will be used in animal studies, but it has not been established whether this new concentrator would be satisfactory for a human exposure study at NYU. Second, reports from the laboratories at EPA, Southern California, and the University of Rochester have provided evidence that few, if any, significant effects are observed in normal, healthy subjects exposed to concentrated ambient PM at concentrations greater than those we originally proposed. Thus, our small project, with 10 healthy subjects exposed to concentrated ambient PM, was unlikely to be fruitful. Therefore, with the agreement of the External Advisory Committee, the Project was terminated and the Center resources were made available for research needs.

In Vitro Studies with Airway Epithelial Cells. Due to the fact that human exposure project was not moving forward during Year 2, Drs. Reibman, Chen, and Gordon concentrated their efforts in examining the *in vitro* response of human bronchial epithelial cells to size-fractionated ambient PM. Because of the significant association between ambient PM and exacerbation of allergic asthma, we examined the potential for airway epithelial cells (primary culture) to modulate the immune system. Size-fractionated ambient PM was collected with a MOUDI impactor for 2 week intervals throughout the year and used to treat human bronchial epithelial cells obtained from normal human volunteers. The fraction of particles less than 0.18 μm produced a dose-dependent increase in GM-CSF released from the epithelial cells. GM-CSF is a cytokine that can elicit inflammation in the airways via an effect on eosinophils and can also modulate immune responses via effects on dendritic cells. There was no change in secreted GM-CSF in cells treated with larger size ambient particles or equivalent doses of carbon or Mount St. Helen dust particles, thus suggesting that the human epithelial cell response was not due to a general particle effect. Moreover, treatment of epithelial cells with endotoxin had no effect on GM-CSF. Further experiments with inhibitors demonstrated that MAPK pathways are involved in the ambient particle effects on GM-CSF secretion by epithelial cells. This research has resulted in publication of 2 manuscripts and was used as preliminary data in a successful National Institute of Environmental Health Sciences (NIEHS) grant application by Dr. Reibman in Year 2. These studies have progressed under Dr. Reibman's R0-1 grant.

Multi-Site Ambient PM Study (MAPS). The overall objective of the MAPS study was to collect particles from several different geographical regions, characterize their physical and chemical properties, and make them available to investigators for *in vitro* and animal toxicology studies. The results of these studies would be used to relate health effects with PM components and ultimately sources. Recent studies suggest that PM derived from different sources may differ in toxicity and that specific PM components may serve as markers for different sources, suggesting an alternative, more efficient way of regulating PM. To directly study this issue, airborne particles in the ultrafine, fine, and coarse thoracic size ranges were collected in eight different locations in the U.S. and Europe. The sites were selected to take advantage of regional differences in PM sources and components. Weekly samples were collected for a period of a month in each location, using a 3 stage particle impactor, developed at Harvard's EPA Center, which is capable of collecting 15 to 100 mg of material at 3 size fractions during a weekly sampling interval. The particles have been assayed for a number of chemical components in collaboration with Dr. Devlin (U.S. EPA- National Health and Environmental Effects Research Laboratory [NHEERL]) and made available to investigators in several different laboratories. Several studies have been completed and results presented so far demonstrate clear particle size and source dependent differences in toxicity. At least four manuscripts (U.S. EPA, University of Rochester, and NYU) are in preparation for submission.

Physicochemical Parameters of Combustion Generated Atmospheres as Determinants of PM Toxicity, R827351C005, L.C. Chen

Objectives. Combustion generated particles often make up a significant portion of ambient PM in many regions. This study examines the hypothesis that the toxicological effects associated with combustion-generated PM depend upon specific physicochemical characteristics of the particles. PM effluents from high temperature processes, such as fossil fuel combustion and pyrometallurgical systems, consist of inorganic materials having a wide size range and chemical composition, including H_2SO_4 and unreacted SO_2. Such effluents have been shown to be toxicologically active. Freshly formed acidic fly ash atmospheres (containing SO_2 and ultrafine particles with transition metals on their surface) produce decrements in lung function (Amdur, et al., 1986; Chen, et al., 1990). Furthermore, sulfuric acid as a coating on particle surfaces has been shown to be 10 fold more potent in producing pulmonary effects than are pure acid droplets of the same H^+ concentration (Amdur and Chen, 1989). Epidemiological data have indicated increased daily mortality to be associated with particulate air pollution indices, and a significant contribution from SO_2 could not be ruled out. Since SO_2, by itself, has low toxicity, it is reasonable to speculate that a synergistic interaction between SO_2 and particles may have been responsible for these observed effects (Amdur and Chen, 1989; Amdur, et al., 1986).

Several human panel studies in the U.S. and the MONICA study in Europe (Gold, et al., 1998; Pope, et al., 1998; Shy, et al., 1998; Peters, et al., 1998), as well as animal studies (Watkinson, et al., 1998; Lovett, et al., 1998; Nadziejko, et al., 1997) have suggested an association between PM and changes in host homeostasis. In this study, cardiopulmonary effects are measured in healthy and compromised animals exposed by inhalation to laboratory-generated particle atmospheres having precisely defined physicochemical characteristics.

This study examined the hypothesis that the toxicological effects associated with combustion-generated PM depend upon specific physicochemical characteristics of the particles and determined the influence of physicochemical parameters of combustion generated PM on the time course, dose response, and persistence of particle-induced cardiopulmonary effects.

Technical Aspects. This project was closely integrated with Project 4 (R827351C006; Nadziejko, PI) in the measurement of cardiopulmonary effects upon exposures to various PM atmospheres. The accomplishments of Project 4 are separately reported by Dr. Nadziejko.

We have developed two furnace systems to produce realistic combustion effluents, and have successfully produced a mixture of carbon, SO_2, and metal (iron or copper). This allows determination of specific components, especially metals, which may be responsible for adverse health effects, and an assessment of whether any effects could be nonspecific, i.e., they follow inhalation of any type of particle. For the work described herein, the electronics for temperature regulations of both furnace systems were updated. To produce Fe (or Cu), and S coated carbon particles, sucrose solutions containing varying concentrations of $Fe(NO_3)_3$ (or $Cu(NO_3)_2$) were produced by a nebulizer and burned in the furnace system previously used to produce coal fly ash. The mass median diameters (MMD, determined by a Mercer impactor) of particles produced by a Collison nebulizer (before combustion) using 10 sucrose solutions (each containing 1117 ppm Fe) were 0.9 μm. When a 10% sucrose solution containing 1117 ppm Fe (or Cu) was burned in the furnace at 750°C in the presence of 1 ppm SO_2, ultrafine particles with a median diameter of 32 ± 1.3 nm (34.0 ± 7.4 nm for Cu) and sg of 1.55 were produced. Number concentrations as high as 1.9×10^7 particles/cc were achieved. XRF was used to measure the concentrations of iron, copper, and sulfur in these particles. At this combustion condition, the particles produced from this furnace contained 35.1% and 3.6% by mass of iron and sulfur, respectively (30.6% copper and 6.9% sulfur when copper was used). It appeared that copper is almost twice as efficient (6.9% vs. 3.6%) in converting sulfur dioxide gas to particle-associated sulfur.

Sprague Dawley rats were exposed to furnace gas or 450 $\mu g/m^3$ of these particles for 3 hours and their lungs were lavaged 24 hr post exposure. A lead oxide diffusion denuder was used to remove SO_2 from the exposure atmospheres. None of the exposure atmospheres produce changes in LDH levels in the lavage fluid. However, those aerosols containing a mixture of iron, SO_2, and carbon produced a 6.8 fold increase over the furnace gas control for the total number of cells in the lavage, whereas particles containing copper, SO_2, and carbon did not produce any change in this parameter. The results are shown in Table 1.

Table 1. Effects of Ultrafine Particles in Rats

Exposure Atmospheres	Total Cell Counts (10^6)	LDH (BB unit)
Furnace Gas	0.70 ± 0.14	95.5 ± 10.2
SO_2 + carbon	1.52 ± 0.31	78.7 ± 6.3
Copper + SO_2 + carbon	1.52 ± 0.23	80.0 ± 13.5
Iron + SO_2 + carbon	4.77 ± 0.41*	113.7 ± 28.2

Values were mean ± SE, (n=4 to 7 per exposure group).
* significantly different than furnace gas control ($p < 0.0001$).

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Objectives. The effect of PM on the cardiovascular system is an increasingly important public health issue. However, the physical and/or chemical properties of PM responsible for these serious health effects are currently unknown. The questions are: 1) What are the biologically active components of PM? 2) What are the mechanisms by which PM affects the cardiovascular system? and 3) What are the sensitive subpopulations? These three questions are inextricably intertwined. Any hypothesis about a mechanism of cardiovascular effects rests on some assumptions that a certain type of constituent of PM is the culprit.

This research focused on particle-associated irritants based in part on the time course of effects reported in recent epidemiological studies. There is consistent evidence from times-series studies that the lag time between elevated levels of $PM_{2.5}$ and increases in cardiovascular-related hospital admissions and death is very short, i.e. one day or less. There is one well-studied physiological mechanism that is consistent with rapid effects of PM on both cardiovascular and pulmonary function, namely stimulation of irritant receptors in the respiratory tract. Irritant receptor activation involves a bimolecular reaction between a protein receptor in the lung and an agonist, which triggers a rapid increase in intracellular calcium (Ca^{++}) leading to activation of nerve fibers that send impulses to the central nervous system. Signals from the central nervous system then cause slowing of respiration and changes in blood pressure and heart rate via neural reflex pathways. The stereotypical response to an inhaled irritant is an immediate change in respiratory rate and heart rate, which returns to normal soon after exposure stops.

