



RESEARCH REPORT

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National Particle Component Toxicity (NPACT) Initiative: Integrated Epidemiologic and Toxicologic Studies of the Health Effects of Particulate Matter Components

Morton Lippmann, Lung-Chi Chen, Terry Gordon, Kazuhiko Ito,
and George D. Thurston

NPACT Study 1. Subchronic Inhalation Exposure of Mice to Concentrated Ambient PM_{2.5} from Five Airsheds

Lung-Chi Chen and Morton Lippmann

NPACT Study 2. In Vitro and in Vivo Toxicity of Exposure to Coarse, Fine, and Ultrafine PM from Five Airsheds

Terry Gordon, Morton Lippmann, Arthur Nádas, and Christina Hickey

NPACT Study 3. Time-Series Analysis of Mortality, Hospitalizations, and Ambient PM_{2.5} and Its Components

Kazuhiko Ito, Zev Ross, Jiang Zhou, Arthur Nádas, Morton Lippmann,
and George D. Thurston

NPACT Study 4. Mortality and Long-Term Exposure to PM_{2.5} and Its Components in the American Cancer Society's Cancer Prevention Study II Cohort

George D. Thurston, Kazuhiko Ito, Ramona Lall, Richard T. Burnett,
Michelle C. Turner, Daniel Krewski, Yuanli Shi, Michael Jerrett,
Susan M. Gapstur, W. Ryan Diver, and C. Arden Pope III



Includes a Commentary by the Institute's NPACT Review Panel

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ABOUT HEI

The Health Effects Institute is a nonprofit corporation chartered in 1980 as an independent research organization to provide high-quality, impartial, and relevant science on the effects of air pollution on health. To accomplish its mission, the institute

- Identifies the highest-priority areas for health effects research;
- Competitively funds and oversees research projects;
- Provides intensive independent review of HEI-supported studies and related research;
- Integrates HEI's research results with those of other institutions into broader evaluations; and
- Communicates the results of HEI's research and analyses to public and private decision makers.

HEI typically receives half of its core funds from the U.S. Environmental Protection Agency and half from the worldwide motor vehicle industry. Frequently, other public and private organizations in the United States and around the world also support major projects or research programs. For the research funded under the National Particle Component Toxicity Initiative, HEI received additional funds from the American Forest & Paper Association, American Iron and Steel Institute, American Petroleum Institute, ExxonMobil, and Public Service Electric and Gas.

HEI has funded more than 280 research projects in North America, Europe, Asia, and Latin America, the results of which have informed decisions regarding carbon monoxide, air toxics, nitrogen oxides, diesel exhaust, ozone, particulate matter, and other pollutants. These results have appeared in the peer-reviewed literature and in more than 200 comprehensive reports published by HEI.

HEI's independent Board of Directors consists of leaders in science and policy who are committed to fostering the public-private partnership that is central to the organization. The Health Research Committee solicits input from HEI sponsors and other stakeholders and works with scientific staff to develop a Five-Year Strategic Plan, select research projects for funding, and oversee their conduct. The Health Review Committee, which has no role in selecting or overseeing studies, works with staff to evaluate and interpret the results of funded studies and related research. For the NPACT studies, a special NPACT Review Panel — comprising Health Review Committee members and outside experts — fulfilled that role.

All project results and accompanying comments by the Health Review Committee are widely disseminated through HEI's Web site (www.healtheffects.org), printed reports, newsletters and other publications, annual conferences, and presentations to legislative bodies and public agencies.

ABOUT THIS REPORT

Research Report 177, *National Particle Component Toxicity (NPACT) Initiative: Integrated Epidemiologic and Toxicologic Studies of the Health Effects of Particulate Matter Components*, presents a research project funded by the Health Effects Institute and conducted by Dr. Morton Lippmann and his colleagues: Drs. Lung-Chi Chen (Study 1), Terry Gordon (Study 2), Kazuhiko Ito (Study 3), and George D. Thurston (Study 4). During the conduct of this project, Drs. Lippmann, Chen, Gordon, Ito, and Thurston were all at the Department of Environmental Medicine, New York University School of Medicine, Tuxedo, New York. This report contains the following sections:

The HEI Statement, prepared by staff at HEI, is a brief, nontechnical summary of the study and its findings; it also briefly describes the HEI NPACT Review Panel's comments on the study.

The Investigators' Report comprises Studies 1 and 2 (toxicologic studies), Study 3 (time-series analysis), and Study 4 (mortality analyses). Each describes the scientific background, aims, methods, results, and conclusions of that portion of the project. Also included is an overall summary of the study and its conclusions.

The Commentary, prepared by members of the HEI NPACT Review Panel (see below) with the assistance of HEI staff, places the study in a broader scientific context, points out its strengths and limitations, and discusses remaining uncertainties and implications of the study's findings for public health and future research.

The Synthesis, also prepared by the HEI NPACT Review Panel, provides a summary evaluation of the NPACT Initiative, which includes this Research Report and the accompanying HEI Research Report 178 by Dr. Sverre Vedal and colleagues, and puts the results of the NPACT Initiative in a broader context.

This report has gone through HEI's rigorous review process. When an HEI-funded study is completed, the investigators submit a draft final report presenting the background and results of the study. The draft report was evaluated by the HEI NPACT Review Panel, an independent panel of distinguished scientists, including some members of the HEI Health Review Committee, who had no involvement in selecting or overseeing these studies. Comments from the Panel were sent to the investigators, who revised their report as they considered appropriate. The revised report was again evaluated by the Panel, which then prepared the Commentary based on the final version of the report.

CONTRIBUTORS

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PREFACE

HEI's Research Program on Particle Component Toxicity

INTRODUCTION

Findings from epidemiologic and controlled-exposure studies about the health effects of particulate matter (PM) have led the U.S. Environmental Protection Agency (EPA) and other regulatory agencies to establish mass-based ambient air quality standards for PM within a specific size range. PM with an aerodynamic diameter $\leq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) is considered to be particularly important because the small particles can be easily inhaled. Because the composition of PM is complex, there has long been a question as to whether some components of the PM mixture are of greater public health concern than others. Obtaining this information would help focus efforts to reduce people's exposure by enabling the control of those sources that contribute most of the toxic components in the PM mixture.

Detailed information on $\text{PM}_{2.5}$ composition began to be collected systematically in the year 1999, in what was then called the Speciation Trends Network (currently the Chemical Speciation Network [CSN]). In an effort to consolidate the available data from several data sources and make them more accessible to researchers, HEI funded the company Atmospheric and Environmental Research (AER) through a December 2003 Request for Proposals (RFP) (titled *To Create a Database of Air Pollutant Components*) to set up and maintain such a database. The resulting HEI Air Quality Database (<https://hei.aer.com>) was launched by AER in September 2005 and comprises data from the EPA's monitoring networks, particularly concentrations of $\text{PM}_{2.5}$ components and gaseous pollutants at and near sites in the CSN and state, local, and tribal air monitoring stations. Currently, the database contains information on speciated PM components and gaseous pollutants at these sites for the years 2000 to the present.

While the Air Quality Database was under construction, HEI issued Request for Applications (RFA) 05-1-A, *Conducting Full Studies to Compare Characteristics of PM Associated with Health Effects*. Its goal was to support integrated multidisciplinary studies — including epidemiology, toxicology, exposure science, and statistics — to investigate the health effects of PM components in

humans and animal models at locations across the United States where PM sources and components differ. The comparison of PM component effects was to be made in the context of the contribution of gaseous copollutants to the air pollution mixture and its health effects, as well as to PM-related toxicity and health effects.

RFA 05-1-A was accompanied by RFA 05-1-B, *Conducting Planning or Demonstration Studies to Design a Major Study to Compare Characteristics of PM Associated with Health Effects*, in order to provide a smaller amount of funding to multidisciplinary study teams that had not previously worked together. These teams would then conduct planning or demonstration studies to gather and analyze the data necessary to design a full study of the toxicity of PM components, similar to those funded under RFA 05-1-A.

DESCRIPTION OF THE NATIONAL PARTICLE COMPONENT TOXICITY INITIATIVE

HEI's National Particle Component Toxicity (NPACT) Initiative was launched in view of emerging evidence that the composition of PM is different in different places as well as that there are geographic differences in the toxicity of PM across the country. Given the complexity and importance of these issues, HEI organized several workshops and held extensive discussions and consultations about the best approaches to investigate these questions. These deliberations resulted in the publication of several RFAs and the funding of two major studies. The primary goal of the NPACT Initiative was to determine if components of PM from various sources are equally toxic to health, or if some components are more toxic than others. A summary of the studies funded under the NPACT Initiative is provided in the table.

HEI funded two major NPACT studies under RFA 05-1-A, which combined coordinated efforts in (1) exposure assessment using advanced techniques, (2) epidemiology focusing on PM components and long-term health effects, and (3) toxicology focusing on endpoints that are relevant to the cardiovascular and other health

effects observed in epidemiologic studies. Each main study comprised several studies, led by co-investigators, looking at different aspects of the questions regarding the cardiovascular and other health effects of short- and long-term exposure to PM components, using exposure assessment, epidemiologic approaches, and toxicologic approaches that would complement each other.

The two major NPACT studies were led by Dr. Morton Lippmann at New York University and Dr. Sverre Vedal at the University of Washington. Dr. Lippmann's study comprised two toxicologic studies led by Drs. Lung-Chi Chen and Terry Gordon and two epidemiologic studies led by Drs. Kazuhiko Ito and George Thurston. Dr. Vedal's study comprised an epidemiologic study of two cohorts, as described below, and a toxicologic study conducted by Drs. Matt Campen at the University of New Mexico and Jacob McDonald at the Lovelace Respiratory Research Institute.

At the time of funding for the two integrated NPACT studies, HEI was already supporting a time-series epidemiologic study of PM components by Dr. Michelle Bell at Yale University (RFA 04-2, *Walter A. Rosenblith New Investigator Award*). Because the topic was very relevant to the NPACT Initiative, HEI decided to include this study under the broader umbrella of NPACT (although the study was reviewed separately and published earlier).

Oversight of the NPACT Studies

Given the complexity of the NPACT studies, the HEI Research Committee formed an NPACT Oversight Committee composed of Research Committee members and additional technical experts. The Oversight Committee met approximately annually with the investigator teams during the conduct of the study and provided advice and feedback on the study design, analytical plans, and progress. The Oversight Committee members are listed on the Contributors page of this report.

In addition, HEI formed an NPACT Advisory Group, which included representatives from the EPA and industry sponsors of the NPACT studies, as well as other interested stakeholders. The advisory group met with the NPACT investigators to discuss study designs, progress, and other key issues.

Study by Lippmann et al.

In one of the two toxicologic studies, Drs. Chen and Lippmann used an animal inhalation approach to evaluate the effect of PM components on cardiovascular endpoints in vivo. They selected a mouse model of the development of atherosclerosis, the underlying cause of

most chronic cardiovascular disease. The mice were exposed for 6 months to fine concentrated ambient particles (CAPs) collected at five different sites across the United States. These CAPs represented ambient pollutant mixtures from diverse locations that are dominated by different source categories, including coal combustion, wood smoke, and traffic. For their comprehensive assessment of the cardiovascular toxicity of PM_{2.5} components, Chen and Lippmann chose two cardiovascular endpoints (atherosclerotic plaque progression and heart rate variability) to represent the long- and short-term effects of CAPs exposure, respectively, as well as a number of additional markers of inflammation, oxidative stress, and cardiovascular changes.

In the second toxicologic study, Dr. Gordon and colleagues used a combined in vitro and in vivo approach to analyze acute toxicity of a large number of PM samples collected at five locations identical or in close proximity to the sites studied by Chen and Lippmann. The overall goal was to examine the toxicity of exposure to PM of varying composition and size. At each location, daily PM filter samples were collected in three size fractions (coarse, fine, and ultrafine) over a 2-week period during two seasons. Each individual sample was tested in a cell culture or administered to mice by aspiration into the lung. In addition, the investigators conducted a longer-term study in two of the five locations, in which 100 daily samples were collected and administered to mice by aspiration.

In the first epidemiologic study, Dr. Ito and colleagues examined associations between short-term exposure to ambient air pollution and mortality (for all ages) and hospital admissions among people 65 years or older, using a multicity two-stage time-series study design. The investigators based their analyses on exposure to ambient concentrations of individual components of PM_{2.5}, using CSN data from 150 cities in the United States and a subset of 64 cities where gaseous pollutant data were also available. The investigators also conducted source apportionment, partitioning the daily PM_{2.5} mass into separate factors attributed to different source categories. Mortality data were available for the years 2000 to 2006, and hospitalization data for the years 2000 to 2008. The investigators ran city-specific analyses and combined the results using second-stage random effects models.

In the second epidemiologic study, Dr. Thurston and colleagues expanded previous analyses of the American Cancer Society's Cancer Prevention Study (CPS-II) cohort. They used data from the cohort to evaluate associations between long-term exposure to speciated

Preface

HEI's NPACT Studies

RFA/RFP ^a Investigator (Institution)	Study or Report Title	Citation or PI
RFP December 2003: To Create a Database of Air Pollutant Components		
Christian Seigneur (AER)	Creation of an Air Pollutant Database for Epidemiologic Studies	https://hei.aer.com
RFA 04-2: Walter A. Rosenblith New Investigator Award		
Michelle Bell (Yale University)	Assessment of the Health Impacts of Particulate Matter Characteristics	Bell 2012
RFA 05-1-A: Conducting Full Studies to Compare Characteristics of PM Associated with Health Effects		
Morton Lippmann (New York University)	National Particle Component Toxicity (NPACT) Initiative: Integrated Epidemiologic and Toxicologic Studies of the Health Effects of Particulate Matter Components	
	NPACT Study 1. Subchronic Inhalation Exposure of Mice to Concentrated Ambient PM _{2.5} from Five Airsheds	Chen
	NPACT Study 2. In Vitro and In Vivo Toxicity of Exposure to Coarse, Fine, and Ultrafine PM from Five Airsheds	Gordon
	NPACT Study 3. Time-Series Analysis of Mortality, Hospitalizations, and Ambient PM _{2.5} and Its Components	Ito
Sverre Vedal (University of Washington)	NPACT Study 4. Mortality and Long-Term Exposure to PM _{2.5} and Its Components in the American Cancer Society's Cancer Prevention Study II Cohort	Thurston
	National Particle Component Toxicity (NPACT) Initiative Report on Cardiovascular Effects. Section 1. NPACT Epidemiologic Study of Components of Fine Particulate Matter and Cardiovascular Disease in the MESA and WHI-OS Cohorts	Vedal
	National Particle Component Toxicity (NPACT) Initiative Report on Cardiovascular Effects. Section 2. NPACT Animal Toxicologic Study of Cardiovascular Effects of Mixed Vehicle Emissions Combined with Non-vehicular Particulate Matter	Campan
RFA 05-1-B: Conducting Planning or Demonstration Studies to Design a Major Study to Compare Characteristics of PM Associated with Health Effects		
JoAnn Lighty (University of Utah)	A planning study to investigate the impacts of dust and vehicles on acute cardiorespiratory responses in the arid Southwest	Lighty et al. 2008 (unpublished report)

^a RFA indicates request for applications; RFP indicates request for proposals.

components of PM_{2.5} and all-cause, cardiovascular, and pulmonary mortality during the years 1982 to 2004. This analysis included approximately 450,000 members of the cohort residing in the 100 metropolitan statistical areas for which measurement data for PM_{2.5} components were available. In primary analyses, the investigators averaged all available measurements of 24-hour

PM_{2.5} component concentrations obtained from the CSN for the years 2000 to 2005. In secondary analyses, they constructed source categories using the PM_{2.5} component data. They also applied a novel approach to estimate the relative impacts of component mixtures in a multipollutant environment, using a total relative risk impact method.

