

Final Technical Report

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Center Name: NYU-EPA PM Center: Health Risks of PM Components

Center Director: Morton Lippmann

Title: Asthma Susceptibility to PM_{2.5} (Particulate Matter $\leq 2.5 \mu\text{m}$)

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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: 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 New York University (NYU)-U.S. Environmental Protection Agency (EPA) PM Research Center (e.g., via controlled exposure studies).

Summary of Findings:

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”=PM₁₀ $\geq 40 \mu\text{g}/\text{m}^3$, while “Low”=PM₁₀ $\leq 20 \mu\text{g}/\text{m}^3$.

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 FEV₁ at the start of the procedure, and after each period of saline inhalation. If the FEV₁ 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), FEV₁ ($\leq 85\%$ and $>50\%$ of predicted), and smoking history (≤ 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, O₃) 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 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 $\mu\text{g}/\text{m}^3$ to nearly 60 $\mu\text{g}/\text{m}^3$. 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 O₃ 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 O₃ levels, although only for O₃ was the association marginally significant. Mean daily O₃ levels were also considered, but the associations were stronger (more positive and significant) for maximum hourly O₃ 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.

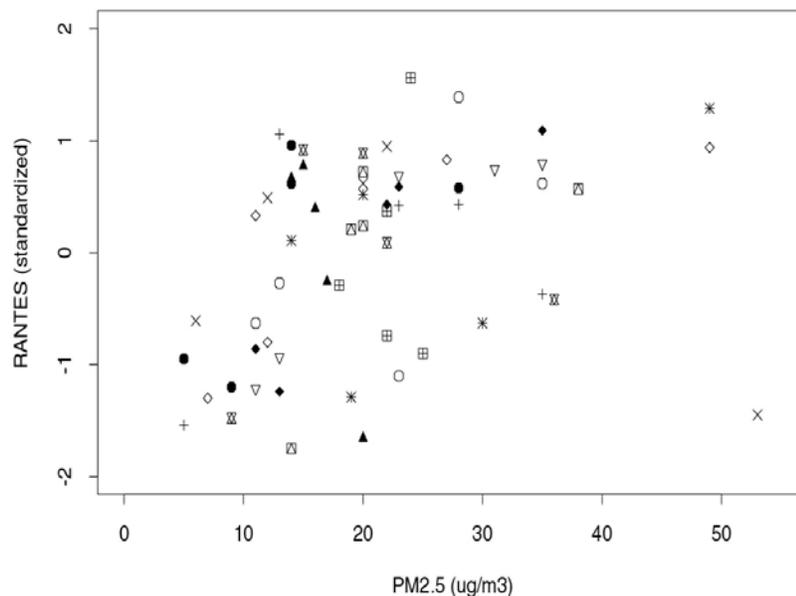


Figure 1. Plot of Serum RANTES Concentrations vs. Ambient PM_{2.5} During 2001

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

$\mu\text{g}/\text{m}^3$), 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 $\text{PM}_{2.5}$), and deviation from the mean (14.1% increase per IQR of $\text{PM}_{2.5}$) 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 $\text{PM}_{2.5}$).

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 $\text{PM}_{2.5}$ 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.

<u>Cytokine Format</u>	<u>Pollutant (Lag)</u>	<u>Analyses</u>	<u>Z Statistic</u>	<u>% Mean</u>	
				<u>Slope</u>	<u>(95% Confidence Intervals)</u>
RANTES (Concentration: pg/ml)					
	$\text{PM}_{2.5}$ (same day)	Fully-Adjusted*	2.13	18.94%	(1.51, 36.36)%
	O_3^\ddagger (same day)	Fully-Adjusted	-0.46	-3.82%	(-20.09, 12.45)%
RANTES (Z-transformed) [‡]					
	$\text{PM}_{2.5}$ (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) [§]					
	$\text{PM}_{2.5}$ (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.

† O₃ = 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.

Supplemental Keywords: NA

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