The objectives of this project were: 1) to examine the time course of effects of concentrated ambient PM (CAPs) on cardiovascular function in sensitive animals to establish the biological plausibility of short lag times between PM exposure and cardiovascular effects; and 2) to expose rats (both normal rats and rat models of cardiac disease) to sulfuric acid aerosols, a known irritant found in PM, to determine whether irritant aerosols cause cardiovascular changes consistent with the adverse health effects of PM. Exposure to carbon black particles was used as a non-irritant control.

Technical Aspects. We examined the effects of various PM air pollutants on rats with surgically implanted ECG and blood pressure (BP) transmitters to determine whether inhaled PM causes immediate physiological effects. Spontaneously hypertensive rats (SHR) with BP transmitters (which measure BP, heart rate and respiratory rate) were exposed to CAPs for 4 hrs. The SHR were also exposed to fine and ultrafine sulfuric acid aerosols because acid is one of the components of PM that could potentially activate irritant receptors and cause effects during exposure. Young and old (> 20 months) Sprague Dawley (SD) rats with ECG transmitters (which measure heart rate and core temperature) were exposed to fine and ultrafine acid aerosols and to resuspended carbon black. Inhalation of CAPs by the SHR caused a striking decrease in respiratory rate that was apparent soon after the start of exposure, and that stopped when exposure to CAPs ceased. The decrease in respiratory rate was accompanied by a decrease in heart rate. Exposure of the same SHR to fine particle size sulfuric acid aerosol also caused a significant decrease in respiratory rate similar to the effects of CAPs. Ultrafine acid had the opposite effect on respiratory rate in SHR as CAPs. In both old and young SD rats, inhalation of fine acid aerosol caused an immediate increase in temperature (compared to air-exposed rats) that ceased when exposure stopped. Ultrafine acid caused an immediate decrease in heart rate and temperature during exposure in young SD rats and no significant effect on old SD rats.

Carbon black inhalation had no significant effect on heart rate or temperature during exposure in either old or young rats. This study showed that inhalation of ambient PM and acid aerosols have immediate effects on cardiopulmonary function during exposure. The pattern of the response to inhaled PM is consistent with activation of irritant receptors in the respiratory tract.

Overall, we did more than 50 experiments exposing rats to CAPs, irritant aerosols, particulate matter surrogates and even some irritant gases. Every experiment involved monitoring of cardiovascular functional data in an air-exposed and pollutant-exposed group before exposure, during exposure and for 48-72 hrs exposure. We did extensive exploratory data analysis while experiments were being performed and solved a number of issues related to quantifying telemetric data. However, it was apparent that there was no suitable statistical method for determining whether there was a significant difference between the treated and control groups because the onset and duration of the effects were unknown. Drs. Nadziejko and Chen, in collaboration with Dr. Jing-Shiang Hwang, a visiting scientist (and statistician) in the NYU PM Center, and Dr. Arthur Nadas, a mathematical statistician in the Department of Environmental Medicine, developed a simple but powerful method of analyzing repeated measures data when the time course of the effect is not known *a priori*. This method, which is called the Fishing License method, has been published and used to analyze all of the telemetry data performed in the PM Center.

Role of PM-Associated Transition Metals in Exacerbating Infectious Pneumoniae in Exposed Rats, R827351C007, J.T. Zelikoff

Objectives. Previous investigations in this laboratory demonstrated that a single 5 hr inhalation exposure of *Streptococcus pneumoniae*-infected male rats to concentrated ambient $PM_{2.5}$ from New York City (NYC) air [at concentrations approximating or greater than the promulgated 24 hr National Ambient Air Quality Standard (NAAQS) for $PM_{2.5}$ (~65 - ~150 vs. 50 $\mu g/m^3$, respectively)], altered both pulmonary and systemic immunity, as well as exacerbated the infection process, in a time- post-exposure-dependent manner. These NYU-PM Center-supported studies were performed to correlate metal content of ambient $PM_{2.5}$ with its *in vivo* immunotoxicity so as to identify and characterize the role of constitutive transition metals for exacerbating ongoing *S. pneumoniae* infections. The central hypothesis of this particular component was that metals (either individually or in combination) associated with inhaled NYC particulates influenced the severity and/or kinetics of pulmonary bacterial clearance induced by concentrated ambient NYC $PM_{2.5}$. By exposing rats previously-infected with *Streptococcus pneumoniae* (i.e., 48 hr prior to PM exposure) to PM-associated soluble metals, at doses representative of those within the original intact parental PM atmosphere, metals that influence the ability of PM to alter host resistance against infectious agents could be defined.

Soluble metals selected for study included zinc (Zn), iron (Fe), copper (Cu), nickel (Ni), and manganese (Mn), which were based upon those immunomodulating metals identified by XRF analyses and atomic absorption spectroscopy from filters collected during the original NYC study. For the first sets of studies, rats were exposed by inhalation (nose-only) to a single metal at a concentration of 100 $\mu g/m^3$. A dose substantially higher than that found on the original PM atmosphere was selected so as to eliminate those metals having no effect on bacterial host resistance.

Three major objectives were originally proposed to test the aforementioned hypothesis:

(1) To determine whether particle size influences PM-induced alterations in the handling (i.e., uptake and/or killing) of an ongoing pulmonary infection with *S. pneumoniae*.

(2) To identify whether the soluble or insoluble portion of a given size fraction of ambient air PM is responsible for exacerbation of an ongoing pneumococcal-associated pneumonia.

(3) To ascertain which transition metals (either individually or in combination) found most active in the previously identified portion of ambient PM play significant roles in exacerbating ongoing pneumococcal-induced pneumonia in PM-exposed hosts.

Unfortunately, due to a number of technical and personnel difficulties encountered throughout the project the originally proposed hypothesis and specific aims were modified shortly after project initiation. The redesigned **working hypothesis** tested the notion that particle solubility, and/or metal constituents of PM play a critical role in mediating PM-associated pneumonia-related morbidity/mortality in exposed individuals. In this case, the role of metals (alone and in combination with each other), as well as the individual physico-chemical attributes of the metals that influence the ability of PM to alter host resistance against infectious agents, could be defined. The major infection endpoint investigated in these studies remained the same as previously proposed. Specific Aim 1 was deleted and the second aim was modified such that composition and solubility of metal particles were determined from within a single PM size range. Since NYC studies could not be performed due to the lack of a "workable" concentrator at that time, Aim 3 was also deleted and the role of solubility and the identification/quantitation of metal constituents in concentrated PM were determined from NYC filter samples collected previously for the Health Effects Institute (HEI) study.

Technical Aspects. Soluble metals selected for study including Zn, Fe, Cu, Ni, and Mn were based upon those immunomodulating metals identified from filters collected during the original NYC study. In the first sets of studies, rats were exposed to a single metal at a concentration of 100 $\mu g/m^3$. A dose substantially higher than that found on the original PM atmosphere was selected so as to eliminate those metals having no effect on bacterial host resistance. Iron, Zn, and Ni proved most biologically active in this capacity. In addition to host resistance, a number of immune parameters important for resistance of the host against infectious bacterial pathogens were also evaluated. These included: pulmonary histology; lung cell numbers and profiles; lavageable lactate dehydrogenase activity; total protein levels and cytokines; macrophage-mediated production of reactive oxygen species; splenic lymphocyte proliferation; and circulating blood cell profiles. Even at this relatively high metal dose, inhalation of either Cu or Mn had little or no effects on these particular immune parameters. Similar to that observed for host resistance, Zn, Fe, and Ni, had the greatest effects on these biological endpoints. Based upon these results, only these three metals were evaluated at more relevant concentrations. In this case, only Fe and Ni altered host resistance at a 10-fold lower concentration (i.e., 10 $\mu g/m^3$); Fe compromised pulmonary bacterial clearance by about 60, while exposure to 10 μg Ni/m^3 actually enhanced clearance by ~30%. Given that PM-associated metals do not exist in isolation, the biological effects of exposure to PM likely depend upon responses to metals in combination,

and that exposure to pollutant combinations often results in responses different from those seen following inhalation of individual materials, mixture studies were performed to examine the interactive toxicity of Zn, Ni, and Fe on anti-bacterial defense mechanisms and the "handling" of ongoing pneumococcal infections. At an equimolar metal concentration of 50 $\mu g/m^3$, rats were exposed simultaneously to Cu plus Ni, Zn plus Ni, or Fe plus Mn. Both Cu and Mn significantly antagonized the pulmonary toxicity of Ni and Fe, respectively. On the other hand, exposure to Zn acted to reverse the "beneficial" effects of Ni alone on pulmonary bacterial clearance; simultaneous exposure of Zn and Ni reduced clearance of Streptococcus by about 30%.

Conclusions. These studies demonstrated that even an acute (5 hr) exposure to PM-associated metals including soluble Fe, Zn, and Ni act to exacerbate an ongoing *S. pneumoniae* infection in particle-exposed rats. Moreover, these same metals in combination can produce responses different from those seen following inhalation of the individual metals alone. This study has provided necessary information as to the particular PM constituents/metal interactions responsible for the observed effects upon host immunocompetence. Taken together, results of these investigations provide biological plausibility for the role of certain PM-associated transition metals to worsen the outcome of an ongoing pulmonary infection.