Study by Vedal et al.

Dr. Vedal's epidemiologic study assessed the effects of long-term exposure to fine PM components and emission sources on cardiovascular endpoints, using data from two established cohort studies sponsored by the National Heart, Lung, and Blood Institute (specifically, the Women's Health Initiative-Observational Study [WHI-OS] and the Multi-Ethnic Study of Atherosclerosis [MESA]). The WHI-OS cohort included more than 93,000 postmenopausal women, between 50 and 79 years old, from 46 U.S. cities. They were evaluated at baseline between 1994 and 1998 for cardiovascular disease risk factors and followed annually through 2005. In addition, two five-year extension studies were initiated in 2005 and 2010. The MESA cohort study recruited more than 6000 participants without known heart disease between 2000 and 2002. Participants were from diverse ethnic or racial groups (major categories were white non-Hispanic, African American, Chinese American, and Hispanic), were between 45 and 84 years old, and lived in one of six metropolitan areas: New York, New York; Los Angeles, California; Chicago, Illinois; Winston-Salem, North Carolina; St. Paul, Minnesota; and Baltimore, Maryland.

The specific aim of the Vedal team's analyses within both cohorts was to identify the chemical components of ambient PM that contribute to the incidence of cardiovascular events. The major hypothesis was that PM_{2.5} components in primary emissions from motor vehicles have a greater effect on long-term cardiovascular toxicity than do inorganic or crustal components in secondary PM.

In the WHI-OS analyses, Vedal and colleagues assessed associations of concentrations of PM_{2.5} and four major components — elemental carbon, organic carbon, sulfur, and silicon measured at government monitoring sites across the United States — with cardiovascular health outcomes. These outcomes included myocardial infarction, stroke, mortality due to cardiovascular or cerebrovascular diseases, hospitalization for coronary heart disease or angina pectoris, and coronary revascularization procedures including bypass surgery and angioplasty.

In the MESA analyses, Vedal and colleagues assessed associations of concentrations of PM_{2.5} and its components and subclinical markers of atherosclerosis, primarily coronary artery calcification and carotid intima-media thickness. The investigators developed a spatio-temporal exposure model that included estimates at the individual home level and a national spatial exposure model that used data from numerous monitoring sites

to estimate spatial variability of specific PM_{2.5} components in the six metropolitan areas. The investigators focused some of their analyses on data from MESA-Air, an ancillary MESA study funded in 2004 by the EPA, which included monitoring at three additional locations: along the coast in Los Angeles, California; inland in Riverside, California; and in Rockland County, New York, a suburban area outside New York City.

The toxicology component of the Vedal study consisted of an animal inhalation study in which Apo-E knockout mice were exposed to a mixture of diesel and gasoline engine exhaust (MVE) or to non-vehicular particles (specifically, sulfate, nitrate, and road dust filtered to include only fine PM), or to combinations of MVE and non-vehicular particles. In addition, the investigators evaluated mice exposed to MVE gases only — with the particles filtered out — or to combinations of MVE gases with each of the non-vehicular particles listed above. The mice were exposed for 50 days and evaluated for markers of oxidative stress, inflammation, and cardiovascular outcomes. Responses were compared among mice exposed to the various mixtures and mice exposed to filtered air, providing an indication of which mixtures may be more toxic than others. In addition, the investigators used a statistical approach to evaluate the role of individual components of the mixtures.

Study by Bell

Dr. Bell evaluated short-term effects of PM components on mortality in more than 187 counties across the United States (as reported in her study *Assessment of the Health Impacts of Particulate Matter Characteristics* [2012]). She was one of the first researchers to make use of the data that would later make up the CSN database, and she applied the time-series approach developed in the National Morbidity, Mortality, and Air Pollution Study (Samet et al. 2000) to look for associations between PM component concentrations and mortality and morbidity outcomes. Bell obtained data on PM_{2.5} total mass and on the mass of 52 chemical components of PM_{2.5} for air monitored in 187 counties in the continental United States for the period 2000 through 2005. She also collected data on daily admissions to hospitals for cardiovascular- and respiratory-related illnesses for the period 1999 through 2005 for Medicare enrollees 65 years or older. She began by characterizing how the chemical composition of PM_{2.5} varies regionally and seasonally in the United States. Subsequently, she evaluated whether the associations between short-term exposure to PM total mass and

health effects followed regional and seasonal patterns, and whether the observed effects could in turn be explained by regional and seasonal variations in the chemical composition of PM_{2.5} (Bell 2012).

REVIEW OF THE NPACT STUDIES

Given the breadth and depth of the two major NPACT studies, HEI convened a special NPACT Review Panel, chaired by members of the HEI Review Committee and comprising twelve experts in medicine, epidemiology, toxicology, statistics, atmospheric chemistry, and exposure. The members of the Panel were not involved in either conducting or overseeing the studies, and they subjected the studies to intensive peer review. The Panel and HEI scientific staff then produced the detailed Commentaries published in the reports to discuss the strengths and weaknesses of the studies, as well as the relevance of the findings to major air quality public health policy questions.

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HEI STATEMENT

Synopsis of Research Report 177

NPACT: Particulate Matter Components Associated with Health Effects

BACKGROUND

Extensive epidemiologic evidence, as well as toxicologic evidence, supports an association between air pollution exposure and adverse health effects, in particular cardiovascular disease (CVD). In order to gain an insight as to whether certain components of the particulate matter (PM) mixture may be responsible for its toxicity and human health effects, HEI funded the National Particle Component Toxicity (NPACT) Initiative. The Initiative consisted of coordinated epidemiologic and toxicologic studies to evaluate the relative toxicity of various chemical and physical properties of PM and selected gaseous copollutants. The lead investigators were Drs. Morton Lippmann (this report) and Sverre Vedal (HEI Research Report 178). Given the well documented associations between ambient PM concentrations and cardiovascular mortality and morbidity, the NPACT studies focused primarily on health outcomes and biologic markers related to CVD.

APPROACH

Lippmann and colleagues at New York University conducted four toxicologic and epidemiologic studies to determine short- and long-term health effects associated with PM and its components. Study 1, led by Lung-Chi Chen, analyzed heart rate variability (HRV) and atherosclerosis in mice exposed for 6 months by inhalation to fine concentrated ambient particles (CAPs) in five geographic regions in the United States. Study 2, led by Terry Gordon, measured acute changes in markers of inflammation and oxidative stress in mice and in human cell lines exposed to a large number of PM samples collected at the same five locations as in the Chen study, focusing on metal composition and PM size classes (coarse, fine, and ultrafine). Study 3, led by Kazuhiko Ito, used data from the U.S. Environmental Protection

Agency's Chemical Speciation Network (CSN) in a time-series analysis of all-cause mortality and hospital admissions associated with specific source categories of $PM \leq 2.5 \mu m$ in aerodynamic diameter ($PM_{2.5}$) in 150 U.S. cities. Study 4, led by George Thurston, also used CSN data to evaluate associations between long-term exposure to PM components and mortality from CVD, respiratory disease,

What This Study Adds

- In this comprehensive and ambitious study, Lippmann and colleagues performed coordinated epidemiologic and toxicologic studies of the health effects of PM and its components. They conducted studies in mice and in human cell lines exposed to ambient PM and epidemiologic studies of short- and long-term cardiovascular effects. These studies mark an important addition to research on air quality and health.
- This study has provided new insights into the toxicity of components and source categories, and identified the Coal Combustion, Residual Oil Combustion, Traffic, and Metals source categories as most consistently associated with health effects. However, other components and source categories could not be definitively excluded as having no adverse effects.
- Better understanding of exposure and health effects is needed before it can be concluded that regulations targeting specific sources or components of $PM_{2.5}$ will protect public health more effectively than continuing to follow the current practices of targeting $PM_{2.5}$ mass as a whole.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Morton Lippmann at New York University Medical Center, Tuxedo, N.Y., and colleagues. Research Report 177 contains the detailed Investigators' Report, a Commentary on the study prepared by the Institute's NPACT Review Panel, and a Synthesis by the Panel discussing the results of this study and those of HEI Research Report 178.

and lung cancer for participants in the Cancer Prevention Study II (CPS-II) cohort maintained by the American Cancer Society.

Lippmann and colleagues used source-apportionment techniques to evaluate which specific components and source categories might be contributing most to the health effects associated with exposure to PM. Studies 1, 2, and 3 used basic factor analysis; Study 4 used absolute principal component analysis to further apportion PM_{2.5} mass to the source categories.

RESULTS AND INTERPRETATION

Study 1 Chen and Lippmann observed that mice exposed to CAPs for 6 months showed greater plaque development in the arteries than mice exposed to filtered air at Manhattan and Tuxedo, New York, and East Lansing, Michigan. In contrast, no differences between the control and CAPs-exposed mice were seen at Seattle, Washington, and Irvine, California. They found that CAPs exposures were associated with acute increases in heart rate and decreases in HRV at Manhattan and, to a lesser extent, at Tuxedo. Very few significant associations for HRV were seen at the other locations. The investigators concluded that the effects on plaque progression were most likely attributable to a Coal Combustion source category, and that the Residual Oil Combustion, Coal Combustion, and Traffic source categories contributed most to the observed acute cardiac effects.

In its independent review, the HEI NPACT Review Panel noted that the results of Study 1 are consistent with evidence from earlier studies that exposure to CAPs leads to acute changes in heart rate and HRV, as well as chronic changes in atherosclerotic plaques and markers of inflammation. Presumably, the effects observed at Tuxedo resulted from long-range transport of pollutants from other areas. Surprisingly, few changes were observed at Seattle and Irvine, two major urban areas dominated by traffic-related pollution. The Panel was not persuaded by the investigators' interpretation that the Residual Oil Combustion and Coal Combustion source categories were the most important contributors to health effects, however. It remains unclear to what extent the larger responses observed in some locations might have reflected higher CAPs exposures, rather than differences in PM composition. There is also uncertainty in assigning source categories in the factor analyses,

and it remained unclear why plaque progression in mice exposed to CAPs at Seattle and Irvine was the same as that in mice exposed to filtered air.

Study 2 Gordon and colleagues observed small differences in the production of reactive oxygen species (ROS) in human epithelial and endothelial cell lines according to location, season, and size fraction, with the highest ROS production for samples from Manhattan and Los Angeles. ROS responses to ultrafine PM samples from all sites were higher than responses to coarse and fine PM samples (on an equal mass basis); responses were higher in summer for fine and ultrafine samples but higher in winter for coarse samples. Strong correlations were observed between ROS production and copper, antimony, vanadium, cobalt, beryllium, and nickel. The investigators observed an increase in neutrophils, a sign of inflammation, in the lungs of PM-exposed mice. They noted a larger neutrophil response to the coarse fraction of PM than to the fine and ultrafine fractions, but those changes did not correlate well with in vitro ROS production for the same PM sample. The investigators concluded that the composition of PM samples pointed to the Traffic and Residual Oil Combustion source categories as contributors to the observed effects.

The Panel noted that Gordon and colleagues had conducted a large and systematic effort to evaluate the relative toxicity of PM samples and found some differences according to size fraction, season, and location. However, the Panel thought that the differences were relatively small and therefore the possible toxicity of any particular components or size classes could not be ruled out. A limitation of the study is that it did not evaluate organic carbon (OC), elemental carbon (EC), or other organic components of PM.

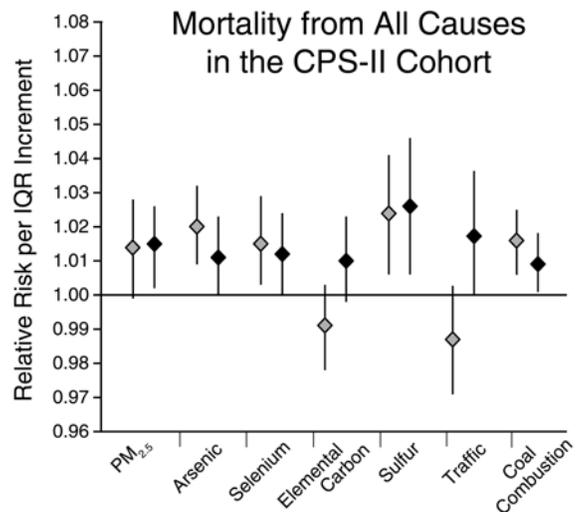
Study 3 Ito and colleagues evaluated associations between PM components or source categories and daily deaths and hospital admissions in 150 U.S. cities and in a subset of 64 cities for which data on both PM components and gaseous pollutants were available. In city-specific analyses, they reported many associations across a variety of statistical models, although associations with individual PM_{2.5} components were not particularly consistent. The most consistent associations were with total PM_{2.5} mass itself and with the Traffic source category. However, the Panel noted that this could be in part because

PM_{2.5} was measured more frequently than its components were, and Traffic was more often identified as a source category than were other categories. In nationwide analyses, significant associations were observed most consistently between all-cause mortality and sulfate, weekday excess PM_{2.5}, lead, and carbon monoxide; between cardiovascular hospitalizations and copper, nickel, and vanadium; and between respiratory hospitalizations and copper, nitrogen dioxide, and silicon. In two-pollutant analyses, the inclusion of total PM_{2.5} in the models with the individual components in many cases appeared to decrease the effect estimates.

The Panel noted that the results of Study 3 support associations of daily mortality and hospital admissions with both traffic-related pollutants and secondary aerosols. The Panel emphasized that some results should be interpreted with caution because a high proportion of the data for important PM components (e.g., nickel, arsenic, copper, and vanadium) was below the limit of detection or had low monitor-to-monitor correlations. The patterns of correlations between pollutants were complicated, and it was difficult to interpret their potential effects on associations with health effects.

Study 4 In this cohort study, Thurston and colleagues found the strongest associations for mortality with the Coal Combustion and Traffic source categories and with sulfur, which strongly contributed to both of those categories, and EC, the primary contributor to Traffic. The associations of Traffic and EC with mortality were, however, highly sensitive to the inclusion of ecologic covariates in the analyses and to the use of a random effects Cox model instead of a standard Cox proportional hazards model (see the figure). The investigators concluded that long-term exposure to PM_{2.5} and the Coal Combustion source category explained most of the associations of exposure to PM_{2.5} with all-cause, ischemic heart disease, and lung cancer mortality (but not respiratory mortality).

The Panel noted that Study 4 yielded many important results during the extended follow-up period of the CPS-II cohort. However, the Panel was not convinced that the study has definitively demonstrated that long-term exposure to components of PM_{2.5} is more important than exposure to total PM_{2.5} in causing adverse effects. Although the Panel agreed that the investigators found the most consistent associations with the Coal Combustion source category,



Relative risks of mortality from all causes in the CPS-II cohort associated with PM_{2.5} and selected components and factors. Results presented are those that demonstrated the most consistently positive associations; the remaining results were not positive or significant. Gray and black diamonds depict results from the random effects Cox models without and with contextual ecologic covariates, respectively. Note that the IQR (interquartile range) varied by pollutant; e.g., the IQRs for PM_{2.5} and sulfur were 3.13 µg/m³ and 0.53 µg/m³, respectively.

the Panel disagreed with the investigators' conclusion that exposure to coal combustion emissions is more strongly associated with mortality than exposure to traffic emissions, because traffic is one of the most important contributors to within-city differences in PM_{2.5} exposure; however, this is not well captured by the limited number of monitors within a city. The Panel also noted other issues, such as a decreasing trend in coal combustion emissions over the past decades.

Although the Total Risk Index analysis provided some interesting results that suggested that exposure to combinations of components and gases in pollutant mixtures is potentially more toxic than exposure to PM_{2.5} mass alone, the Panel thought that the approach, although promising, had some problems that precluded considering these results to be more than suggestive.

CONCLUSIONS

Lippmann and colleagues conducted a comprehensive research program to evaluate the relative toxicity of PM_{2.5} components and source categories. The findings identified Coal Combustion, Residual Oil

Combustion, Traffic, and Metals source categories as most consistently associated with health effects. However, the Panel thought that the study has not shown conclusively that specific components or sources were more definitively associated with health outcomes than other components or sources.

SYNTHESIS OF NPACT STUDIES BY LIPPMANN AND VEDAL

This section looks broadly at the approaches and results of the reports by Drs. Lippmann and Vedal and considers whether there is coherence and consistency in the epidemiologic and toxicologic results.

Both studies found that adverse health outcomes were consistently associated with sulfur and sulfate (markers primarily of coal and oil combustion) and with traffic-related pollutants, although the relative importance of the latter remains unclear because exposure to traffic-related pollutants varies within metropolitan areas and thus is more subject to uncertainty than exposure to pollutants from other source categories. The results for sulfur and sulfate may have been more consistent because their concentrations were more accurately estimated (due to their spatial homogeneity) than concentrations of other pollutants.

Biomass combustion, crustal sources, and related components were not generally associated with short- or long-term epidemiologic findings in these studies, but there were only a limited number of cities where these sources and components were likely to be measured consistently. The possibility remains that biomass combustion contributed to OC concentrations, and thus to its associations with cardiovascular outcomes. There were few consistent

associations with other components or sources, although the Panel cautions that this is not conclusive evidence that these components and sources do not have adverse health effects. Further analyses of some of these sources are warranted.

Both studies highlight how important the CSN is to research on the health effects of components of air pollution and to air quality management. Neither study could have been performed without CSN data, although the studies highlighted some limitations that suggest that further efforts would be helpful to characterize EC, OC, and metals (i.e., combustion- and traffic-related components); to lower the detection limits of some components; and to collect daily measurements.

The NPACT studies, which are to date the most systematic effort to combine epidemiologic and toxicologic analyses of these questions, found associations of secondary sulfate and, to a somewhat lesser extent, traffic sources with health effects. But the Panel concluded that the studies do not provide compelling evidence that any specific source, component, or size class of PM does not play a role in toxicity. If greater success is to be achieved in isolating the effects of pollutants from mobile and other major sources, either as individual components or as a mixture, more advanced approaches and additional measurements will be needed so that exposure at the individual or population level can be assessed more accurately. Such enhanced understanding of exposure and health effects will be needed before it can be concluded that regulations targeting specific sources or components of PM_{2.5} will protect public health more effectively than continuing to follow the current practice of targeting PM_{2.5} mass as a whole.

National Particle Component Toxicity (NPACT) Initiative: Integrated Epidemiologic and Toxicologic Studies of the Health Effects of Particulate Matter Components

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ABSTRACT

Particulate matter (PM*), an ambient air criteria pollutant, is a complex mixture of chemical components; particle sizes range from nanometer-sized molecular clusters to dust particles that are too large to be aspirated into the lungs. Although particle composition is believed to affect health risks from PM exposure, our current health-based air quality standards for PM are limited to (1) the mass concentrations of PM_{2.5} (particles 2.5 μm or smaller in aerodynamic diameter), which are largely attributable to combustion products; and (2) PM₁₀ (10 μm or smaller), which includes larger-sized mechanically generated dusts. Both of these particle size fractions are regulated under the National Ambient Air Quality Standards (NAAQS) and both have been associated with excess mortality and morbidity.

We conducted four studies as part of HEI's integrated National Particle Component Toxicity (NPACT) Initiative research program. Since 1999, the Chemical Speciation Network (CSN), managed by the U.S. Environmental Protection Agency (U.S. EPA), has routinely gathered air monitoring

data every third or sixth day for the concentrations of numerous components of PM_{2.5}. Data from the CSN enabled us to conduct a limited time-series epidemiologic study of short-term morbidity and mortality (Ito study); and a study of the associations between long-term average pollutant concentrations and annual mortality (Thurston study). Both have illuminated the roles of PM_{2.5} chemical components and source-related mixtures as potentially causal agents.

We also conducted a series of 6-month subchronic inhalation exposure studies (6 hours/day, 5 days/week) of PM_{2.5} concentrated (nominally) 10× from ambient air (CAPs) with apolipoprotein E-deficient (ApoE^{-/-}) mice (a mouse model of atherosclerosis) (Chen study). The CAPs studies were conducted in five different U.S. airsheds; we measured the daily mass concentrations of PM_{2.5}, black carbon (BC), and 16 elemental components in order to identify their sources and their roles in eliciting both short- and long-term health-related responses. In addition, from the same five airsheds we collected samples of coarse (PM_{10-2.5}), fine (PM_{2.5-0.2}), and ultrafine (PM_{0.2}) particles. Aliquots of these samples were administered to cells in vitro and to mouse lungs in vivo (by aspiration) in order to determine their comparative acute effects (Gordon Study).

The results of these four complementary studies, and the overall integrative analyses, provide a basis for guiding future research and for helping to determine more targeted emission controls for the PM components most hazardous to acute and chronic health. Application of the knowledge gained in this work may therefore contribute to an optimization of the public health benefits of future PM emission controls.

The design of each NPACT study conducted at NYU was guided by our scientific hypotheses, which were based on our reviews of the background literature and our experience in conducting studies of associations between ambient PM and health-related responses. These hypotheses guided the development and conduct of the four studies.

This Investigators' Report is one part of Health Effects Institute Research Report 177, which includes a Commentary by the NPACT Review Panel, an HEI Statement about the research project, and a Synthesis of the NPACT Initiative relating this report to Research Report 178. Correspondence concerning the Investigators' Report may be addressed to Dr. Morton Lippmann, New York University School of Medicine, Department of Environmental Medicine, 57 Old Forge Road, Tuxedo, NY 10987; morton.lippmann@nyumc.org.

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* A list of abbreviations and other terms appears at the end of the Investigators' Report.

Hypothesis 1. Coarse, fine, and ultrafine PM are each capable of producing acute health effects of public health concern, but the effects may differ according to particle size and composition. (Applies to all studies.)

Hypothesis 2. Long-term PM_{2.5} exposures are closely associated with chronic health effects. (Applies to studies 1 and 4.)

Hypothesis 3. The source-apportionment techniques that we have developed and refined in recent years provide a useful basis for identifying major categories of sources of PM in ambient air and specific chemical components that have the greatest impacts on a variety of acute and chronic health effects. (Applies to all studies.)

Hypothesis 4. The health effects due to ambient PM exposures can best be seen in sensitive subgroups within overall human populations and in animal models of such populations. (Applies to studies 1, 3, and 4.)

Overall, the studies have demonstrated that the toxicity of PM is driven by a complex interaction of particle size range, geographic location, source category, and season. These findings suggest that the components of PM — associated with certain categories of sources — are responsible for the observed adverse health effects. Most importantly, the responsible components and source categories vary with the health-related endpoints being assessed.

Across all studies, fossil-fuel combustion source categories were most consistently associated with both short- and long-term adverse effects of PM_{2.5} exposure. The components that originate from the Residual Oil Combustion and Traffic source categories were most closely associated with short-term effects; and components from the Coal Combustion category were more closely associated with long-term effects.

INTRODUCTION

HISTORICAL IDENTIFICATION OF AIR POLLUTION COMPONENTS THAT AFFECT HEALTH

Smoke generated by coal combustion has been considered a nuisance, and sometimes an intolerable one, for many centuries (Evelyn 1661). An early belief that acidic fog was deleterious to health, especially in relation to respiratory disease, was widespread (Russell 1924), but quantitative data on exposure and health impacts were lacking. On the other hand, some artists found the effects of coal smoke on light scatter and the reduction of visibility to be aesthetically interesting. Claude Monet produced over 100 canvases of scenes in London in the 1870s and at the turn of the 19th century to illustrate the highly variable appearance of the dense smoke and fog.

A quantitative estimate of the mortality impact of an air pollution episode in the Meuse Valley in Belgium in 1930 was made by Firket (1936). He attributed 60 excess deaths (in a population of ~6000) to the dense smoke from an atmospheric inversion and estimated that a comparable inversion in London could cause 3000 excess deaths (in a population of ~800,000). When a particularly dense smoke enveloped London for 3 days in December 1952 (which made surface transportation impossible), the hospitals and morgues were overloaded with people suffering with or dying from acute bronchitis. A detailed report estimated that ~4000 excess deaths within the 4 weeks that followed the smoke episode were associated with the very large increase in the concentrations of black smoke and sulfur dioxide (SO₂) (Ministry of Health 1954). A later reexamination of data records that extended the time period of effect to 4 months after the pollution episode concluded that there were ~12,000 excess deaths (Bell et al. 2004).

Concern about adverse health effects due to the smoke exposures led Doctors Patrick J. Lawther and Robert E. Waller at St. Bartholomew's Hospital in London to establish, before the December 1952 episode, a laboratory program to monitor pollution exposures; during the episode they extended it to daily measurements. The program involved a method for collecting smoke particles on a filter paper disc and measuring to what extent the reflectance of white light from the filter was reduced. The method was periodically calibrated against the increase in mass of the particles on the filter. Since it was known that the air was acidic, they also measured the pH change in a scrubbing solution of a parallel sampling stream; acidity was expressed in terms of the concentration of SO₂ that would produce the same acidity.

The measurement of black smoke in London continued for many years and served as a monitoring system to gauge the effectiveness of the control measures taken to reduce smoke exposures. The primary control measure in the early years was a ban on burning bituminous coal for space heating; the coal was replaced with coke in order to reduce exposure to the volatile components within the coal and to the secondary PM components they produced. The efficacy of this control approach was demonstrated when an inversion comparable to the one in 1952 occurred in December of 1962. The excess mortality within 4 weeks was ~700, compared with ~4000 in the 1952 episode. The control efforts, which extended to all parts of the United Kingdom (UK), also reduced the annual mortality rates (Chinn et al. 1981).

The most notable early air pollution episode that involved a quantitative analysis of human health impacts in the United States occurred in 1947 in Donora, Pennsylvania, a small steel-mill town in the Monongahela River

Valley. The concentrations of smoke components and SO_2 were not measured, but it was determined that 43% of the population were affected; of those, 10% required medical assistance and 60 excess deaths were recorded (Schrenk et al. 1949). Subsequent analyses of PM collected on air cleaning intake filters in operation during the episode indicated that the PM included unusually high proportions of sulfates and transition metals (i.e., metals that have several oxidation states, such as iron [Fe], and thus may easily react with other compounds). In a follow-up study 10 years later, Ciocco and Thompson (1961) reported higher rates of mortality, heart disease, and chronic bronchitis in those who had had acute responses to the smoke in comparison with those who had not.

Amdur and coworkers (1978) later conducted toxicologic research related to the Donora episode to determine the roles of the PM components on the pulmonary system. They demonstrated that aerosol acidity and zinc compounds were associated with the excess mortality and pulmonary effects that had been observed.

For the epidemiologic studies of the Donora episode, the investigators' analyses were limited to crude indices of PM (light reflectance or mass concentration). However, we now know that PM in ambient air is a complex mixture that varies greatly in chemical composition and particle size due to the nature of its origin; thus, studies with such limited methods were inherently incapable of identifying PM components that may have had disproportionate health impacts.

Ambient air PM can include a vast array of components from many sources. Primary solid particles are dispersed into the air by mechanical processes that break up solid surface materials by crushing or grinding; particles (mostly larger than $\text{PM}_{2.5}$) are resuspended by wind action. Much smaller primary particles, such as soot that results from incomplete combustion in diesel engines, are emitted from exhaust pipes. Power plants that burn fossil fuels emit (1) coarse mineral ash particles composed of non-volatile elements and their oxides, and (2) ultrafine and fine particles with aggregates formed by condensation from metals that are volatile at flame temperatures. Reactive gases undergo chemical reactions within the ambient air to form secondary particles that remain suspended as fine particles and can be composed of both inorganic and organic chemicals. In consideration of the varying particle sizes (which largely determine where they deposit in the respiratory tract after inhalation) and their chemical composition (which largely determines whether they are toxic at the deposition sites or at more distant sites in the body after translocation or dissolution into body fluids), the biological effects can vary from negligible to severe.

One of the first studies to go beyond crude indices of PM in order to examine the associations of PM components with health effects was that of Özkaynak and Thurston (1987). They used the concentrations of $\text{PM}_{2.5}$ components — largely trace element signatures measured in EPA's Inhalable Particulate Network (IPN) — to develop profiles indicative of source categories; they then conducted a cross-sectional study in which they compared the source strengths with annual mortality in U.S. cities. They reported that mortality was significantly and most strongly associated with the Coal Combustion source category and less associated with the Traffic and Metals Industry categories. This pioneering study had a limited impact because it could not account for individual-level variations in personal risk factors in the various communities; however, the current Thurston study reported here features more recent speciation data from the EPA's CSN, more recent annual mortality data from the American Cancer Society's (ACS) Cancer Prevention Study-II (CPS-II) cohort, and an analysis that accounted for personal risk factors at the individual level.