Immunomodulation by PM: Role of Metal Composition and Pulmonary Phagocyte Iron Status, R827351C008, M.D. Cohen

Objectives. Particulate matter ≤ 2.5 μm in diameter ($PM_{2.5}$) has been shown to induce/exacerbate infectious lung disease and alter the manner by which lungs handle bacteria. This research project sought to validate the hypotheses that: 1) $PM_{2.5}$ modulates lung phagocyte antibacterial function by altering cellular iron (Fe) status; 2) metals (rather than organics, biomatter) in $PM_{2.5}$ underlay any change in lung leukocyte Fe status; and 3) relative Fe content in $PM_{2.5}$ governs these effects. With respect to the latter, in entrained $PM_{2.5}$ with a high relative Fe content, phagocyte uptake and subsequent dissolution of any associated insoluble Fe—combined with an increase in cellular deposition of any soluble Fe (via transferrin [Tf] activity)—will lead to Fe overload and decreased antibacterial function. Conversely, with low relative Fe content-$PM_{2.5}$, presence of relatively greater levels (with respect to Fe) of competitors for Tf binding (e.g., aluminum [Al], manganese [Mn], and vanadium [V]) will bring about Fe deficit and reduced antibacterial function due to inhibited transport of endogenous Fe to cells.

To test the hypotheses, the objectives were: 1) in cooperation with the Los Angeles and Seattle PM Centers, to collect daily $PM_{2.5}$ samples in each metropolitan region over a 3-mo period to characterize patterns of proportionality of Fe to Al, Mn, and V; 2) to determine *in vitro* if a presence of Al, Mn, and V impacted on Fe homeostasis in a rat lung macrophage cell line (i.e., NR8383) when varying doses of each metal (reflective of relative amounts in each city's $PM_{2.5}$) were used; and 3) to examine effects from each city's $PM_{2.5}$ on lung Tf status, Fe status, and antibacterial function of their local macrophages.

Technical Aspects. Daily regional $PM_{2.5}$ samples in NYC, Los Angeles (LA), and Seattle were collected and analyzed by XRF to determine elemental composition and, specifically, the relative and absolute Fe, Al, Mn, and V contents. Results indicated that: the $PM_{2.5}$ in each city had disparate metal compositions; there were wide variations in absolute and relative Fe, Mn, Al, and

V content; and, there were significant differences in the relative ratios of each competitor to Fe. Based on these differences, *in vitro* studies with NR8383 cells sought to characterize if each competitor (at levels that would be encountered in a given day's $PM_{2.5}$) could alter cell Fe homeostasis and, ultimately, which competitor was most potent in inducing the effect.

Our initial studies using induction of iron response protein (IRP) binding to iron response element (IRE) sequences as an indicator of shift in cellular iron balance indicated that if cells were treated with Fe (as Fe^{3+}) alone or with V, Al, or Mn (individually or in combinations) at levels equivalent to those expected in 500 µg of a given $PM_{2.5}$ sample, each competitor caused Fe deficit in the cells. By employing increasing molar ratios of competitor to Fe, the determination was made that V had the greatest effect on Fe status and Mn the least. Studies using combinations of two or all three competitors indicated that there was a synergistic effect when V and Mn were both present; co-presence of Al with Mn or V had little impact.

To determine if effects observed with varying molar ratios of Al, Mn, and V would reflect what might be occurring with actual $PM_{2.5}$ in the three cities, IRP studies were performed using cells treated with Fe alone and with Al, Mn, and V at levels that would be present in a 500 µg sample of a given day's $PM_{2.5}$. Using treatments that were based on the $PM_{2.5}$ of three randomly-selected days in each city, it was found that levels of IRP activation (compared to that obtained with Fe alone) were greatest in cells treated with the combination of Fe + V + Al + Mn that would be found in NYC. Effects from co-treatments using levels of the metals found in $PM_{2.5}$ from Seattle or Los Angeles were minimal.

Because a presence of nitric oxide (NO) might affect the levels of IRP activation assayed, studies were done to assess inducible nitric oxide synthase (iNOS) levels in the cells. Analyses of ERK-1 and -2 activation were performed concurrently as these MAP kinases are believed to play a role in iNOS formation. Only increasing amounts of Al had significant effects on iNOS expression; treatment with increasing molar ratios of V and Mn failed to induce iNOS to levels significantly above that of Fe alone and below that of iron chelating desferroxamine (DFX). This would suggest that the observed effects from V on IRP activity were unadulterated in that there was no significant increase in NO levels that could enhance IRP-1 binding activity. Results of the ERK studies indicated that increasing molar ratios of V and Al both caused significant increases in phosphorylation (and so, activation) of ERK-1 (p44); only V appeared to increase ERK-2 (p42) activation. Studies to better discern the meaning of these three sets of observations (i.e., changes in IRP activity, iNOS expression, and ERK-1/2 activation) are needed. For now, the results clearly indicate that at least two of these PM-associated metals induce effects on cell Fe homeostasis regulatory mechanisms (i.e., the IRPs—in either a direct or indirect manner) even when there is a level of Fe present that should keep the cell Fe-sufficient.

In light of these results, a re-examination of the three city IRP studies indicated that selected days for Los Angeles had relatively high Al:Fe molar ratios (i.e., > 3.0). As these values fell into the range predicted to cause significant iNOS induction in the NR8383 cells, it is possible that any expected IRP activation was masked by increased NO formation. In contrast, NYC samples had Al:Fe, Mn:Fe, and V:Fe molar ratios that routinely fell into the previously-determined optimal ranges (e.g., 0.75-1.50, 0.04-0.08, and 0.1-0.2, respectively) for inducing enhanced IRP activity in the cells. Samples from Seattle tended to have fairly low V:Fe molar ratios even

while having values for Al:Fe and Mn:Fe expected to induce IRP activation. From these results, and the previous observations on IRP activation using varying molar ratios of these competitors for Tf binding, we concluded that it is the relative amounts of V to that of Fe that are most critical in determining whether a given PM sample is likely to modify the Fe status of a lung macrophage. Furthermore, in PM that contains moderate-to-high amounts of Al, while effects on Fe status are likely, use of the IRP marker as an indicator of this outcome is not practical due to confounding effects introduced by effects on NO formation induced by Al ions.

Performing the *in vivo* exposure studies outlined for Aim 3 was ultimately not possible due to the limitations in the total amount of any given day's sample of $PM_{2.5}$. Instead, the information expected to be gleaned from those studies was obtained, in part, from a concurrent National Institutes of Health/National Institute of General Medicine Sciences (NIH/NIGMS)-funded study. Rats were exposed 5 hr/d for 5 d to atmospheres containing physico-chemically distinct forms of V (or other $PM_{2.5}$ metals) and their lung fluids were then analyzed for total Fe content, ferritin and Tf levels. Antibacterial activity in the lungs of exposed cohorts, reflecting the functional status of local macrophages, was also examined. These studies indicated that prior to the start of a lung infection, exposure to pentavalent V—the most common form found in PM—caused significant increases in lavage fluid Fe and ferritin levels, but had less overall effect on total Tf levels. Effects from soluble V were greater than those from an insoluble counterpart. These same result patterns were seen in the ability of the exposed rats to clear a viable bacterial challenge from their lungs, i.e., rats that inhaled soluble V had the most significantly reduced resistance against a pathogen as compared to controls.

These rat study results, taken together with those of the *in vitro* studies performed here, suggest that soluble V ion-induced alterations in the ability of Tf to bind Fe can lead to increases in the levels of free Fe in the airways and, concurrently, less Fe delivery to resident phagocytes. With both more Fe available for sustenance, and local immune cells less capable of performing their normal sentinel duties, the survival of most common bacterial pathogens that invade the lungs would then be greatly enhanced. The specific mechanisms hypothesized and then validated in these studies now allow us to better explain the means by which $PM_{2.5}$—and more importantly, its specific constituents—act to induce or exacerbate infectious lung diseases in exposed populations.

Conclusions

These studies showed that:

- Select metals within a given sample of $PM_{2.5}$ can cause altered cellular Fe homeostasis.

- The effects of $PM_{2.5}$ with respect to altered macrophage Fe homeostasis—from region to region, or site to site in a given region—are governed by the relative content relationships between Fe and at least three co-constituent metals, e.g., V, Mn, and Al. Of these three modulants, V is the most potent effector on this parameter.

- Analysis of IRP activity can be an effective way to examine effects of a wide variety of criteria pollutants upon iron homeostasis in the lungs. But, investigators need to monitor for

effects on NO formation by the pollutants to determine if their measured effects on IRP are being adulterated.

Except where indicated above, the project was found to be technically feasible to conduct.

Lung Hypoxia as Potential Mechanisms for PM-Induced Health Effects, R827351C010, M.D. Cohen, K. Salnikow

Objectives. Ambient PM often contains relatively high levels of transition metals, predominantly iron (Fe), nickel (Ni), vanadium (V), aluminum (Al), and chromium (Cr), with levels of each varying depending on the particle source. At-risk individuals with pre-existing hypertensive disease or atherosclerosis, appear to have overtly negative responses to PM. However, both the mechanisms underlying these effects and the constituents that might be causing these outcomes remain unclear. As atherosclerosis has more recently been designated a chronic inflammatory process, it has been accepted that circulating levels of interleukin (IL)-6 may reflect the intensity of occult plaque inflammation and vulnerability to rupture. Both monocyte chemoattractant protein-1 (MCP-1) and IL-8 may also play a crucial role in initiating and promoting atherosclerosis by enhancing development of atherosclerotic lesions and plaque. Several atherogenic factors induce these cytokines in cardiovascular tissues, primarily through activation of transcription factors such as NF-κB or peroxisome proliferator-activated receptors. Hypertensive patients are at particular risk of cardiovascular complications, possibly related to endothelial damage or abnormal angiogenesis. These pathophysiologic processes correlate with plasma levels of vascular endothelial growth factor (VEGF); plasma VEGF levels are higher in hypertensive patients compared with controls and correlate significantly with age, systolic and diastolic BP, cardiovascular disease, and cerebrovascular accident risk scores.