CSN data have also been used to investigate the influence of $\text{PM}_{2.5}$ components on daily mortality (Franklin et al. 2008) and on hospital admissions (Bell et al. 2009) in time-series studies, but such studies have been limited by the CSN's sampling frequency (only every third day in most cities). Some of these limitations have been overcome in the time-series analyses conducted as part of the current Ito Study 3.

Toxicologic studies that have investigated the effects of ambient air $\text{PM}_{2.5}$ mixtures were reviewed by Lippmann and Chen (2009). In those studies laboratory animals were exposed by inhalation to CAPs, and samples of the exposure atmospheres were analyzed to determine the concentrations of the $\text{PM}_{2.5}$ components. The earliest studies of CAPs, conducted at Harvard University, Boston, Massachusetts (Clarke et al. 1999, 2000), consisted of short-term exposures and acute effects, whereas subsequent studies at NYU involved daily exposures over several months, such as the series of 6-month studies in Tuxedo, New York, that have been described by Lippmann and associates (2005a,b, 2006).

Under the NPACT Initiative, we conducted two complementary toxicologic studies of both short- and long-term in vivo responses with mice after several weeks or months of daily exposure to either CAPs via inhalation or to coarse, fine, and ultrafine PM by aspiration at five different sites across the United States. In Gordon Study 2 the samples of coarse, fine, and ultrafine PM were collected in both summer and winter; this study also included in vitro cellular assays.

METHODS FOR THE SOURCE APPORTIONMENT OF PM_{2.5} MASS

The term “source apportionment” is used to describe several available analytical methods to characterize the contributions of various emission sources to PM mass concentrations measured at a monitor or a “receptor” (hence, it is sometimes called “receptor modeling”). The goal of source apportionment, in this study, was to associate the measured levels of PM_{2.5} components with different types of source categories (which represent source emissions). By analyzing the resulting source categories relative to health outcomes, we were able to reduce the number of variables in a multipollutant atmosphere and facilitate interpretation of the results in terms of source emissions.

Source apportionment analyses are conducted using data for chemically speciated PM (often consisting of over a dozen components) from PM filters analyzed by laboratory measurement methods, such as x-ray fluorescence (XRF). Using chemically speciated data, researchers have a choice of two broad types of source apportionment methods: chemical mass-balance (CMB) analysis or multivariate factor analysis. The CMB approach requires source profiles (i.e., the relative mass fractions of chemical species) of known emission sources as input for the analysis. This approach may be useful for determining the impact of a specific source (e.g., an industrial plant), whose profile is well characterized in a given city. But such an approach was not suitable for this project because our objective was to determine the health effects of major source categories in many cities.

Multivariate factor analysis is a data-driven approach in which resulting factors (i.e., groups or categories) are interpreted by researchers using external information (e.g., knowledge of key signature species — tracers — for each source category). These methods have an advantage over CMB methods in that they can readily include other PM components that are not used as tracers for source categories, such as collocated gaseous and vapor concentrations, that may aid in identifying and quantifying the components of a pollution source category. Therefore, our four studies used multivariate methods based on factor analysis.

Several multivariate methods are currently available for conducting source apportionment. Factor analysis and a related approach, Principal Component Analysis (“components” here means “a group of correlated pollutant measurements”), are two commonly used multivariate methods to elucidate an underlying set of factors. For example, correlations among 20 variables (such as PM_{2.5} components and pollutant gases) could be summarized by an underlying set of five factors if each variable could be expressed as a linear combination of the factors. Thus, factor analysis can be a

pragmatic means to reduce the dimensionality of data, but it also fits the concept that observed variations of many PM chemical species could be explained by the variation of emissions from a smaller number of source categories (given additional data for source emissions). However, whereas factor analysis and PCA reduce the dimensionality of the data, they themselves do not produce quantitative apportionment of mass unless they are followed by regression of PM mass on the factors.

In the 1980s and 1990s, researchers in the air pollution field developed specialized versions of multivariate methods that would emphasize the condition that underlying factors (i.e., variations in emissions from sources) must take positive values because they represent mass concentrations of pollutants. These methods included Absolute Principal Component Analysis (APCA; Thurston and Spengler 1987), Positive Matrix Factorization (PMF; Paatero 1997), and UNMIX (Henry and Kim 1989). To address the uncertainties associated with applying these different source apportionment methods, eight research groups independently conducted source apportionment with the same data sets from two cities — Phoenix, Arizona, and Washington, District of Columbia — using various methods (Thurston et al., 2005; Hopke et al., 2006). When they compared results at a U.S. EPA workshop on source apportionment in 2003, they found some differences in how specific subcategories of sources, such as gasoline vs. diesel engines, were identified. Nevertheless, the major source categories defined by different investigators and analytical approaches (e.g., Traffic [which combined gasoline and diesel engines], Residual Oil Burning, and Soil) were highly correlated (Hopke et al., 2006). Furthermore, the time-series analysis of associations between source categories and mortality found that source categories were a significant predictor of mortality risk estimates, whereas the differences between results obtained by different groups of investigators were not important (Thurston et al. 2005).

The four studies in this NPACT project had different study designs, so we decided to use relatively straightforward factor analysis methods for all four studies. This was partly because factor analysis can be conducted using general statistical software (SAS, S-Plus, and R), rather than the highly specialized software required for some other methods; this choice would minimize the complexity of source apportionment as applied in the different study designs. Only in Thurston Study 4 was PM_{2.5} mass apportioned to the identified source categories using APCA (explained below), which is essentially a factor analysis followed by regression of PM_{2.5} mass on the factors; therefore the types of methods used in the four studies were essentially the same.

Another important consideration common to these studies was what degree of orthogonality (independence) in the identified factors (source categories) would produce the most useful results. In source apportionment analysis, the factors can be “rotated” obliquely to allow correlation among factors. Allowing factors to be correlated sometimes yields a more interpretable result, for example when temporal variations of mass concentrations from different source categories are driven by common weather conditions. In other methods, such as PMF and UNMIX, the constraint to produce source categories with only positive values generally yields correlated factors (source categories). Although such correlated source categories may be more realistic, especially for short-term variations, they also can make it difficult to interpret the health effects of an individual source category. Therefore, we chose to use orthogonal (independent) rotations so that the resulting source categories would be uncorrelated. The only exception was the factor analysis in Chen Study 1, in which oblique rotations yielded more clearly interpretable source categories.

Here we summarize the different ways source apportionment was used because of the study designs; method details are provided in the report for each study.

In Chen Study 1 (mouse exposure to concentrated ambient particles [CAPs]), factor analysis of daily $PM_{2.5}$ chemical components was conducted at each of the five sites; the identified source categories were examined for their associations with changes in heart rate and heart rate variability in the mice exposed to the concentrated $PM_{2.5}$.

In Gordon Study 2 (mouse and tissue exposure to three sizes of particles), factor analysis of chemical components from ultrafine, fine, and coarse particles was conducted using all the samples of the three size fractions collected at the five sites during two seasons (summer and winter). The contrast in the clustering of elements for different source categories could be maximized when the data were analyzed together. Note also that Gordon Study 2 used the more sensitive inductively coupled plasma mass spectrometry (ICP-MS) method for speciation, whereas the other three studies used data measured by the XRF method. Because ICP-MS yields better signal-to-noise ratios for some elements (e.g., antimony [Sb]) than the XRF method, the components analyzed in Study 2 are also slightly different from those analyzed in the other three studies.

Ito Study 3 (time-series analysis of mortality and elderly hospitalizations) is analogous to Chen Study 1 in that factor analysis was conducted in individual cities. However, because it was not feasible to conduct a detailed factor analysis in 64 cities that would consider each city’s unique source characteristics, the analysis was conducted

in two stages. First, a nationwide factor analysis for the entire data set for the 64 cities was performed using the deviations from monthly means to remove seasonal trends and spatial variation from the exposure data. Second, based on the major source categories identified from this nationwide analysis, factor analyses for individual cities were then conducted using a computer program to assign source categories to city-level factors identified in the national analysis. Unlike the other three studies, Study 3 also included gaseous pollutants (nitrogen dioxide [NO_2], SO_2 , and carbon monoxide [CO]) in the factor analysis and individually in the health effects analyses in the context of a multipollutant assessment.

As in Study 3, Thurston Study 4 (long-term mortality effects in the CPS-II cohort) used data from the EPA’s CSN. In contrast to Study 3, however, Study 4 exploited both spatial and temporal (day-to-day) variations of the chemical components in the nationwide factor analysis involving over 200 speciation monitors. In addition, because the components associated with secondary aerosols (sulfates, nitrates, and organic carbon [OC]) were regionally distributed and therefore obscured spatial identification of sources, the secondary aerosols (as well as the data for sulfur [S]) were excluded from the factor analysis step to help clarify the source-specific factors. Study 4 also applied a simpler mass apportionment approach by regressing $PM_{2.5}$ mass on a single tracer component (identified from the factor analysis) for each source category (e.g., nickel [Ni] for Residual Oil Combustion, selenium [Se] for Coal Combustion) in order to compare with their APCA results. The two approaches yielded similar results.

Each study includes tables or figures showing “factor loadings” from the factor analyses, which are correlations between individual chemical components and a factor. Note, however, that a factor analysis yields factor loadings without the interpretation of factors as source categories.

The source category names have been derived from (1) the combination of the statistical methods used in the studies, (2) the factor analysis process (which has associated uncertainties), and (3) the interpretations of the identified factors as source categories by the study investigators. Such interpretation is based on the cumulative and existing knowledge of signature elements associated with each source type, some of which is based on summaries and reviews of past source apportionment studies (U.S. EPA 2003; Desert Research Institute 2005) and our involvement in the 2003 U.S. EPA source apportionment workshop mentioned above. Therefore, the labels used in tables and figures of factor loadings are the source categories as interpreted by the various investigators. Despite these possible uncertainties, however, considerable coherence was found

in the source categories identified by the four diverse investigations reported here.

Finally, note that of the four studies, only Thurston Study 4 apportioned PM_{2.5} mass concentrations to source categories using APCA. This is mainly because mass apportionment requires an additional regression step after the factor analysis, and we decided that the determination of PM_{2.5} associated with each source category would be most precisely done in Study 4, which had the largest number of observations, spatially and temporally, for the chemical components. However, interpretation of the statistical associations between the source categories and the health outcomes in these four studies was not substantially affected by mass apportionment, and thus the temporal or spatial variations of the factor scores can be considered as standardized (unitless) representations of source emission behavior.

In this section, we have used the terms “factors” and “source categories” somewhat interchangeably. However, in the rest of the report, we use mostly “source categories” to refer to the factors by their specific source category names, such as Traffic, Soil, Residual Oil Combustion, rather than, for example, “the factor that suggests traffic-related emissions”, which would be more precise but lengthy. We use this less complete shorthand mainly to enhance readability and to simplify interpretation of the various results of the health-effects analyses.

BACKGROUND ON HEALTH EFFECTS OF PM COMPONENTS

Both peak and cumulative exposures to PM in ambient air are significantly associated with adverse health effects and account for increases in mortality and morbidity more than any other regulated environmental pollutant (U.S. EPA 1997b, 2008). In particular, (1) the mass concentration of PM_{2.5} has generally been significantly associated with excess cardiovascular mortality and morbidity in urban areas around the world (U.S. EPA 2008); (2) the mass concentration of PM_{10-2.5} in ambient air has often been significantly associated with excess respiratory mortality and morbidity in urban areas (U.S. EPA 2008); (3) the associations with adverse health effects are usually stronger for combustion products of solid and liquid fossil fuels (e.g., transition metals and BC) than for other components of PM, which suggests that mass concentrations are, at best, crude indicators of health risks (Lippmann 2009b); and (4) interventions that have reduced exposures to metals and BC have led to prompt improvements in public health (Lippmann 2011).

PM includes a wide range of particle sizes and is a complex mixture of chemical components. Particle size and

composition affect the delivered dose and biological responses. However, in the absence of a substantial body of data on the effects of specific chemical components and particle sizes on specific health endpoints, current regulations specified by the National Ambient Air Quality Standards (NAAQS) for ambient PM are still limited to mass concentrations within two ranges of aerodynamic diameter (PM_{2.5} and PM₁₀). Based on the literature review that follows, we conclude that, although the air quality standards have served as guideposts for public health protection, they may not be the most efficient metrics to evaluate further progress. Furthermore, considerable evidence suggests that some PM components have much greater impacts on health-related indices than others (reviewed recently by Lippmann and Chen [2009]). The findings of some key studies of PM exposures of human populations, laboratory animals, and cells in vitro are summarized below. In each case, sufficient data were available on PM composition to demonstrate that some of the constituents were much more influential than others.

Human Exposure–Response Studies

Daily Mortality Related to PM_{2.5} Components Average daily mortality in the U.S. cities studied in the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) was significantly associated with average concentrations of Ni and V, but not with other PM_{2.5} components (Lippmann et al. 2006; Dominici et al. 2007a).

Franklin and associates (2008) modeled EPA air quality speciation data (available for every third or sixth day) and daily mortality data for 25 U.S. cities between 2000 and 2005 to determine how the association between PM_{2.5} and mortality was modified by PM_{2.5} composition. They first determined the association between daily PM_{2.5} and mortality, and then used meta-regression to examine how the pooled association was modified by community and by season-specific PM_{2.5} composition. The association was stronger when the PM_{2.5} mass had a higher proportion of aluminum (Al), arsenic (As), sulfate (SO₄²⁻), silicon (Si), or Ni. The extent to which the intercity heterogeneity in the association could be explained was greatest for Al (45%), Ni (41%), and, in a multivariate model, for a combination of Al, Ni, and SO₄²⁻ or Al, Ni, and As (100%). These findings suggest that the sources of Soil Dust (indexed by Al and Si), Residual Oil Combustion (indexed by Ni), and Coal Combustion (indexed by As or SO₄²⁻) are especially influential.

In Seoul, South Korea, Son and coworkers (2012) studied 14 months of 1-hour average concentrations of PM_{2.5}, EC, OC, and of several ions in PM_{2.5} (magnesium [Mg²⁺], sodium [Na⁺], potassium [K⁺], calcium [Ca²⁺], chlorine [Cl⁻], ammonium [NH₄⁺], SO₄²⁻, and nitrate [NO₃⁻]) to

assess associations with total, cardiovascular, and respiratory mortality. The average number of total deaths per day was 92, of which 22.4 were classified as due to cardiovascular causes, and 5.4 as due to respiratory causes. For cardiovascular mortality, nearly significant associations ($P < 0.1$) were found for an interquartile change in NH_4^+ , SO_4^{2-} , and NO_3^- ; for respiratory mortality, the only ions with $P < 0.1$ were Cl^- and Mg^{2+} .

Annual Mortality Among U.S. Military Veterans Related to $\text{PM}_{2.5}$ Components Lipfert and colleagues (2006) studied associations between $\text{PM}_{2.5}$ components and all-cause annual mortality for men in the Veteran's cohort for the years 1976–2001. The $\text{PM}_{2.5}$ components that were significantly associated with annual mortality were annual average urban area concentrations of Ni and vanadium (V), along with traffic density (Lipfert et al. 2006).

Annual Mortality Among Residents of California's Central Valley Related to $\text{PM}_{2.5}$ Components Cahill and associates (2011a) described a substantial decrease in annual mortality from IHD in Bakersfield, CA (at the southern end of California's 400-mile-long Central Valley) between 1989 and 1991. The decline in IHD mortality was associated with the 1990 switch at the local oil refinery from combustion of crude oil to the use of natural gas for generating steam to enhance heavy petroleum recovery. Measured concentrations of fine particle S, Ni, V, zinc (Zn), and lead (Pb) at Bakersfield between 1974 and 1976 (in ng/m^3) averaged 1685 for S, 38 for Ni, 19 for V, 61 for Zn, and 1714 for Pb; concentrations were much lower in the more northern parts of the Valley. The corresponding concentrations in 2009 in Bakersfield were 505 for S, 2.3 for Ni, 0.2 for V, 32 for Zn, and 9.4 for Pb. Although there is relatively little variation of climate, elevation, and population demography within the Central Valley, the IHD mortality in 1989–1991 was 60% higher in the area around Bakersfield than in areas further north within the Valley, whereas in 2003–2007, the IHD mortality around Bakersfield had decreased by 30%.

Daily Hospital Admissions Related to $\text{PM}_{2.5}$ Components Average daily hospital admissions for Medicare patients in Atlanta, Georgia, were examined in relation to ambient concentrations of chemically-related groups of $\text{PM}_{2.5}$ components. Significant increases were noted in cardiovascular disease (CVD) admissions associated with exposure to alkanes and to those transition metal oxides (for copper [Cu], manganese [Mn], Zn, titanium [Ti], and Fe) for which > 90% of the measurements were above the limit of detection. For specific disease categories, the transition metals were significantly associated with admissions

for ischemic heart disease (IHD), congestive heart failure, and atrial fibrillation; the alkane group was associated with respiratory-related admissions. By contrast, the aromatic group and the microcrystalline oxide group (As, bromine [Br], Se, Pb, and Si) were associated with fewer admissions related to CVD and respiratory illnesses (Suh et al. 2011).

Daily Hospital Admissions for Cardiovascular and Respiratory Diseases Related to Components of $\text{PM}_{2.5}$

Bell (2011) analyzed data from 187 U.S. counties for 2000–2005 and showed that concentrations of $\text{PM}_{2.5}$ components, daily $\text{PM}_{2.5}$ total mass concentrations, and hospital admissions for cardiovascular and respiratory diseases all vary across counties and regions of the United States as well as across seasons. Statistically significant increases in CVD admissions were associated with increases in same-day $\text{PM}_{2.5}$ total mass in spring and fall, but the associations were strongest in winter. Bell (2012) observed regional differences in the associations, with the strongest associations observed across the 108 northeastern U.S. counties. Increases in respiratory hospitalizations were most pronounced on the second day after exposure to $\text{PM}_{2.5}$. Of the components that made up the largest fractions of $\text{PM}_{2.5}$ mass, only variability in elemental carbon (EC) explained the observed regional variation. For the remaining components studied, Bell (2012) reported that higher concentrations of Ni and V in $\text{PM}_{2.5}$ were associated with larger effect estimates for both cardiovascular and respiratory hospitalizations. Associations between PM_{10} mass and total nonaccidental mortality were also larger in regions and seasons with higher fractions of V and particularly Ni in $\text{PM}_{2.5}$.

Exacerbation of Childhood Asthma Related to $\text{PM}_{2.5}$ Components

Gent and associates (2009) studied 149 children with physician-diagnosed asthma and symptoms or medication use within the previous 12 months who were living in and around New Haven, Connecticut. Air sampling filters were collected daily and analyzed for trace elements by x-ray fluorescence (XRF) and for black carbon (BC) by light reflectance. Using factor analysis, they identified six source categories of $\text{PM}_{2.5}$: Motor Vehicles, Road Dust, Sulfur (a marker for regional $\text{PM}_{2.5}$), Biomass Combustion, Residual Oil Combustion, and Sea Salt. They attributed 42% of the $\text{PM}_{2.5}$ to the Motor Vehicles category and 12% to the Road Dust category. Increased likelihoods of symptoms and inhaler use were largest for exposures averaged over 3 days: a 10% increased likelihood of wheeze per $5\text{-}\mu\text{g}/\text{m}^3$ increase in PM attributed to the Motor Vehicle source category and a 28% increased likelihood of shortness of breath associated with the Road Dust category.

There were no associations with increased health outcome risks for PM_{2.5} itself, or for the other source categories.

Patel and coworkers (2009) studied 3-month averages of PM_{2.5} — and the Ni, V, Zn, and EC within the PM_{2.5} — as they related to longitudinal reports of symptoms of asthma in children who lived near EPA speciation sites and were part of the Columbia Center for Children's Environmental Health birth cohort in Manhattan and the Bronx in New York City. Symptoms for the prior 3 months had been collected every 3 months from 3 to 24 months of age. (About 90% of the children were on Medicaid.) Of all subjects, 30% were reported as having or possibly having asthma based on a doctor's evaluation at 24 months. Symptoms of wheeze and cough were significantly associated with Ni, V, and Zn exposure; EC was associated with only cough; and PM_{2.5} mass concentration was not associated with either symptom.

Mortality and Hospital Admissions During a Utah Valley Steel Mill Strike Related to Content of Metals in PM

Community rates of mortality and hospital admissions were significantly lower during a 14-month strike at a local steel mill in 1986 than in the preceding and following years (Pope 1989, 1991; Pope et al. 1992). In the same period, concentrations of metals on air sampling filters were also lower and these decreases corresponded to in vitro and in vivo toxicity analyses of the metal extracts from the filters (Frampton et al. 1999; Ghio and Devlin 2001; Dye et al. 1997, 1999, 2001).

Hong Kong Intervention to Reduce Sulfur in Fuel Oil

A mandated reduction in the sulfur content of fuels for electric power production in Hong Kong led to a sharp and persistent decrease in the airborne concentrations of SO₂, Ni, and V, but not in the airborne concentrations of other criteria pollutant gases or metals. Decreases in SO₂, Ni, and V were associated with corresponding decreases in monthly mortality and hospital admissions for bronchial hyperactivity (Hedley et al. 2002, 2004).

Long-Term CAPs Exposures in Animal Inhalation Studies

Increases in Heart Rate and Decreases in Heart Rate Variability Related to Metal Concentrations in PM_{2.5}

In a previous study in our laboratory, ApoE^{-/-} mice underwent 6-hour weekday inhalation exposures for 6 months to CAPs in Tuxedo, New York. On 14 days when the exposure atmospheres were characterized by relatively high concentrations of Ni, chromium (Cr), and Fe, and unusually low concentrations of all other measured components, the animals had significant increases in heart rate (HR) (Hwang et al. 2005) and decreases in HR variability

(HRV) (Chen and Hwang 2005). An analysis that used wind direction and back trajectories indicated the influence of a Ni smelter in Sudbury, Ontario, which was located upwind of the laboratory (Lippmann et al. 2006).

In Vitro Exposures to Different Ambient Air PM Mixtures

NF-κB Activity in BEAS-2B Human Lung Cells

Related to PM_{2.5} Sources and Component Exposures

Daily PM_{2.5} samples, collected in Tuxedo, New York, over 6 months in a Biosampler impinger, were analyzed for PM_{2.5} components; aliquots were used to expose BEAS-2B lung cells, which were then analyzed for NF-κB activity. The only significant positive association with a PM_{2.5} source category was for the Residual Oil Combustion category ($r = 0.32$), and it accounted for only 2% of the PM_{2.5} mass (Maciejczyk and Chen 2005). Further analyses of the roles of individual PM_{2.5} components showed significant associations for Ni (averaging 38 ng/m³), barium (Ba; 13 ng/m³), Mn (9 ng/m³), and Fe (500 ng/m³) (Maciejczyk et al. 2010).

NF-κB Activity in Mouse Microglial Cells Related to

Exposure to PM_{2.5} Elemental Components

The composite PM_{2.5} samples collected for the NYU 6-month CAPs exposure study to assess NF-κB activity in lung cells (described in the previous paragraph; Maciejczyk and Chen 2005) were also used to expose mouse microglial cells in a dose-response study. The only elements that had significant dose-related associations were Ni and V (Sama et al. 2007).

OVERALL SPECIFIC AIMS AND APPROACHES FOR THE NPACT PROJECT AT NYU

This section describes the general approach, specific aims, and hypotheses for each of the four studies included in the NYU NPACT project as well as the overall integration of the studies' results.

Study 1 led by Lung-Chi Chen. In Study 1, we conducted 6-month subchronic PM_{2.5} CAPs inhalation studies with ApoE^{-/-} mice at five U.S. sites that differ substantially in PM_{2.5} chemical composition, using the same experimental protocols at each location. The specific aims were to (a) relate the differences in short-term responses to the local daily variations in ambient PM_{2.5} composition at each site, and (b) relate the differences in the long-term responses to variations in the mean PM_{2.5} compositions among the five sites. We conducted elemental speciation analyses on filter samples collected on each exposure day and analyzed the data using source apportionment analyses to relate PM_{2.5} composition and possible source categories to the biologic endpoints. We tested the overall

hypothesis that biological responses to $PM_{2.5}$ exposure are driven by specific chemical components rather than by overall $PM_{2.5}$ mass concentration.

Study 2 led by Terry Gordon. In Study 2, we collected high-volume samples of ambient air PM in coarse, fine, and ultrafine particle size ranges in summer and winter at the same five sites where the 6-month subchronic CAPs inhalation studies were performed. We conducted elemental speciation analyses on samples collected; and administered aliquots of the samples to mouse and human cells in vitro (epithelial, endothelial, and cardiomyocytes) and to mice in vivo by aspiration. The specific aims were to (a) identify biological responses and their daily and long-term variations by particle-size range, collection site, and season, and (b) relate the observed responses to PM elemental composition. We tested the overall hypothesis that biological responses are driven by particle-size range and local variations in climate, as well as by specific chemical constituents rather than by overall $PM_{2.5}$ mass concentration.

Study 3 led by Kazuhiko Ito. In Study 3, we performed time-series analyses of the associations of daily mortality and hospital admissions, by cause, with the daily concentrations of $PM_{2.5}$ mass and its elemental components. This was done (a) in 150 U.S. cities that have CSN $PM_{2.5}$ compositional data collected every third day, and (b) in 64 of these cities that also have data on the gaseous NAAQS pollutants. The specific aim was to estimate short-term risk of mortality and hospital admissions and to model city-to-city variation in risk as a function of city-specific characteristics. We tested the overall hypothesis that daily mortality and morbidity rates are driven by specific chemical constituents rather than by overall $PM_{2.5}$ mass concentration.

Study 4 led by George D. Thurston. In Study 4, we evaluated the annual mortality data from the CPS-II cohort in relation to the annual average concentrations of $PM_{2.5}$ components or source-related components in the communities that have CSN $PM_{2.5}$ compositional data. The specific aim was to investigate the association between mortality and long-term exposure to components of $PM_{2.5}$ in the United States. We tested the overall hypothesis that an increase in mortality is driven by specific chemical constituents rather than by overall $PM_{2.5}$ mass concentration.

Integration of Studies. In the Overall Summary of Results section of the main report, we integrate, to the extent possible, the findings of the four studies outlined above. Our specific aims were to (a) evaluate short-term outcomes, i.e., the influence of $PM_{2.5}$ and its elemental components on short-term biological responses of cells in vitro and of organ systems in animals in vivo (Studies 1 and 2), and on daily morbidity and mortality data for human populations (Study 3); and (b) evaluate long-term outcomes, i.e., the influence of $PM_{2.5}$ and its elemental components on long-term plaque progression in mouse aorta (Study 1) and on mortality data for a human cohort (Study 4).

We tested the overall hypotheses that (1) the increase in mortality from CVD is related to plaque progression, and that both plaque progression and CVD are driven by specific chemical components rather than by overall $PM_{2.5}$ mass concentration; (2) that the $PM_{2.5}$ components that drive short-term responses may differ from those that drive long-term responses; and (3) that the components of coarse, fine, and ultrafine PM may differ in their capacity to produce short-term responses.

These four cross-cutting hypotheses are addressed in several studies:

Hypothesis 1. Coarse, fine, and ultrafine PM are each capable of producing acute health effects of public health concern, but the effects may differ according to particle size and composition.

Hypothesis 2. Long-term $PM_{2.5}$ exposures are closely associated with chronic health effects.

Hypothesis 3. The source-apportionment techniques that we have developed and refined in recent years provide a useful basis for identifying major categories of sources of PM in ambient air and specific chemical components that have the greatest impacts on a variety of acute and chronic health effects.

Hypothesis 4. The health effects due to ambient PM exposures can best be seen in sensitive subgroups within overall human populations and in animal models of such populations.

NPACT Study I. Subchronic Inhalation Exposure of Mice to Concentrated Ambient PM_{2.5} from Five Airsheds

Lung-Chi Chen and Morton Lippmann

ABSTRACT

BACKGROUND

PM_{2.5}* is associated with alterations in the autonomic nervous system and in cardiac function, but there are significant variations in response. Regulators and the broader public would like to know which of the various components of PM_{2.5} are most responsible for the adverse health effects that occur. For the United States as a whole, suspicion to date has focused on fossil fuel combustion products found in PM_{2.5}, such as those in emissions from motor vehicles and power plants. Recognizing that the chemical composition of PM_{2.5} varies by region, we performed inhalation studies in mice, using subchronic exposures to CAPs at five sites representing five airsheds across the United States, in order to identify the PM_{2.5} components that are most responsible for acute and cumulative effects on the cardiovascular system.