We hypothesized here that select PM constituents (e.g., Al, V, Ni, Mn) act on lung epithelial cells and resident macrophages to stimulate release of proinflammatory cytokines/chemokines that may have a role in initiation or promotion of atherosclerosis. We further hypothesized that these metals contribute to the above noted deleterious responses in patients with pre-existing hypertensive disease/atherosclerosis by: 1) *a priori* altering the Fe status of these cells that, in turn, and 2) results in increased intracellular accumulation of hypoxia-inducible factor-1α (HIF-1α). As a result of the latter, lung epithelial cells and macrophages are stimulated to increase their formation/release of proinflammatory cytokines, such as IL-6, IL-8, tumor necrosis factor (TNF)-α, as well as MCP-1 and VEGF, that are then transported to the heart in relatively high (undiluted) concentrations. The underpinning of our hypotheses was our earlier studies that showed that exposure to soluble and insoluble Ni agents strongly activated hypoxia-inducible pathways. These findings were not surprising in that: 1) HIF-1α induction and activation depends on the level of oxygen and activity of Fe-containing hydroxylases; 2) Fe regulation is critical in hypoxia-inducible gene expression; and 3) rat lung macrophage treatment with Ni ions caused significant shifts in cellular Fe levels. To validate our hypotheses, lung macrophage and airway epithelium cell lines were exposed to varying amounts of Fe alone or in combination with Al, V, Mn, or Ni (at levels relevant to those found in ambient urban PM from NYC) and levels of each of the above-cited cytokines/chemokines are measured by ELISA, Northern, and Western analyses (the latter to increase the degree of detectability of effects from the treatments). These

results could then be used as a baseline of effects for later comparison against the actual formation of these proteins in the lungs of rodents exposed to the "parent" NYC $PM_{2.5}$ samples.

Technical Aspects. Since it was shown earlier that water soluble Ni was responsible in large part for the pulmonary injury caused by residual oil fly ash (ROFA) and that the local inflammation induced was reproducible with instilled soluble forms of Ni (i.e., nickel chloride, sulfate, or sulfide) (Benson, et al., 1986; Dreher, et al., 1997; Kodavanti, et al., 1997, 1998), our initial studies examined the potential effects of Fe and Ni (alone and in combination) on hypoxic stress induction and IL-8 production in the human lung epithelial 1HAEo- cell line. Exposure to Ni produced hypoxic stress as measured by the induction of inducible NDRG-1 and IL-8 production. Similar degrees of hypoxic stress and IL-8 production were induced by the Fe chelator desferroxamine (DFX), suggesting that Ni interfered with cell Fe status. Interestingly and unexpectedly, IL-1β, IL-6 and TNFα secretions were not affected. It is possible that the Ni may have become substituted for Fe in Fe-bearing enzymes/proteins, leading to inhibition of their activity and, subsequently, to activation or induction of HIF-1 transcription factors. It is also possible that Ni caused Fe entry into cells to be impacted, much in the manner seen with other PM-associated metals in Project 8 (R827351C008). Those studies using the IRP activation parameter indicated that treatment of the rat lung macrophage cell line NR8383 (NR) with PM-relevant levels of Ni in conjunction with Fe caused a cellular Fe deficit.

To assess if treatment with PM-associated metals caused an altered Fe homeostasis that should, in turn, induce HIF-1α expression, cultures of 1HAEo- or NR were treated overnight with various amounts of soluble forms of Ni, V, or Al in combination with a fixed amount of Fe. Each level of metal tested was equivalent to that amount that would have been present in a representative 500 μg sample of NYC $PM_{2.5}$ during the month of October in 2001). Western analysis of cellular products indicated that increases in the ratio of V:Fe caused significant increases in HIF-1α expression (to values similar to those from DFX). Exposure to increasing amounts of Ni also caused increased HIF-1α expression, but less so than with V. Al treatments seemed to have no effect on HIF-1α expression; however, as these exposures caused more cell death and proteolytic damage to materials isolated from viable cells, these measurements may have been biased from those that would be seen at more realistic levels of exposure.

Analyses of HIF-1α-inducible cytokines/chemokines indicated that Al, Ni, and V each induced VEGF formation in NR cells. Induction levels were all equal-to-greater than that from DFX and the strength of effect was Al ≥ Ni > V. Western analyses of MIP-2 levels were inconclusive, primarily due to difficulties in trying to adequately resolve the 8 Kd protein in the cell lysates. ELISAs yielded indications that Al had the strongest impact among the metals on MIP-2 release. With the 1HAEo- line, ELISAs of culture supernatants from treated cells suggested that at the higher metal:Fe levels tested, each metal caused a strong induction in IL-8 formation/release. In contrast, any stimulation of VEGF production appeared to only occur with increasing levels of Ni. Western blot analyses were attempted to confirm these patterns, but problems of poor specificity by the antibodies precluded adequate determinations. Because of these technical problems, NR mRNA levels of respective HIF-1α-inducible genes were measured to obtain an indication of any induced shift in cytokine/chemokine formation. RT-PCR analyses showed that Al and Ni induced significant levels of VEGF mRNA and that the effect was ratio (i.e., Al:Fe,

Ni:Fe)-related; oddly, the effect of V treatment was nominal. Similar results were also noted for IL-6 gene induction. With MCP-1, MIP-2, and TNFα, none of the metals tested had any effect.

The results with the rat macrophage line (as compared to the human lung epithelial line) suggest that there may be potentially species-/cell type-related differences in responses to PM-metal-induced alterations in Fe homeostasis. The data also suggest that the relationship between metal-induced HIF-1α expression and levels of select cytokine/chemokine products may not necessarily be linear. However, this latter finding needs to remain tentative as effects of Al, Ni, and V on several relevant processes in cells need to be refined, i.e., differential effects of each metal on the timeframe of induction/maintenance of elevated gene expression in response to increased HIF-1α levels need to be examined.

Conclusions

These studies showed that:

- Select metals within a given sample of $PM_{2.5}$ can cause altered cellular Fe homeostasis and this effect appears to be governed by the relative content relationships between Fe and at least three co-constituent metals, e.g., Ni, Al, and V.

- Among these metals (that alter Fe homeostasis), Ni and Al (more so than V) also cause changes in the release of select cytokine/chemokine factors that could impact initiation or promotion of atherosclerosis. This effect also is related to relative content relationships between Fe and these metals.

- The observed effects on the inducible release of these factors seem, for now, to be both species- and cell type-dependent.

Except for the above-noted problems encountered during some of the Western blot analyses, the project was found to be technically feasible to conduct.

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Urban $PM_{2.5}$ Surface Chemistry and Interactions with Bronchoalveolar Lavage Fluid (BALF), R827351C011, M. Kendall

Objectives. The objective of this research project was to investigate the surface chemistry of urban fine particles ($PM_{2.5}$), and to quantify the adsorbed and desorbed species exposed to bronchoalveolar lavage fluid (BALF).

Technical Aspects. Urban background and roadside $PM_{2.5}$ samples of different mass concentration and total weight were collected in triplicate in the South Bronx region of New York City. Simultaneously, the concentrations of other atmospheric pollutants (CO, NO_x, SO_2, O_3, EC) were measured, and weather conditions recorded. The collected $PM_{2.5}$ samples underwent one of three treatments; no treatment, treatment *in vitro* with BALF, or treatment in a saline solution (control). The surfaces of untreated, saline and BALF treated $PM_{2.5}$ samples were then analyzed using X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). These results were then compared with ambient air pollutant concentrations, weather variables, selected BALF characteristics, and results from a previous London study conducted using identical methods.

Both surface techniques were useful in detecting surface species and observing changes in surface concentrations. The surface of untreated urban $PM_{2.5}$ consisted of 79 to 87% carbon and 10 to 16% oxygen with smaller contributions of N, S, Si and P in the samples from both locations. A wide variety of other inorganic (metals, Cl^-, NH_4^+) and organic species (aliphatic and aromatic hydrocarbons) were detected with ToF-SIMS. The surface characteristics of particles from the roadside and background sites were very similar, except for higher ($p<0.05$) nitrate concentrations at the roadside $PM_{2.5}$ that were attributable to higher roadside NO_x concentrations. Comparable species and quantities were identified in a previous study of London $PM_{2.5}$, but $PM_{2.5}$ surface chemistry differed considerably from other sources, particularly in surface concentrations of oxygen and trace species.

After treatment with BALF, the N-C signal detected by XPS analysis increased by an average of 372±203%, indicating significant surface adsorption of protein or other N-containing biomolecules. Lower N-C signals were observed for BALF from smokers. ToF-SIMS data confirmed N adsorption after BALF treatment, and also indicated an adsorption of phospholipid on the $PM_{2.5}$ surfaces in terms of increased fragment ions characteristic of phospholipid adsorption. The primary phospholipid in BALF is DPPC, although positive identification was not possible. Oxygen content of $PM_{2.5}$ surfaces was the most significant determinant of both N-C and phospholipid adsorption. The XPS signal of the soluble species NH_4^+, NO_3^{2-}, Si and S decreased in both saline and BALF treated samples, showing that these species may be bioavailable in the lung.

Thus, we have shown that $PM_{2.5}$ surface chemistry can be analyzed and differentiated using two sensitive surface analytical techniques, XPS and ToFSIMS. $PM_{2.5}$ surfaces in New York City are

similar in overall composition to $PM_{2.5}$ surfaces analyzed in London. Distinct differences in surface chemistry were also found comparing urban $PM_{2.5}$ from different types of locations. In particular, surface oxygen concentrations increased with "aged" $PM_{2.5}$, so that clean air $PM_{2.5}$ was > NYC and London $PM_{2.5}$, which was > tobacco smoke $PM_{2.5}$. It is proposed that such a difference may be an important—and hitherto unconsidered—determinant in the health effects of $PM_{2.5}$ exposure. The wide variations in carbon:oxygen ratios detected could be used to distinguish smoke, urban and "clean air" $PM_{2.5}$. In this study, we also confirmed results from previous studies that $PM_{2.5}$ surfaces interact strongly with BALF over very short periods, and that $PM_{2.5}$ immersed in BALF are desorbed of particular components and coated with bio-molecules. We showed that consistently large increases of the N-C signal from $PM_{2.5}$ surfaces occur as a result of interactions with BALF, and we attribute these increases to protein adsorption.

Long Term Health Effects of Concentrated Ambient $PM_{2.5}$, R827351C013, L.C. Chen, M. Lippmann

Objectives. The Harvard Six Cities Study (Dockery, et al., 1993) and American Cancer Society (ACS) Cohort Study (Pope, et al., 1995) have shown substantially increased mortality in cities with higher average $PM_{2.5}$ concentrations that was not explained by other risk factors. The validity and robustness of the Six Cities and ACS mortality studies findings were confirmed by the Health Effects Institute (HEI) Reanalysis project (Krewski, et al., 2000). The association between chronic exposure to $PM_{2.5}$ and increased mortality was also significant when the ACS and Six Cities studies were extended for an additional 9 years (Pope, et al., 2001; Laden, et al., 2001). The PM effect size estimates reported in these studies are much larger than the cumulative effects reported for acute PM exposure and mortality (EPA, 2001). This finding indicates that people who live in areas with elevated PM experience cumulative adverse health effects in addition to acute transient effects. Increased mortality is not the only adverse health effect associated with PM exposure; several cross-sectional studies of children (Dockery, et al., 1989; Raizennne, et al., 1996) have shown that children who live in cities with higher average $PM_{2.5}$ concentrations have more respiratory symptoms and decreased pulmonary function. Moreover, the Children's Health Study in Southern California has shown that chronic exposure to increased PM pollution is also associated with slower cumulative lung growth (Peters, et al., 1999a; b; Gauderman, et al., 2000; Avol, et al., 2001).

Based on these findings, the NYU PM Center has conducted the first ever subchronic animal inhalation study using concentrated air particles (CAPs) in order to provide supplementary and complementary data analogous to that developed in the human cohort studies in cities with varying levels of fine PM. The studies began in 2002, with daily 6-hour exposures to CAPs for 5 days/week over a six month period. The main focus of this subchronic inhalation study was on the direct and indirect cardiopulmonary effects of PM. **This study tested the hypothesis that subchronic exposure of normal and compromised mice to CAPs will cause cumulative adverse affects on the respiratory and cardiovascular systems.**

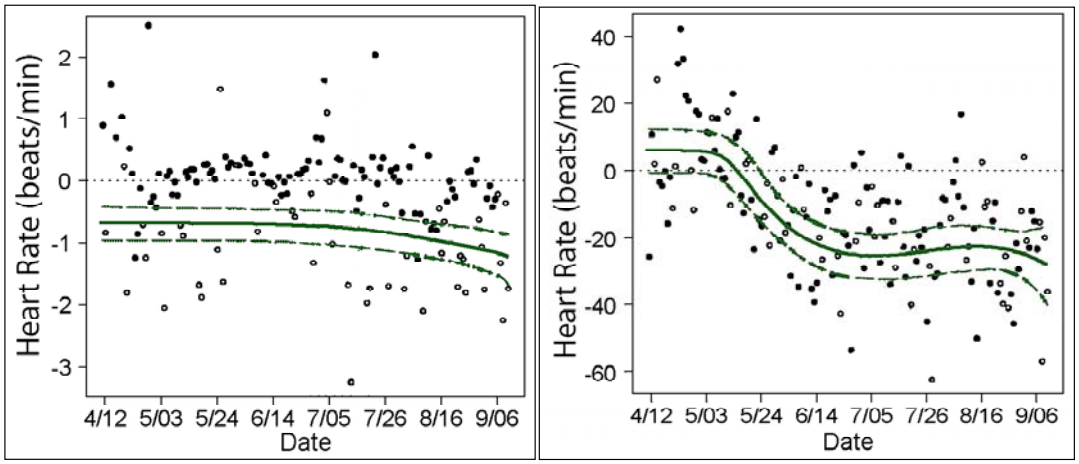
The objectives of this study were to:

1. Determine the effects of subchronic CAPs exposure on pulmonary histopathology and lavage fluid biomarkers of lung inflammation and lung injury.

2. Determine whether subchronic CAPs exposure accelerates the development of atherosclerotic plaques in a mouse model of human atherosclerotic cardiovascular disease (apoE-/- LDLr-/- mice).

Technical Aspects. Drs. Chen and Lippmann conducted the first subchronic animal inhalation screening study using FPM CAPs. A modified VACES (Maciejczyk, et al., 2005) was used to expose mice to at NYU's Sterling Forest Laboratory to a ten-fold concentration of Northeastern regional background FPM CAPs daily for 6 hr/d, 5 d/wk for up to 6 months. A cohort of C57BL/6 (C57) mice was used to investigate effects on the respiratory system. Other cohorts, i.e., C57 (n=6), and $ApoE^{-/-}$ (ApoE knock-out) mice, implanted with ECG transmitters (DataScience), were used to investigate the effects of FPM CAPs on the cardiovascular system. A separate cohort of double knockout mice (DK, ApoE and LDLr knockout) was also included to investigate histopathological changes and gene expression patterns of the cardiovascular and pulmonary systems. The overall mean concentration during the 30 hr/wk FPM CAPs exposure was 110±79 $\mu g/m^3$ (19.6 $\mu g/m^3$ normalized annually). Detailed descriptions were published of the experimental design (Lippmann, et al., 2005a,b), modification of the exposure system (Maciejczyk, et al., 2005), and the observed effects in heart rate (HR), heart rate variability (HRV), atherosclerotic plaques on endothelia, gene expression, and brain cell distributions (Chen and Hwang, 2005; Chen and Nadziejko, 2005; Gunnison and Chen, 2005; Hwang, et al., 2005; Maciejczyk and Chen, 2005; Veronesi, et al., 2005), as well as an overall summary (Lippmann et al., 2005a). Brief descriptions of the study design and some key findings follow.

1. FPM CAPs Induced Alterations in HR and HRV. We used our recently developed non-parametric statistical method (Nadziejko, et al., 2004) to estimate the times that mean heart rates, body temperature, and physical activity differed significantly between the FPM CAPs and sham exposed groups. FPM CAPs exposure most affected HR between 1:30-4:30 AM, and the greatest effects were seen at the end of the 5-month exposure. We used a two-stage modeling approach to obtain the estimates of chronic and acute effects on these three response variables. As shown in Figure 1, there were significant decreasing patterns of HR (reaching a reduction of 33.8 beats/min in HR in FPM CAPs exposed $ApoE^{-/-}$ mice compared to air exposed $ApoE^{-/-}$ controls), body temperature, and physical activity in $ApoE^{-/-}$ mice over the five months of FPM CAPs exposure, with smaller and non-significant changes in C57 mice (Hwang, et al., 2005). In addition, there was a 10 beats/min per 100 $\mu g/m^3$ decrease in HR during the daily exposure period for the $ApoE^{-/-}$ mice that was not seen in the C57 mice.



A B

Figure 1. A: The Posterior Means (Solid) and 95% Equal-Tail Credible Intervals (Dotted) of HR During Exposure (Between 11:00-13:00); and B: Between 1:00 and 4:00 AM for ApoE$^{-/-}$. The circles in the plots are daily crude effects estimated in the 1st stage (full for exposure day and empty for non-exposure day).

At the same time, there was a quite different pattern of change for HRV (SDNN and RMSSD) (Chen and Hwang, 2005). There was a prolonged elevation, peaking at about two months into the study, a decline to below the initial levels by 4 months, and a relatively modest change in the last month of the exposure series. There were no HRV effects seen in normal C57 mice exposed to the same atmospheres. The response patterns indicated a perturbation of the homeostatic function in the cardiovascular system with initial stimulation (enhancement) and later depression of the HRV parameters. Bidirectional response to a low level pollutant challenge in a biological system is not uncommon. For example, the rate of clearance of inert particles from the lung conducting airways in response to sulfuric acid and cigarette smoke have been observed in both humans (Lippmann and Schlesinger, 1984; Lippmann, 2000) and in experimental animals (Chen and Schlesinger, 1983). A transient rise in HR was seen in the first month of FPM CAPs exposure, followed by a substantial decline in the second and third month, and a continued depression to the end of the exposure period. In addition, changes in HR fluctuation (HRF), a measure of variations in HR analogous to HRV, were markedly progressing at the end of the 5-month exposure period (Hwang, et al., 2005). Since the autorhythmicity of the cardiovascular system is modulated by many factors (Stauss, 2003), our results in HR, HRF, and HRV suggest that prolonged exposure to FPM CAPs may be necessary to alter the homeostatic function of the cardiovascular system. The need for prolonged FPM CAPs exposure to induce changes in these cardiac parameters also explained, at least in part, the absence of reported HRV alteration in many of the previous short-term studies involving animal exposures to FPM CAPs and other particles.

Using source apportionment analysis, four major FPM source categories were found in SF FPM CAPs, i.e., secondary sulfate (SS), resuspended soil (RS), residual oil (RO) combustion, and other, largely due to motor vehicle traffic (Maciejczyk and Chen, 2005). We then examined associations between these FPM components and both HR and HRV for three different daily time periods: during exposure, the afternoon after exposure, and late at night (Lippmann, et al., 2005b). For HR there were significant transient associations for RS during exposure, and for SS

in the afternoon after exposure. For HRV, there were comparable associations with RO in the afternoon after exposure and for both SS and RS late at night. The biologic bases for these associations and their temporal lags are not known but may be related to the differential solubility of the biologically active PM components at the respiratory epithelia and their access to cells that release mediators that reach the cardiovascular system. Clearly, further research to elucidate the underlying processes is needed. We will address this issue in our proposed HEI study by having more diverse FPM mixtures for exposure and, as we expect, greater variations in the responses. Using our daily measurements of the FPM components, source apportionments, and regression analyses, we expect to identify the FPM component(s) responsible for the acute and chronic changes in the biological measures of response.