METHODS

A mouse model of atherosclerosis (ApoE^{-/-}), previously used in our laboratory, was used in this study. We

first conducted simultaneous exposures to CAPs of mice at Mount Sinai School of Medicine, in Manhattan, New York, and at our facility in Sterling Forest State Park, an exurban park in Tuxedo, New York. At the Manhattan site, substantial increments of Ni, EC, and OC are found in the PM_{2.5}; long-range transported PM_{2.5} is present at both sites. Subsequent studies using the same experimental and analytic protocols and animal model were conducted at the University of Washington in Seattle, Washington, a major seaport with high levels of ambient wood smoke in winter; at Michigan State University in East Lansing, Michigan, which is located in a region with high levels of ambient coal combustion emissions; and at the University of California at Irvine, California, where the PM_{2.5} is generally dominated by traffic-related emissions. Groups of ApoE^{-/-} mice were exposed either to filtered air or to CAPs for 6 hr/day, 5 days/wk for 6 months. Mass concentrations were determined gravimetrically, and the concentrations of 35 chemical elements were measured during each 6-hr exposure by XRF. The principal source categories of the PM_{2.5} were identified by factor analysis. Electrocardiogram (ECG) tracings were made using telemetry ($n = 8$ to 12/group), and atherosclerotic plaque progression ($n = 8$ to 12/group) was assessed by ultrasound biomicroscopy.

RESULTS

The mean CAPs mass concentration in the exposure chamber was highest at the Irvine site (138 µg/m³), followed by Tuxedo (136 µg/m³), Manhattan (122.9 µg/m³), East Lansing (67.8 µg/m³), and Seattle (60.5 µg/m³). For CAPs mass and component concentrations, we examined associations with changes in three ECG measures (HR; standard deviation of normal-to-normal intervals [SDNN], a measure of total HRV; and the root mean square of successive differences in beat-to-beat intervals [RMSSD], a measure of short-term changes in HRV) for three lag days (0, 1, and 2), four times of day (during CAPs exposures, afternoons, late evenings, and overnight), and 16 elemental

This Investigators' Report is one part of Health Effects Institute Research Report 177, which includes Investigators' Reports of three other studies, a Commentary by the NPACT Review Panel, an HEI Statement about the research project, and a Synthesis of the NPACT Initiative relating this report to Research Report 178. Correspondence concerning this Investigators' Report may be addressed to Dr. Lung-Chi Chen, New York University School of Medicine, Department of Environmental Medicine, 57 Old Forge Road, Tuxedo, NY 10987. lung-chi.chen@nyumc.org; chenl@env.med.nyu.edu.

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* A list of abbreviations and other terms appears at the end of the Investigators' Report.

components of PM_{2.5}. Significant differences were found in HR, SDNN, or RMSSD between the CAPs-exposed and control mice — including 29 such differences at Manhattan, 26 at Tuxedo, 9 at East Lansing, 5 at Seattle, and 3 at Irvine — suggesting that certain components of PM_{2.5} (such as S) that were found in much lower concentrations at East Lansing, Seattle, and Irvine were the most efficacious at producing these ECG changes, either directly or by potentiating overall PM toxicity. The regressions of the five-site mean component concentrations with the most significant responses in terms of HR, SDNN, and RMSSD were Ni ($r^2 = 0.96$), followed by Al ($r^2 = 0.81$), EC ($r^2 = 0.79$), P ($r^2 = 0.77$), S ($r^2 = 0.65$), V ($r^2 = 0.35$), Mg ($r^2 = 0.30$), Zn ($r^2 = 0.27$), and Se ($r^2 = 0.19$). The components with the lowest r^2 values were Cu ($r^2 = 0.00$), Cr ($r^2 = 0.02$), Si ($r^2 = 0.02$), Fe ($r^2 = 0.02$), Br ($r^2 = 0.03$), Na ($r = 0.06$), Ti ($r^2 = 0.08$), and OC ($r^2 = 0.08$). Over the 6 months of the CAPs exposures, significant brachiocephalic artery plaque progression was found in the mice exposed at Manhattan, Tuxedo, and East Lansing (the three sites where coal combustion emissions account for a significant fraction of ambient air PM_{2.5}) compared with plaque progression in control mice, but not at Seattle or Irvine, suggesting that Coal Combustion as a source category had more influence on plaque progression than other PM_{2.5} source categories did.

CONCLUSIONS

Subchronic CAPs exposures at locations with different PM_{2.5} compositions produced different effects on ECG measures in the ApoE^{-/-} mice. In terms of strength of associations, components attributable to source categories for Residual Oil Combustion (Ni, V, and S), Coal Combustion (S and Se), and Traffic (EC, Al, and P) appeared to be the most influential. In terms of atherosclerotic plaque progression, the small number of sites limited our ability to identify the most influential components or source categories, but it was notable that plaque progression occurred at the three sites with exposure to Coal Combustion emissions and was lacking at the two sites without such exposure.

INTRODUCTION

Regulators and the general public need to know which of the various components of PM_{2.5} are most responsible for its adverse health effects. For the United States as a whole, suspicion to date has focused on the components of PM_{2.5} found in fossil fuel combustion products (see, for example, Pope et al. 2002), especially motor vehicle tailpipe emissions, including nitrogen oxides (i.e., nitric oxide and nitrogen dioxide [NO₂]); products of incomplete

combustion, such as EC and OC; and power plant emissions, including SO₂, transition metals, and heavy metals. For more local areas, there are concerns about emissions from smelters, waste transfer and incineration facilities, other large industrial facilities, seaports, airports, and resuspended road dust. Identification of the most causal components or source-related mixtures in the PM_{2.5} responsible for most of the adverse health effects would permit more of a focus on controlling emissions of these components or their sources in order to reduce their health effects.

The current study addresses each of the overall NPACT study's four initial hypotheses in terms of *in vitro* responses in an animal model. Re-stated in these terms, the hypotheses are the following:

1. PM_{2.5} is capable of producing acute health effects of public concern but that the effects might differ according to the chemical composition of the PM_{2.5};
2. Long-term PM_{2.5} exposures are closely associated with chronic health effects;
3. The source-apportionment techniques that have been developed and refined in recent years provide a useful basis for identifying the principal PM_{2.5} air pollution source categories and specific chemical components of PM_{2.5} that have the greatest impacts on a variety of acute and chronic health issues; and
4. The health effects caused by ambient PM_{2.5} exposures are more likely to be observed in animal models that represent sensitive subgroups within overall human populations.

Our mouse inhalation studies involved subchronic exposures to CAPs of a mouse model (ApoE^{-/-} mice) bred to develop atherosclerotic plaque similar to that observed in humans with atherosclerosis. Our prior subchronic inhalation studies using CAPs in Tuxedo found acute and persistent changes in HR (Hwang et al. 2005) and HRV (Chen and Hwang 2005), enhancement of aortic plaque size (Chen and Nadziejko 2005; Sun et al. 2005), and changes in brain cell distribution and function (Veronesi et al. 2005). The biologic plausibility of PM_{2.5} exposures contributing to premature human mortality and excess morbidity in the United States at current ambient concentrations was enhanced by these findings in view of the fact that the long-term average concentrations used in our subchronic inhalation exposures were close to the current NAAQS.

Concern about PM_{2.5} components being the cause of adverse health effects has centered on the components found in fossil fuel combustion products as well as in inorganic compounds containing metals. Most of the mass of these metals in ambient air, especially for the transition metals, is in PM_{2.5}. The majority of the evidence pointing to the biologic effects of metals, EC, and OC has come from

studies involving exposures of laboratory animals in vivo or of cells in vitro. Health concerns have focused on transition metals, such as Cr, Cu, Fe, Ni, V, and Zn, and on polycyclic carbonaceous compounds because of their ability to generate reactive oxygen species (ROS) in biologic tissues (e.g., Costa and Dreher 1997; Kodavanti et al. 1998; and Dye et al. 2001).

Recognizing that the chemical composition of PM_{2.5} varies from region to region across the United States, HEI's NPACT study at NYU has supported inhalation studies of subchronic CAPs exposure in mice at various U.S. locations in order to identify the PM_{2.5} components that are most responsible for acute and cumulative cardiovascular-system health effects. A mouse model of atherosclerosis (ApoE^{-/-}), previously used in this laboratory (and which had responded to CAPs at Tuxedo in past subchronic inhalation studies [Lippmann et al. 2005a,b, 2006]), was first used for simultaneous exposures to CAPs at Tuxedo, 30 miles northwest of Manhattan, and at Mount Sinai School of Medicine, in mid-city Manhattan. At Manhattan, substantial concentrations of Ni, EC, and OC were found in the PM_{2.5}; long-range transported PM_{2.5} of similar composition is present at both Tuxedo and Manhattan. Subsequent studies using the same experimental and analytic protocols and animal model were conducted at Michigan State University in East Lansing, which is located in a region with high levels of ambient Coal Combustion emissions (Jack Harkema, collaborator); at the University of Washington in Seattle, a major seaport with high levels of ambient wood smoke in winter (Daniel Luchtel, collaborator); and at the University of California at Irvine, where the PM_{2.5} is generally dominated by traffic-related emissions (Dr. Michael Kleinman, collaborator).

EXPERIMENTAL DESIGN

Our plan was to create exceptionally rich databases of selected measures of air quality and cardiac function and to assess their associations for groups of mice exposed daily for 6 months to CAPs in five airsheds that differed substantially in the composition of their pollutant mixtures. In Manhattan, ambient air quality is heavily affected by pollutants associated with traffic congestion and ambient metals associated with the combustion of residual oil (a low-grade oil that remains after petroleum has been distilled) for space heating. At Sterling Forest in exurban Tuxedo, ambient air quality is dominated by pollutants associated with the long-range transport of pollutants originating in the Eastern megalopolis and the Midwest. In East Lansing, air quality is dominated by pollutants associated with coal-burning power plants. In Seattle, the main

sources of ambient air pollutants are residential wood burning and seaport activities. In Irvine, air quality is heavily affected by photochemical air pollution.

Having data on the daily concentrations of BC and various elements in these five airsheds was a unique asset of the study. This allowed us to go well beyond the conventional source-apportionment approach (which assumes the presence of mixtures that are influenced by the various sources) in order to directly compare the strengths of the associations of ECG measures with individual elements, source categories, and the conventionally used markers of the larger mass components of PM_{2.5} (i.e., S and EC). For associations with individual elements, we elected to focus on elements for which we had both a high proportion of days with concentrations above the detection limits as well as indications, from our previous studies of subchronic CAPs inhalation at Tuxedo (Maciejczyk and Chen 2005), that these elements were associated with changes in ECG measures. In addition to examining the acute effects of peak exposures on a daily basis, we were also able to examine the cumulative effects of the daily exposures on ECG baseline measures throughout the 6 months of the exposures as well as on aortic plaque progression at periodic intervals using non-invasive ultrasound measurements in anesthetized mice.

MATERIAL AND METHODS

ANIMAL MODEL

Male ApoE^{-/-} mice (Taconic, Germantown, NY), 12 weeks of age at the beginning of the exposures, were fed with normal chow and water ad libitum throughout the experiment except during exposure. Assignments to CAPs versus filtered-air exposures were randomized, and the exposures were performed on weekdays for a total of 6 months. At each of the five sites, the mice were housed one per cage in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The sites were maintained at conditions of constant temperature and humidity. All procedures were approved by the Animal Care and Use Committees of the NYU School of Medicine, Mt. Sinai School of Medicine, the University of Washington, Michigan State University, and the University of California at Irvine.

Several ApoE^{-/-} mice at each site developed skin lesions during the exposures, as ApoE^{-/-} mice normally do because of subcutaneous cholesterol deposition. Following Institutional Animal Care and Use Committee regulations, we killed these otherwise healthy mice at various

times in the study. The following group sizes were used in the final analysis: at Manhattan, $n = 7$ for CAPs and $n = 5$ for controls; at Tuxedo, $n = 6$ for CAPs and $n = 4$ for controls; at Seattle, $n = 10$ for CAPs and $n = 9$ for controls; at East Lansing, $n = 7$ for CAPs and $n = 7$ for controls; and at Irvine, $n = 8$ for CAPs and $n = 6$ for controls.

Some of the mice were implanted with ECG transmitters (Data Sciences International, St. Paul, MN) at NYU by the investigators for the Manhattan, Tuxedo, and Seattle studies 3 weeks before the start of the exposures and by the mouse vendor for the East Lansing and Irvine studies. Baseline ECG tracings and HRs were monitored continuously for 1 week before the first exposures and continued throughout the duration of the study, including during the 6-hour daily exposures, as described previously (Lippmann et al. 2005a,b, 2006; Chen and Hwang 2005). Additional mice were used for other assays, as described below.

SITE SELECTION

Exposure protocols were optimized to be consistent with the principal goals of the overall NPACT study, namely to elucidate the roles of the chemical and physical properties of $PM_{2.5}$ that contribute to the adverse acute and chronic health effects with which $PM_{2.5}$ is associated. In the study described here, we simultaneously compared the effects of CAPs in Manhattan (at Mount Sinai School of Medicine, 10 East 101st Street, between Fifth and Madison Avenues, in New York City, New York) with those of CAPs in Tuxedo, New York (at the NYU campus in Sterling Forest State Park). Long-range transported $PM_{2.5}$ is also present at both locations.

Sterling Forest is a largely undeveloped woodland state park that is ~30 miles northwest, and generally upwind, of Manhattan. The NYU laboratory at Sterling Forest is located near the center of the park on a relatively lightly traveled two-lane road that bisects the park. There are no large power generators or industrial operations within 20 miles of the site. The ambient $PM_{2.5}$ here is therefore representative of the regional background $PM_{2.5}$ aerosol of the Eastern megalopolis that extends from Virginia to Maine. Results of simultaneous gravimetric filter measurements of $PM_{2.5}$ from June through August 2001 at the site and at a second-story rooftop on the campus of Hunter College at First Avenue and East 26th Street in Manhattan showed that $PM_{2.5}$ concentrations at the two sites were highly correlated during that period (Lippmann et al. 2003). For brevity's sake, this site is called Tuxedo throughout the report.

Our East Lansing site is on the campus of Michigan State University in a region that was considered the heart of the industrial Midwest. Much of the industry, however, has

been idled in recent years, including a former Oldsmobile assembly plant in nearby Lansing. The principal sources of $PM_{2.5}$ today are the primary emissions from nearby coal-burning electric utility plants, and secondary sulfates and associated metals from more distant coal-burning plants located to the northwest, west, southwest, and south.

Our Seattle site on the University of Washington campus, adjacent to Lake Washington, is close to the Port of Seattle on Puget Sound to the west and is surrounded by residential areas that burn wood for space heating in winter.

Our site in Irvine is on the campus of the University of California at Irvine in an area that has light industrial, suburban residential, and considerable traffic-related sources of primary pollutants. It is also notable for its sunshine and therefore has a greater proportion of secondary photochemical pollution than any of the other sites.