In the 2nd subchronic study conducted between Feb. and May 2004, we investigated the effects of FPM CAPs exposure on the autonomic nervous system (ANS). In this study, we developed a new method to quantify the linear HRV parameters in a nonlinear Poincare plot (Li, et al., Submitted). We used two strains of mice that have been shown to be sensitive to PM exposure in terms of cardiac function changes, i.e., old AKR mice to model an elderly population susceptible to heart failure, and younger ApoE-/- mice to model a younger population susceptible to atherosclerosis. Two exposure groups of each strain (n=8/group) were exposed to SF FPM CAPs or filtered air for 6 hr/d, 5 d/wk from Feb. 10th to May 7th, 2004. Ten second ECG, body temperature, and activity data were sampled throughout the study from each mouse every 5 minutes using implanted ECG transmitters.

By analyzing the baseline pre-exposure ECG waveform data for each mouse for 14 days, we were able to rank their mortality risk levels within each strain. We found that: 1) while no spontaneous deaths occurred in the ApoE-/- group, those AKR mice that died spontaneously, or became very sick during the experiment (based on ECG criteria and visual observation) had higher baseline risk levels (based on their pre-exposure data); 2) exposure to FPM CAPs significantly increased AKR mortality risk; and 3) mice with higher baseline risk deteriorated faster than those with lower baseline risk during exposure to FPM CAPs.

2. FPM CAPs Exposure Enhance Atherosclerotic Lesions. The lungs, the hearts, the aortas, the brains, and the upper airways of all mice in the first FPM CAPs study were harvested for histopathological examination. For all mice, one lung was lavaged for biochemical and cellular endpoints. The other was perfused and stored in with 4% paraformaldehyde, embedded in paraffin, and serial sections prepared for hematoxylin and eosin staining. The heart and thoracic and abdominal aorta of the DK mice were removed *en bloc*, fixed in 4% paraformaldehyde and shipped to Dr. Douglas Taatjes of University of Vermont for quantitative immuno-histochemistry and morphometric analysis of the atherosclerotic lesions of the aorta roots (Chen and Nadziejko, 2005).

The cross sectional area of the aorta root of DK mice was examined morphologically using confocal microscopy for the severity of lesion, extent of cellularity, and lipid contents. Aortas from the arch to the iliac bifurcations were also sectioned longitudinally and lesion areas were stained with Sudan IV (Chen and Nadziejko, 2005). All DK mice, regardless of exposure, had developed extensive lesions in the aortic sinus regions, with lesion areas that covered more than 79% of the total area. In male DK mice, the lesion areas in the aortic sinus regions appeared to

be enhanced by FPM CAPs, with changes approaching statistical significance (p=0.06). In addition, plaque cellularity was increased by 28% (p=0.014) whereas there was no FPM CAPs associated changes in the lipid content in these mice.

When examining the entire aorta opened longitudinally, both the ApoE$^{-/-}$ and DK mice had prominent areas of severe atherosclerosis covering 40% or more of the lumenal surface. Visual examination of all images suggested that plaques tend to form in clusters concentrating near the aortic arch and the iliac bifurcations. Quantitative measurements showed that FPM CAPs exposure increased the percentage of aortic intimal surface covered by grossly discernible atherosclerotic lesion by 57% in the ApoE$^{-/-}$ mice (p=0.03). Changes produced by FPM CAPS in male (10% increase) or female DK mice (8% decrease) were not statistically significant. Thus, subchronic exposure to FPM CAPs in mice prone to develop atherosclerotic lesions had a significant impact on the size, severity, and composition of aortic plaque. Effects of FPM CAPs on non-susceptible C57 mice were minimal.

In the 3rd subchronic study, in collaboration with Drs. Rajagopalan and Sun at Mt. Sinai School of Medicine, we confirmed that FPM CAPs exposure can indeed enhance atherosclerosis in ApoE$^{-/-}$mice and that the effects were dramatically enhanced by feeding mice with a high fat chow (HFC) (Sun, et al., 2005). As shown in Figure 2, at an average exposure concentration of 85 µg/m^3 (14.8 µg/m^3 normalized annually), in the FPM CAPs-HFC group, the mean composite plaque area was 41.5% vs. 26.2% in the filtered air (FA)-HFC group; while plaque area was 19.2% and 13.2% in the FPM CAPs-normal chow (NC) and FA-NC groups, respectively. Lipid content in the aortic arch as measured by oil red-O staining, revealed a 1.5 fold increase in FPM CAPs$_5$-HFC mice vs. the FA-HFC mice.

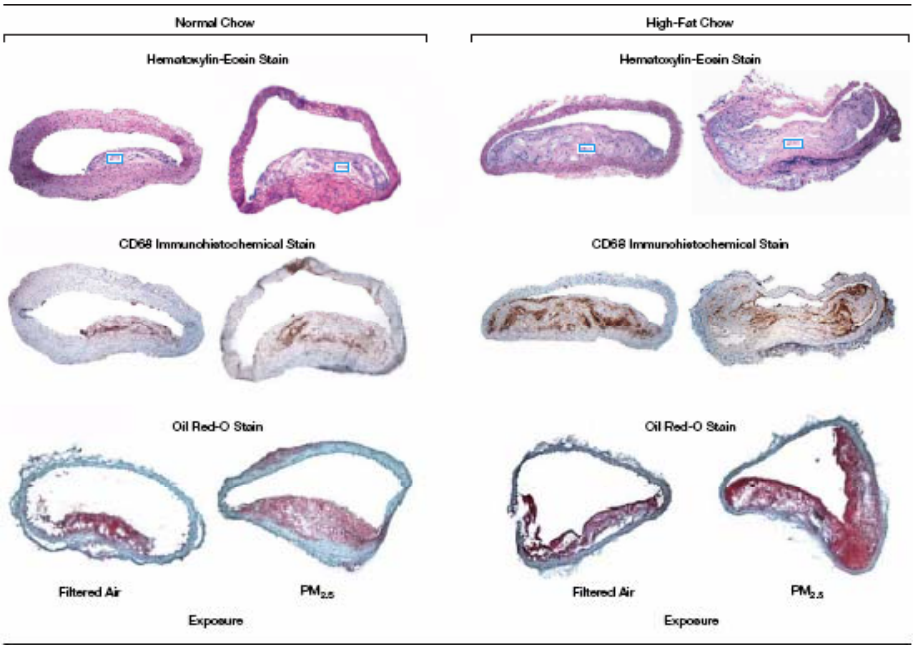


Figure 2. Representative Photomicrographs of Hematoxylin-Eosin Staining, CD68 Immunohistochemical Staining, and Oil Red-o Staining of Aortic Sections.

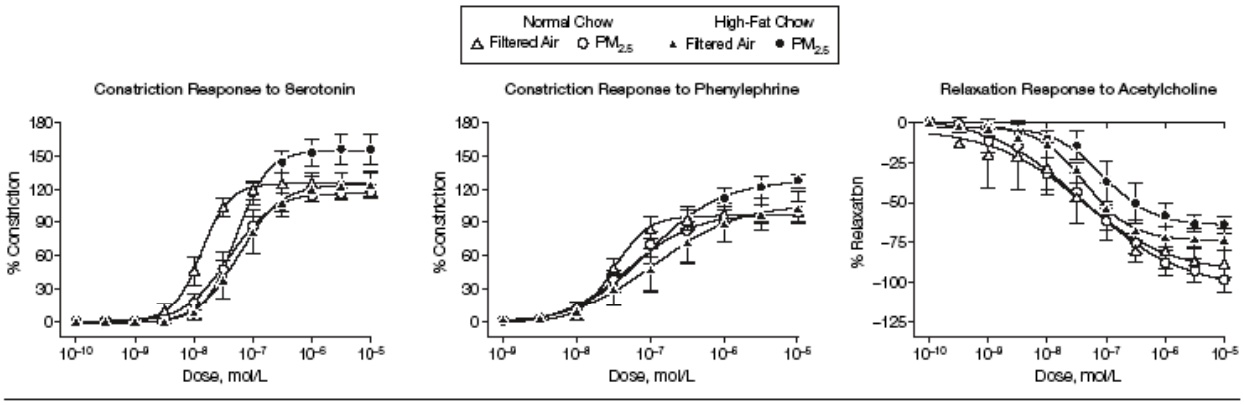


Figure 3. Mean Vasoconstriction of Aortic rings in Response to Serotonin and Phenylephrine, and Vasorelaxation in Response to Acetylcholine. Error bars represent SE. Values represent responses to graded doses of serotonin or phenylephrine expressed as a percentage of the peak response to 120 mEq/L of potassium chloride solution, or responses to graded doses of acetylcholine expressed as a percentage of preconstricted tension in response to serotonin. For serotonin and phenylephrine, *P*=0.03 for mice exposed to CAPs and fed high-fat chow vs. other 3 groups. For acetylcholine, *P*=0.04 for half-maximal dose for dilation vs. all other groups.