PROTOCOL FOR EXPOSURE TO CAPS

CAPs were produced using a versatile aerosol concentration enrichment system (adapted at NYU from a Sioutas particle concentrator) that employs the principle of condensational growth of ambient fine particles followed by virtual impaction to concentrate the $PM_{2.5}$ (as previously described by Maciejczyk and Chen [2005]). A schematic diagram of our inhalation exposure system is shown in Figure 1. Two identical systems were set up for use in Manhattan and Tuxedo and subsequently reconditioned for later use at East Lansing, Seattle, and Irvine. The mouse exposures, monitoring of the exposure atmospheres and ambient aerosol particles, and exposure concentration calculations were performed as previously described (Chen and Hwang 2005; Sun et al. 2005). At all locations, the mice were exposed to CAPs at nominal 8–10× ambient concentrations for 6 hr/day, 5 days/wk, for a total of 6 months (May to November 2007 at Manhattan and Tuxedo; June to December 2010 at East Lansing; January to July 2009 at Seattle; and August 2010 to February 2011 at Irvine). The control mice were exposed to an identical protocol, except that a high-efficiency particulate air (HEPA) filter was positioned in the inlet valve to remove all of the CAPs in the filtered-air stream. Because there was no way to normalize the exposures at the various sites for the concentrations of $PM_{2.5}$ mass (or of its components), we elected to use the same concentration multiple at each site, recognizing that there would then be different $PM_{2.5}$ mass concentrations at each site that varied from day to day and by month. We also made no attempt to remove pollutant gases from either the CAPs or filtered-air streams. During non-exposure periods, the mice were kept in the animal facilities with HEPA-filtered air at all locations.

SOURCE-APPORTIONMENT ANALYSIS

A description of our factor analysis was provided in the overall Introduction to this report. Briefly, Drs. Ramona Lall and George Thurston performed the analyses using factor analysis in the statistical software package S-Plus. Given the small number of trace elemental data points per site, oblique rotation ("oblimin" in the S-Plus package) was found to provide the most distinguishable and interpretable source categories and was therefore used in all factor analyses for this study. Factor loadings (the correlations between the trace elements and the factor scores) were used to identify source categories at each study site, and factor scores calculated by the model were used to test for associations with toxicologic health endpoints. Trace elements and PM components considered in the source-apportionment analysis included Al, Ba, Br, Ca, Cu, Fe, K, Mn, Ni, Pb, S, Se, Si, V, Zn, and EC. Based on our findings from previous source-apportionment studies conducted for New York City (Ito et al. 2004; Lall and Thurston 2006; Qin et al. 2006), traffic is one of the major contributors of PM_{2.5}. Because of the lack of one or more unique tracer components associated with the Traffic source category, NO₂ data for New York City (a marker of local combustion in the city) were obtained from the EPA database and included in the source-apportionment analysis at Manhattan in order to aid in the identification and separation of a Traffic source category.

ANALYSIS OF ECG TELEMETRY DATA

The times (in milliseconds) of occurrence of two consecutive R waves in the ECG channel (i.e., the R-R interval) were calculated on a beat-to-beat basis using physiologic-data acquisition and analysis software (Dataquest A.R.T. Analysis, Data Sciences International). Only normal-to-normal intervals between 100 and 200 msec with normal-to-normal ratios between 0.8 and 1.2 were included in the analyses. Two time-domain HRV indices were measured — SDNN and RMSSD. Frequency-domain HRV indices that were measured included the low-frequency (LF) and high-frequency (HF) domains as well as the ratio of the LF and HF domains, which reflects the balance of the sympathetic and parasympathetic components of the nervous system.

For each of the HR and HRV measures, the daily averages for each mouse at each of four assigned time periods (9:00 AM–1:59 PM, 7:00 PM–9:59 PM, 10:00 PM–12:59 AM, and 1:00 AM–3:59 AM) were calculated separately, based on the recorded five-minute R-R intervals within each hour. For each of the time periods, the mean of the daily average outcome for each mouse was calculated and treated as the outcome baseline for that mouse and that time period. For

each mouse, the outcome baseline was then subtracted from all the daily average outcomes for the same time period. These daily baseline-subtracted outcomes, hereafter called daily outcomes, were the data used for statistical modeling.

In notation, let y_{ijt} be the t th daily outcome at a time period for the j th mouse in the i th exposure group, where $i = 1$ (control) or 2 (CAPs exposure). The complete data for mice in the control groups and mice in the CAPs groups were included for modeling health effects in the following equation. The proposed mixed-effects model, including chronic effects and acute effects associated with daily exposure levels, can be written as

$$y_{ijt} = a_{ij} + \nu \times E_t + f_\alpha(t) + f_\beta(t) \\ \times G_i + \sum_{l=0}^q \sum_{k=1}^K \gamma_l C_{k,t-l} \\ \times G_i + \varepsilon_{ijt},$$

where a_{ij} is normally distributed with mean 0 and a constant variance representing the outcome deviation of the j th mouse in the i th group from the overall mean outcome caused by sampling effects, and the error term ε_{ijt} is assumed to be an autoregressive process of order 1 for all the mice to account for the autocorrelations of repeated measurements from the same animal. The indicator (dummy) variable E_t was defined as 1 when exposure was implemented on day t and as 0 otherwise. The parameter ν was therefore interpreted as the overall difference caused by "experiment effect" on all mice between exposure days and non-exposure days. Another indicator variable, G_i , was defined as 1 when mouse i was in an exposure group and as 0 otherwise. The polynomial function of degree g , $f_\alpha(t) = \alpha_0 + \alpha_1 t + \dots + \alpha_g t^g$, was used to model the overall trend of the outcomes among all the mice across the exposure experiment. The polynomial function of degree h , $f_\beta(t) = \beta_0 + \beta_1 t + \dots + \beta_h t^h$, represented patterns of chronic exposure effects across the experiment. The concentration of the k th source on the t th day with an l -day lag was denoted by $C_{k,t-l}$, where $l = 0, 1, \dots, q$, letting $C_{k,t}$ be 0 if day t was not an exposure day. The parameters $\gamma_0, \gamma_1, \dots, \gamma_q$ were interpreted as the acute effects caused by exposure on the current day (lag 0), previous day (lag 1), through lag day q , respectively. Akaike information criterion and Bayesian information criterion methods of model selection were applied to determine the degrees of the polynomial functions and the number of lag days.

QUANTIFICATION OF PLAQUE BY ULTRASOUND IMAGING

We measured the progression of atherosclerosis in separate groups of ApoE^{-/-} mice during CAPs exposures at 3 and 6 months in Manhattan and Tuxedo ($n = 8/\text{group}/\text{site}$) using non-invasive ultrasound biomicroscopy (Vevo 770, VisualSonics, Toronto, ON), as described previously in papers demonstrating that biomicroscopy measurements agreed with histologic measurements (Chen et al. 2010; Quan et al. 2010). On the basis of the substantial changes observed at 3 months at these two sites, we also made comparable measurements at 2, 4, and 6 months at East Lansing, Seattle, and Irvine.

For these measurements, each mouse was anesthetized using continuous 1.5% isoflurane (EZ-Anesthesia, Euthanex, Palmer, PA) and placed supine on a physiologic platform. Ten B-mode movies (in the cross-sectional position) were obtained along the brachiocephalic artery (BA) and left common carotid artery (LCCA) at approximately 333- μm intervals. From each of the second, third, and fourth movies, three pictures were selected, based on the criteria that they were clear, representative of the entire movie, and ECG-synchronized. For each picture, plaque area was measured using a freehand drawing and then image processing and analysis software (National Institutes of Health, IH Image, National Institutes of Health, Bethesda, MD); it was expressed as a percentage of plaque area relative to the cross-sectional area of the vessel cavity. For each artery, the percentage area of the three locations was then averaged. One-way analysis of variance (ANOVA), followed by the Dunnett test, was used to assess differences in the effects of exposure to CAPs and filtered air.

MAPPING ARTERIAL PLAQUE SURFACE AREA

Groups of 7 CAPs-exposed and 7 control mice at Seattle and groups of 12 CAPs-exposed and 17 control mice at Irvine were killed after 6 months of exposure. Mouse aortas were dissected out from the aortic root to the iliac bifurcation and immersion-fixed overnight in 3% neutral PBS buffered paraformaldehyde at 4°C. Using methods described by Palinski and colleagues (1994), the aortic tree was cleaned of fatty tissues, opened longitudinally with extremely fine microscissors (Fine Science Tools, Foster City, CA), and pinned flat on a black wax surface in a dissecting pan with 0.2-mm (diameter) stainless steel pins (Fine Science Tools). The aorta was then stained with Sudan IV (a fat-soluble dye that stains triglycerides and protein-bound lipids red) for 5 minutes and rinsed. Images of the aorta were captured using a Canon SD550 digital photography system through a dissecting scope. Because

of the aorta's length, two images were taken for each aorta and stitched together using graphics-editing software (Adobe Photoshop CS6, Adobe Systems, San Jose, CA). The images of the pins and surrounding black wax background were removed using Photoshop retouching tools for subsequent quantitative image analysis using image-processing software (ImageJ 1.62, National Institutes of Health, Bethesda, MD, <http://rsb.info.nih.gov/ij/>). The density slide tool of ImageJ was used to isolate and calculate the area stained with Sudan IV using the red channel of the acquired image. The data were expressed as percentage of area covered by atherosclerotic lesion for each animal, and the *t* test was used to test for statistical differences between CAPs exposures and control exposures at both sites.

OTHER CARDIAC-FUNCTION MEASUREMENTS

We made measurements of other cardiac functions, such as ejection fraction, fractional shortening, and cardiac wall thickness, at Manhattan and Tuxedo. However, because these measurements showed no exposure-related responses at either site, we did not make them at the other three sites.

MEASUREMENT OF SERUM BIOMARKERS

Serum samples were taken at 3 and 6 months at Manhattan and Tuxedo and, at the suggestion of the HEI NPACT Oversight Committee, at 2, 4, and 6 months for the subsequent studies at East Lansing, Seattle, and Irvine. Concentrations of serum C-reactive protein; interleukins 6, 10, and 12 (IL-6, IL-10, and IL-12); and tumor necrosis factor-alpha (TNF- α) were determined by enzyme-linked immunosorbent assays (ELISA) for samples collected at Manhattan and Tuxedo.

Because the concentrations of these cytokines and markers were determined to be very low, we decided to use more sensitive electrochemiluminescence assays (Meso Scale Discovery, Gaithersburg, MD) to measure these markers for samples collected at Seattle and Irvine (serum samples were used up for other assays at East Lansing and were not available for this analysis). A special Meso Scale Discovery cytokine panel with seven markers (IL-6, IL-10, IL-13, monocyte chemoattractant protein 1, macrophage inflammatory protein 1, macrophage inflammatory protein 1 beta, and TNF- α) and a prototype Meso Scale Discovery panel with the three other markers (granulocyte-macrophage colony-stimulating factor [GM-CSF], matrix metalloproteinase 9, and vascular endothelial growth factor A [VEGF-A]) were used. These assays were performed by Dr. Andy Ghio at the EPA.

ADDITIONAL STUDIES NOT FUNDED BY HEI

For additional studies not funded by HEI, we also collected tissues that were analyzed by collaborating scientists, including brain tissue collected for Dr. Michael Kleinman for his supplemental study supported by the California Air Resources Board, and liver tissue collected for Dr. Joseph Odin at Mt. Sinai School of Medicine and Dr. Kezhong Zhang at Wayne State University. The results of these studies were not available at the time this report was written.

Dr. Qinghua Sun and colleagues at Ohio State University performed additional studies at 2, 4, and 6 months using the mice exposed at East Lansing that were not implanted with ECG transmitters and not used for ultrasound measurements. For the 2-month exposures at East Lansing, the following additional studies were conducted: PM effects on oxidative stress, alterations of mitochondria, gene expression in brown and white adipose tissues, liver endoplasmic reticulum, and vascular remodeling (Xu X et al. 2011 [Appendix E, which is available on the HEI Web site]; Xu Z et al. 2011; Laing et al. 2010); and PM effects on vascular remodeling (Xu X et al. 2011). For the 4- and 6-month exposures at East Lansing, the investigators at Ohio State evaluated PM effects on liver endoplasmic reticulum stress and on oxidative stress (Laing et al. 2010).

For the studies on liver endoplasmic reticulum stress and oxidative stress mentioned above, the experiments included gene expression analysis, immunohistochemistry staining, protein level, and activity analyses. In addition, measurements were made of lipid profiles, regulation of lipogenesis, fatty acid oxidation, and glucose metabolism in the liver of the mice (including lipid and fatty acid profiling, glucose synthesis, and usage [energy] analysis). The Ohio State investigators also examined hepatic inflammation and liver fibrosis in the mice exposed to CAPs or filtered air, including hepatic inflammation scoring, signal transduction analysis, and liver fibrosis scoring.

RESULTS

CAPS CONCENTRATIONS

Table 1 shows the mass concentrations of ambient $PM_{2.5}$, CAPs in the exposure chambers, and selected CAPs components at each of the exposure sites.

Figure 2 shows daily (top panel) and monthly (bottom panel) average concentrations of CAPs and ambient $PM_{2.5}$ for the 6 months of exposures at the five sites. The mean CAPs concentration was highest at Irvine ($138 \mu\text{g}/\text{m}^3$), followed by Tuxedo ($136 \mu\text{g}/\text{m}^3$), Manhattan ($123 \mu\text{g}/\text{m}^3$),

East Lansing ($68 \mu\text{g}/\text{m}^3$), and Seattle ($61 \mu\text{g}/\text{m}^3$). Month-by-month variations were found in both the ambient $PM_{2.5}$ and CAPs concentrations at all sites. At Manhattan and Tuxedo, ambient $PM_{2.5}$ and CAPs concentrations were highest in June and July and decreased significantly in fall. During the summer months, ambient $PM_{2.5}$ concentrations at Manhattan were 15% higher on average than those at Tuxedo, reflecting an added traffic component at Manhattan. During the fall months (i.e., October and November), ambient $PM_{2.5}$ concentrations at Manhattan were significantly (68%) higher than those at Tuxedo. In East Lansing, CAPs concentrations varied substantially during the exposure period, especially in September, October, and November, when it rained or snowed and concentrations were much lower. An unusual dip was found in both ambient $PM_{2.5}$ and CAPs concentrations at East Lansing in October that was associated with frequent rain at the site in that month. In Seattle, CAPs concentrations were highest in January and decreased to 52% of that observed in January for the remaining months of exposure. At Irvine, CAPs concentrations were lowest in November, and there were more sharp concentration peaks. Strong seasonal variations were seen at all sites other than Irvine.