In addition, FPM CAPs exposure also attenuates responsiveness to an endothelium-dependent agonist and heightens vasoconstrictor responsiveness. Figure 3 depicts responsiveness to the vasoconstrictors serotonin, phenylephrine, and the endothelium dependent agonist acetylcholine in thoracic aortic segments. Furthermore, vascular inflammation and protein nitration are prominent aspects of FPM CAPs-mediated effects on the vasculature. A 2.3-fold higher iNOS (inducible nitric oxide synthase) content was apparent in the FPM CAPs-HFC group compared with the FA-HFC group, and a 4.0 fold increase in the FPM CAPs-NC compared with the FA-NC group, whereas no significant difference was observed between the groups for eNOS (endothelial NOS) staining. In parallel with elevated iNOS expression, more 3-nitrotyrosine was detected in the plaque from FPM treated mice in both HFC mice and in NC mice.

Our results suggest that even seemingly low concentrations of $PM_{2.5}$ exposure may have detrimental effects on the vasculature and bolster emerging data suggesting progression of carotid intima media thickening, a commonly used surrogate for atherosclerosis (Kunzli, et al., 2005). The concentration used in our study (although enriched) when normalized over a 24-hour/7-day period is well within the range of $PM_{2.5}$ concentrations that individuals living in urban areas such as New York City are exposed to, and thus has implications for the long-term impact of FPM exposure on urban populations. Potentiation of atherosclerosis with FPM was noted in both the thoracic and abdominal aorta and was especially higher in response to high-fat feeding. Furthermore, the percentage increase in plaque burden with $PM_{2.5}$ precisely paralleled the increase in macrophage and fatty infiltration noted in aorta, suggesting that these processes might be related. Thus, our most recent findings provide a potential biological basis for the association between atherosclerosis-related events noted in time-series analysis and prospective population cohort studies (Pope, et al., 2004, Peters, et al., 2004, Dockery, et al., 1993). In the proposed HEI study, we will expand our study to different areas of the U.S. having diverse FPM

compositions and, by using state-of-the-art source apportionment techniques, to identify the source categories or FPM components that are responsible for these effects.

3. CAPs Induced Lesions in CNS. In the 1st subchronic study, the brains of DK mice were preserved in 4% for subsequent histopathology of the brain. Microscopic examination of coronal sections of the brain, immunocytochemically stained for dopaminergic neurons, indicated that the number of neurons in the substantia nigral nucleus compacta were significantly reduced by 29% in FPM CAPs exposed $ApoE^{-/-}$ relative to air exposed $ApoE^{-/-}$ controls. In addition, statistical increases in astrocytes were noted. The dopaminergic neurons of the nucleus compacta is specifically targeted in Parkinson's disease. Our study expands the list of biological tissues affected by PM to include the brain and suggests an environmental role in the development of neurodegeneration in oxidative stress-susceptible individuals (Veronesi, et al., 2005).

4. Gene Expression Levels of Lung and Heart Tissues. At the termination of the 1st subchronic study, the lavaged lungs with the heart attached were removed and the tip of the heart was severed, frozen in liquid nitrogen, stored at -70^{o} C and total RNA was extracted from these tissues, amplified, biotin-labeled and fragmented for hybridization and staining on Affymetrix mouse GeneChips® (430A). Data were normalized using the Robust Multiarray Average (RMA (Irizarry, et al., 2003)) method available in GeneTraffic™ (Iobion Informatics LLC) software and analyzed by the SAM (Significance Analysis of Microarrays) statistical technique (Tusher, et al., 2001) to identify genes that were up- or down-regulated in FPM CAPs-exposed mice relative to sham-exposed (control) mice (Gunnison and Chen, 2005).

Among the lists of heart and lung genes that might be affected by subchronic FPM CAPs exposure, the largest functional category is heat shock and other stress response genes. These genes respond to various stimuli such as elevated temperature, hypoxia, ischemia, hypothermia, free radicals, and certain chemicals. Several heat shock protein genes were down-regulated in FPM CAPs-exposed lungs (Dnaja1, Hspa8, Hsp105, Hspa1a, Hspa1b) and one of these (Hspa1b) also in heart tissue of exposed mice.

In addition to heat shock protein activity, certain other biological processes/molecular functions were affected by FPM CAPs exposures. Among these processes are DNA binding and regulation of transcription (Dbp, Cebpd, Sox4, Anp32a), defense responses (Ngp, Ccr2, Igh-6, Il1b, Igk-V5), proteolysis (Mmp8, Mmp9, Adam8), inflammatory response (Ccr1, Reg3g), and signal transduction and signaling pathways (Il1r2, Ccr1, Ccr2, Iigp, Agtrl1).

One gene, Dbp, that is up-regulated in the lung is of special interest because it has been associated with circadian rhythm, and there is evidence that PM exposure affects cardiac circadian rhythm. The Dbp gene is believed to be either a "clock-controlled" gene or a gene that regulates the output of the "clock", i.e., the suprachiasmatic nucleus of the hypothalamus, which controls the circadian rhythm of physiological processes (Lopez-Molina, et al., 1997; Cheng, et al., 2002). As described earlier, we have shown evidence of perturbation of heart rate circadian rhythm due to FPM CAPs exposures. Therefore, the up-regulation of Dbp in lung tissue of two of the three FPM CAPs-exposed mice in this study merits closer evaluation to investigate a possible connection.

Most of the results of our 2nd (3 winter months) and 3rd (6 summer and fall months) subchronic CAPs exposure studies have not yet been published. In the first paper on results from our 3rd study, using our atherosclerotic mouse model, we showed that CAPs (av. = 85 μg/m^3) enhanced atherogenesis in mice fed with a high-fat diet, with accompanying increases in lipid content, enhanced vasoconstrictor responses to phenylephrine and serotonin challenge in the thoracic aorta, attenuated relaxation to the endothelium dependent agonist against acetylcholine, and marked increases in macrophage infiltration, inducible isoform of nitric oxide synthase, generation of reactive oxygen species, and immunostaining for the protein nitration product 3-nitrotyrosine (Sun, et al., 2005). In ApoE$^{-/-}$ mice on a normal fat diet, some of these effects did not reach a level of statistical significance. Other analyses of results from this third subchronic CAPs inhalation study, i.e., on daily and long-term changes in cardiac function over the six-month exposure period in the ApoE$^{-/-}$ mice on a high fat diet, are included in a paper submitted to *Environmental Health Perspectives*.

During our analysis of the daily variations in cardiac function in our 3rd subchronic CAPs inhalation study, we noted the presence of a number of dramatic changes in cardiac function on certain days in the fall months. These observations led us to analyze the influence of daily variations in FPM component elemental concentrations on acute responses to ambient air FPM in terms of cardiac function in our mouse model of atherosclerosis. We found strong correlations with three metals (Ni, Fe, Cr) that generate reactive oxygen species (ROS).

Unusually high excursions of HR during November and December, 2004, not seen in the previous subchronic mouse CAPs inhalation studies, were noted, and we proceeded to examine the associations of HR and HRV with the FPM mass and elemental concentrations that were measured each exposure day. We found that the closest associations were with Ni and Cr, and that the days with high Ni and Cr had unusually low FPM mass concentrations. Figure 4 summarizes the differences between the 14 days with unusually elevated HRs and all of the other exposure days in terms of the exposure chamber concentrations of FPM, Al, S, V, Cr, Fe, Ni, Se, and Br, along with the average difference in HR and HRV between the CAPs exposed and air-sham exposed ApoE$^{-/-}$ mice. Assuming that the Ni, Cr, and Fe were associated with sulfate, they accounted for 12.4% of the FPM mass on those 14 days, and only 1.5% on the other days.

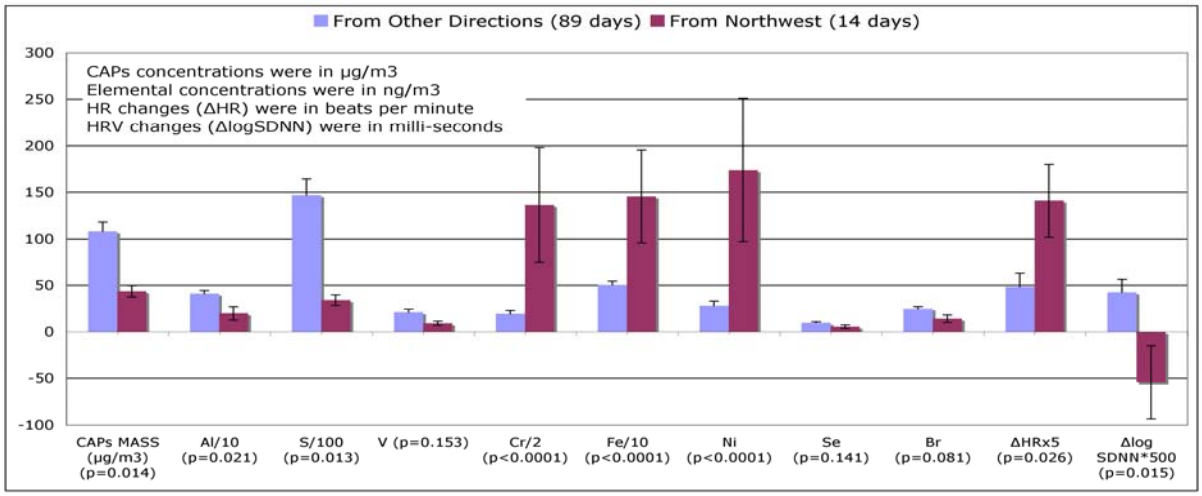


Figure 4. Average Elemental Concentrations and HR and HRV for 14 days When Winds were from the Northwest (right bar) and that for the 89 Days with Winds from all Other Directions and the Differences in Heart Rates of $ApoE^{-/-}$ Mice Exposed to CAPs and Filtered Air. CAPs concentrations were in $\mu g/m^3$, elemental concentrations were in ng/m^3, HR in beats/min, HRV (as log SDNN) in milliseconds. Error bars are ± SE.

Back Trajectory Analyses. We next obtained back trajectory maps for the 14 days with the most notably elevated HRs, which all were associated with high-altitude winds from the northwest (see Figure 4). The 72-hour back trajectories from Sterling Forest for these 14 days appear to avoid population centers and industrial areas other than the Ni smelter near Sudbury, Ontario, which discharges its airborne effluents through a very tall stack.

The results reported here, from our third subchronic CAPs inhalation study in a mouse model of atherosclerosis indicate that inhalation exposure to Ni, more than V, is a more likely causal factor for the exacerbation of cardiac disease. If Ni inhalation, at current ambient air concentrations, does appreciably affect cardiac function and mortality in humans, the reader may wonder why it has not previously been recognized. One reason may be that the increment in cardiovascular mortality that Ni may produce is a relatively small part of the very large cardiovascular mortality. Also, the statistically significant transient and progressive changes that we have seen in cardiovascular function in our mice are relatively subtle, require advanced analytical techniques for their detection, and are unlikely to be detected in the kinds of short-term exposure studies that have previously been undertaken in laboratory animals.

In terms of environmental relevance, it is important to recognize that the peak Ni concentrations in the CAPs were only ~175 ng/m^3 on the peak Ni exposure days, and only 26 ng/m^3 on the 89 other days, and there were no pronounced peaks for V (average ~17 ng/m^3). Thus, Ni appears to be the component most likely to be causal for acute cardiac responses. The long-term average ambient air level of Ni in the U.S. is 1.9 ng/m^3, and the highest, in NYC, is 19 ng/m^3. Biological mechanisms that could account for the significant associations between Ni and the progression of cardiovascular disease in the mice, or with cardiovascular mortality in people exposed at low, environmentally relevant, ambient air concentrations is unknown, and warrants further, mechanistically oriented research in animals in vivo and cells in vitro.

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PM Components and NYC Respiratory and Cardiovascular Morbidity, R827351C014, K. Ito

Objectives. This project took advantage of the continuous PM_{10} and $PM_{2.5}$ data, as well as the newly available $PM_{2.5}$ chemical speciation data, collected in NYC. The project aimed to identify key PM components and source types that are associated with respiratory and cardiovascular morbidity, and to provide excess risk estimates for sub-populations that are characterized by age group, diagnoses, and sub-areas within NYC.

Summary of Findings. We retrieved all the relevant air pollution variables: particulate matter less than 2.5 μm, ($PM_{2.5}$) collected by the 24-hr filter samples using Federal Reference Method (FRM), $PM_{2.5}$ chemical speciation data, $PM_{2.5}$ and PM_{10} data measured by the Tapered Element Oscillating Microbalance (TEOM), ozone (O_3), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and carbon monoxide (CO). $PM_{2.5}$ data were available from 1999, and we therefore evaluated the influence of $PM_{2.5}$ and other co-pollutants for the years 1999-2002. All the pollution data were retrieved from the EPA's Air Quality System (AQS). Hourly readings were available for the gaseous pollutants and the TEOM PM data. Past studies have used O_3 exposure indices with varying averaging time (e.g., daily maximum of 1-hr averages, daily 24-hr average, and daily maximum of 8-hr averages). Since the current O_3 standard is set for the daily 8-hr maximum (the average of the fourth highest of which over three years is not to exceed 80 ppb), to facilitate easier interpretation, we focus on the daily 8-hr maximum values but also examined the 1-hr maximum and the 24-hr average values. For other pollutants, we also used the exposure indices that were used for the air quality standards (i.e., 24-hr average for $PM_{2.5}$ and SO_2; daily maximum of 1-hr average for CO). There is no daily standard for NO_2, and we used the 24-hr average values. The data from all the air quality monitors within a 20-mile radius from the geographic center of NYC were obtained, and the averages for multiple monitors were computed for each day. There were 17 O_3 monitors with this inclusion criterion, but the data from a monitor at the top of the World Trade Center was excluded because of its height (it read higher readings than the nearby monitors on the ground level). There were 33 monitors for the FRM $PM_{2.5}$; 18 monitors for CO; 15 monitors for NO_2; and 19 monitors for SO_2. There were five sites where both $PM_{2.5}$ and PM_{10} were measured by co-located TEOM monitors. Therefore, to

estimate the coarse mass fraction, $PM_{10-2.5}$ we subtracted the $PM_{2.5}$ from PM_{10} for each site, and averaged across the five sites. $PM_{2.5}$ chemical speciation data were available from three sites: 1) New York Botanical Gardens (NYBG) in Bronx; 2) I.S. 52 in Bronx; and 3) Queens College (QC) in Queens. The data are available starting from 2000 for NYBG and from 2001 for I.S. 52 and QC. Available PM components were: 1) $PM_{2.5}$ particulate mass; 2) anions (sulfate, nitrate) and cations (particulate ammonium, sodium, and potassium) by ion chromatograph; 3) trace elements (about 20 key elements from sodium through lead on the periodic table) by energy dispersive X-ray fluorescence (EDXRF); and 4) total carbon including organic, elemental, and carbonate carbon by thermal optical analysis. To adjust for weather effects on morbidity outcomes, we considered daily 24-hr average temperature and daily maximum relative humidity from La Guardia airport.

First, we examined the associations between each of the size-fractionated PM indices and asthma emergency department (ED) visits from the 11 NYC municipal hospitals for the years 1999-2002. The data were analyzed for the full-year period (excluding September and October to avoid the influence of the fall peaks in asthma ED visits), as well as for warm (April-August) and cold (November-March) season subsets. A total of 167,900 asthma ED visits were analyzed for the four-year period. A Poisson Generalized Linear Model (GLM) was used to estimate the impact of the average of 0- and 1-day lags of $PM_{2.5}$ and $PM_{10-2.5}$ on the asthma ED visits, adjusting for weather effects, temporal trends, and day-of-week. We modeled immediate and potentially non-linear temperature effects by including a smooth function (natural spline) of same-day temperature with three degrees of freedom. Likewise, we included a smooth function of the average of past 2- and 3-day temperatures with three degrees of freedom to adjust for any delayed and possibly non-linear effects of temperature. To model the effects of interaction of heat and humidity effects, an indicator variable was included for hot (temperature > 78°F) and humid (relative humidity > 80%) days. Relative risks were computed per 5th-to-95th percentile increments of concentration (i.e., representing "low" to "high" levels) of O_3 or $PM_{2.5}$. Both $PM_{2.5}$ (RR = 1.11; 95% CI: 1.04, 1.18, per 5th-to-95th percentile increment) and $PM_{10-2.5}$ (RR = 1.12; 95% CI: 1.06, 1.18) were associated with asthma ED visits in the full-year data. In the warm season, the risk estimate for $PM_{10-2.5}$ (RR = 1.25; 95% CI: 1.15, 1.36) was somewhat higher than that for $PM_{2.5}$ (RR = 1.15; 95% CI: 1.03, 1.27). In the cold season, $PM_{10-2.5}$ was not associated with asthma ED visits, and $PM_{2.5}$ was only weakly associated. We concluded that coarse summertime PM may be an important component that exacerbates asthma in NYC.

We also conducted regression analysis using the source-apportioned $PM_{2.5}$ that we constructed in Ito, et al. (2004). Two sets of source-apportioned $PM_{2.5}$ data were available, one using Absolute Principal Component Analysis (APCA) and the other using Positive Matrix Factorization (PMF). As described in Ito, et al. (2004), four major source types were identified using both methods: secondary sulfate, traffic-related particles, residual oil combustion/incineration effluents, and re-suspended soil. For each set, we averaged the source-apportioned $PM_{2.5}$ across the three monitors. The same Poisson GLM model described above was employed, except that 0- and 1-day lagged PM component variables were examined separately because of the every-3rd-day sampling frequency. Unfortunately, because of this sampling frequency, the total available number of days (< 180 days) for the study period did not provide sufficient statistical power for the expected magnitude of risk estimates. Most of the source-apportioned $PM_{2.5}$'s risk estimates were positive, but none were statistically significant. Generally, the risk estimates were larger

for warm season than for year-round or cold season. For example, the relative risks per 5th-to-95th percentile of source-apportioned $PM_{2.5}$ at lag 0 day using the APCA set were: 1.15 (95% CI: 0.83, 1.59); 1.03 (95% CI: 0.88, 1.22); 1.02 (95% CI: 0.92, 1.14); and, 1.00 (95% CI: 0.94, 1.067) for residual oil combustion/incineration, secondary sulfate, soil, and traffic, respectively.

Gaseous pollutants were also analyzed using the same regression models. In the warm season, O_3 (RR=1.13; 95% CI: 1.03, 1.23]), NO_2 (RR=1.17; 95% CI: 1.08, 1.25), and CO (RR=1.12; 95% CI: 1.02, 1.22) were each associated with asthma ED visits, and O_3 and NO_2 associations appeared to be independent of $PM_{2.5}$. In the cold season, gaseous pollutants' associations with asthma ED visits were generally weaker.

While we had a sufficient number of observations to examine the effects of size-fractionated PM, the sample size for the $PM_{2.5}$ speciation data was less than desirable.

Conclusion. We found that both $PM_{2.5}$ and $PM_{10-2.5}$ were associated with asthma ED visits in this data set. $PM_{10-2.5}$ in warm season may be an important component that exacerbates asthma in NYC. NO_2 and O_3 also appear to independently contribute to excess asthma ED visits in warm months. An examination of specific PM chemical component(s) responsible for the observed associations requires a larger sample size (more days).

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Supplemental Keywords: NA

Relevant Web Sites: http://www.med.nyu.edu/environmental/
http://es.epa.gov/ncer/science/pm/centers.html