BLACK CARBON, ELEMENTAL CARBON, AND ORGANIC CARBON CONCENTRATIONS IN CAPS

Table 1 shows that, as expected, ambient BC concentrations were higher at the locations having higher traffic volumes, such as Manhattan ($2667 \text{ ng}/\text{m}^3$), Irvine ($1422 \text{ ng}/\text{m}^3$), and Seattle ($1028 \text{ ng}/\text{m}^3$), than at Tuxedo ($785 \text{ ng}/\text{m}^3$) and East Lansing ($526 \text{ ng}/\text{m}^3$). EC correlated very well with BC at Irvine ($r = 0.96$) and Manhattan ($r = 0.76$) but poorly in the other sites ($r = 0.51$ at Tuxedo and $r = 0.40$ at East Lansing). Similarly, OC concentrations (which are also associated with traffic) were highest at Manhattan ($8343 \text{ ng}/\text{m}^3$) and Irvine ($7380 \text{ ng}/\text{m}^3$), followed by East Lansing ($5540 \text{ ng}/\text{m}^3$) and Tuxedo ($3438 \text{ ng}/\text{m}^3$). As shown in Figure 3, there was a clear weekly pattern in BC concentrations at Manhattan, Tuxedo, Seattle, and Irvine but not at East Lansing. The BC concentrations increased from Monday through Thursday, decreased slightly on Fridays (except at Irvine), and were dramatically lower during the weekend days at Manhattan and Tuxedo, and less so at Seattle and Irvine. BC was highest at Manhattan and lowest at East Lansing.

CONCENTRATIONS OF ELEMENTS IN CAPS

Figure 4 shows daily average concentrations of selected CAPs components (as measured by XRF) over the same time period. The five sites had highly different mixtures of elements. In particular, more differences were found

Table 1. Mean Mass Concentrations of Ambient PM_{2.5}, CAPs, and CAPs Components by Site^a

	Manhattan (May 2007–Nov. 2007)			Tuxedo (May 2007–Nov. 2007)			East Lansing (Jun. 2010–Dec. 2010)			Seattle (Jan. 2009–Jul. 2009)			Irvine (Aug. 2010–Feb. 2011)		
	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Ambient PM _{2.5}	122	20.2	13.3	120	16.9	13	121	8	5.8	126	7.1	3.6	93	10.4	5.5
CAPs	122	122.9	81.1	120	135.8	110.5	121	67.8	54.7	126	60.5	35.6	93	138.3	88.5
BC ^b	122	2,667.1	1,520.5	117	784.5	514	121	525.5	310.9	126	1,028	754.8	93	1,422.2	1,188.7
EC ^c	113	1,205.5	764.6	110	375.3	277.1	118	256	241.8	0	0	0	91	709.6	617.2
OC ^c	113	8,343.1	6,030.2	110	3,437.9	2,373.1	118	5,539.5	1,575.5	0	0	0	91	7,380.1	1,794.9
Al	122	1,115.8	622.8	120	930.7	583.5	121	843.6	538.5	126	858	465.9	93	1,006.4	608.2
Br	122	27.4	18.9	120	26.7	21.4	121	25.6	16.3	126	20.4	11.8	93	65.4	37.5
Ca	122	1,203.9	574.2	120	222.5	148.4	121	436.1	419.2	126	501.9	289.8	93	689.5	452.8
Cr	122	16.3	18.2	120	19.3	21.3	121	8.7	15.9	126	25.8	19.2	93	16.7	11.6
Cu	122	63.1	36	120	21.6	18.9	121	5.4	9.9	126	41.3	28	93	86.5	108.1
Fe	122	1,878.8	888.1	120	465.6	362.3	121	302.1	210.2	126	920.8	614.2	93	1,610	1,537.9
K	122	428.9	362.8	120	323.3	300.1	121	297.8	248.6	126	376.2	426.9	93	479.3	214.9
Mg	122	359.5	251.9	120	251.6	201.4	121	290.7	245.4	126	495.3	315.4	93	925	647.7
Mn	122	106.9	74.6	120	14.2	11.9	121	16.4	16.3	126	31.4	28.3	93	35.3	31.6
Na	122	844.9	728.7	120	501.6	587.1	121	226.8	287.2	126	1,733.1	1,596.4	93	5,023.9	4,539.8
Ni	122	69.9	62	120	16	15.8	121	6.5	6.6	126	17.8	19.2	93	14.1	11.9
P	122	256.5	215.8	120	218.7	235.4	121	137.1	204.9	126	66.3	68.3	93	141.7	162.1
Pb	122	152.6	113.9	120	54.7	34.2	121	21.4	27.5	126	42.8	23.3	93	24.6	24.6
S	122	11,258.6	10,432.4	120	17,686.4	19,689.1	121	6,518.1	7,103.1	126	3,841.5	2,702.4	93	7,593.9	5,925.4
Se	122	8.7	8.7	120	10.4	12.1	121	19.6	15.1	126	6	6.4	93	21.4	13.3
Si	122	1,657.8	928.5	120	989.1	798.3	121	796.8	750.4	126	1,399	1,005.2	93	1,911.2	1,514.7
Ti	122	76.8	45.2	120	53.4	33.5	121	20.8	18	126	49.3	34.7	93	83.5	74.1
V	122	41.9	44	120	17.1	16.4	121	19.8	13.4	126	26	35.4	93	46.4	33.5
Zn	122	759.6	1,193.7	120	78.1	97	121	50.7	44.6	126	126.4	89.8	93	100	91.8

^a CAPs in $\mu\text{g}/\text{m}^3$; others in ng/m^3 .^b Measured using an Aethalometer during the exposure period (9:00 AM–3:00 PM).^c Measured using a thermo-optical carbon analyzer on daily PM samples collected on quartz filters.

Mouse Inhalation Exposure to CAPs from Five Airsheds

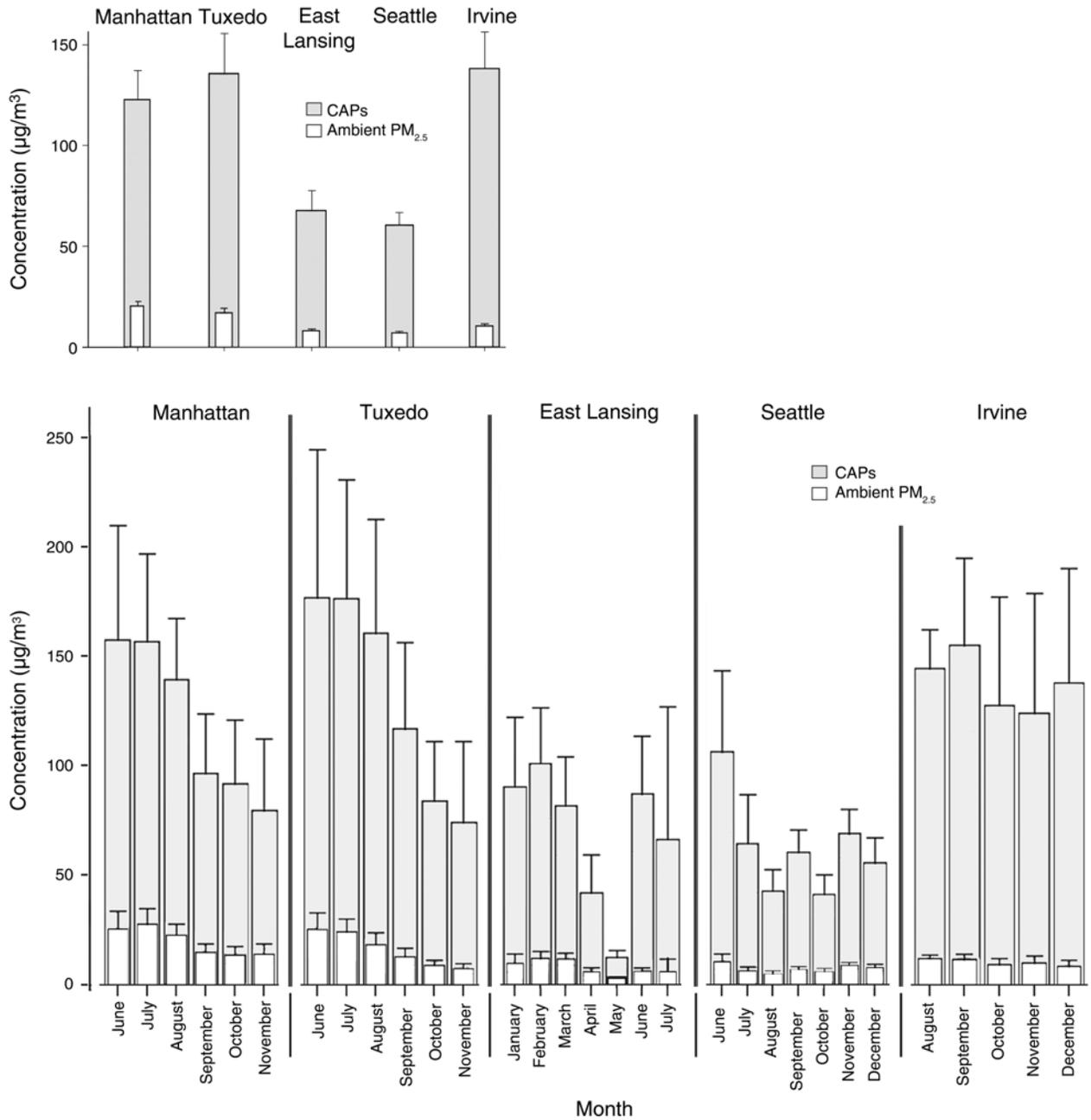


Figure 2. Daily (top) and monthly (bottom) average concentrations (with 95% confidence intervals) of CAPs and ambient $\text{PM}_{2.5}$ by site over the 6 months of exposures. Note that the y-axis scales vary.

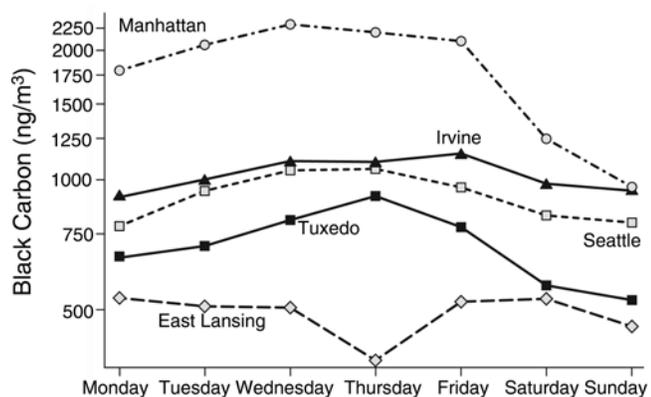


Figure 3. Day-of-week average 24-hour black carbon concentrations (as measured by Aethalometer) by site over the 6 months of exposures. Note that the y-axis scale is logarithmic.

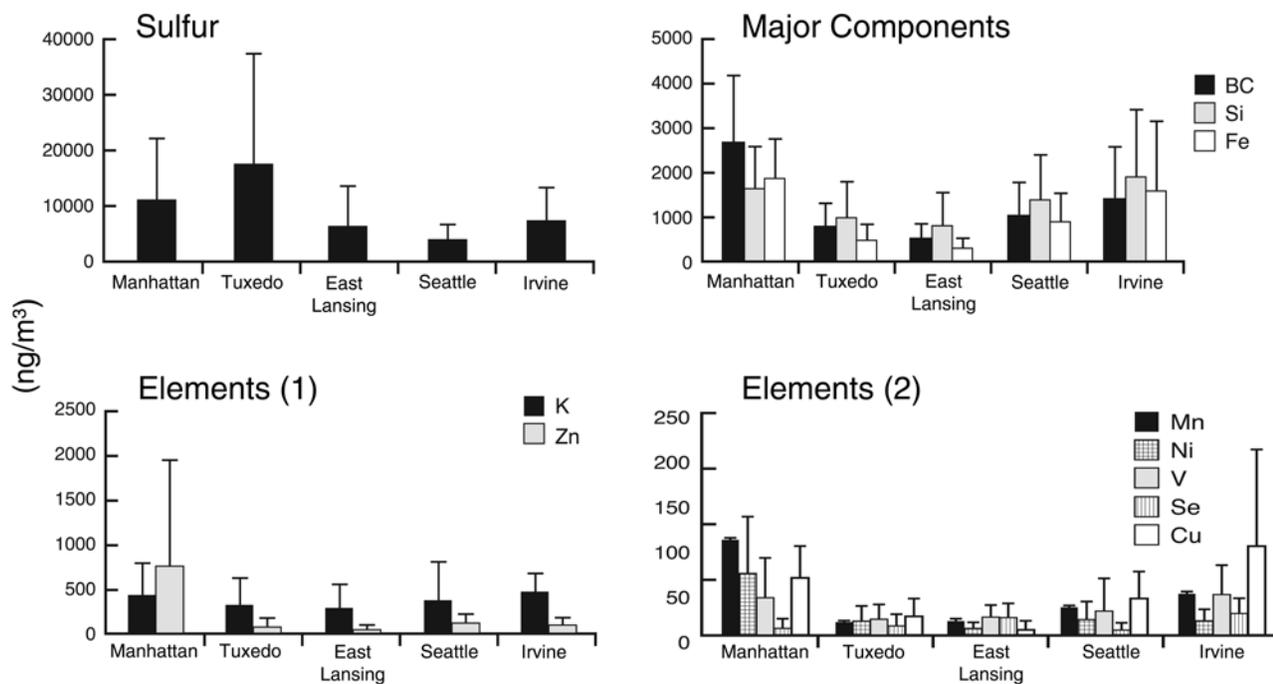


Figure 4. Overall average concentrations of selected CAPs components by site over the 6 months of exposures. Note that the y-axis scales vary.

between Manhattan and Tuxedo than expected, given that both sites lie within an area of the Northeast that is strongly influenced by long-range transport of secondary pollutants. Correlation matrices for CAPs and selected CAPs components are shown for each site in Table 1 and Appendix C (available on the HEI Web site). As shown in Table 1, some notable features of the distributions of elements in CAPs include the following:

- Mean V concentrations in the exposure chambers were highest at Manhattan and Irvine (42–46 ng/m³) and lowest at Tuxedo (17 ng/m³). V correlated well with Ni at sites other than Tuxedo, where the Ni peaks were caused mainly by long-range transport from a Ni refinery in western Ontario rather than from residual oil combustion (Lippmann et al. 2006).
- Mean Ni concentrations were highest at Manhattan (70 ng/m³); much lower at Tuxedo, Seattle, and Irvine (14–18 ng/m³); and lowest at East Lansing (7 ng/m³). However, there were some notable Ni peaks at Tuxedo in June and November and at Seattle in January, May, and June. The ratio of Ni to V was high at Manhattan (1.67), lower at Tuxedo (0.94) and Seattle (0.68), and still lower at East Lansing (0.33) and Irvine (0.30).
- Mean Zn concentrations were much higher at Manhattan than at any other site (760 ng/m³); intermediate at Tuxedo, Irvine, and Seattle (78–126 ng/m³); and lowest at East Lansing (51 ng/m³). Some very pronounced Zn peaks were found at Manhattan in June, July, August, and October. Zn did not correlate with other components at Manhattan, Tuxedo, or East Lansing. At Seattle and Irvine, Zn correlated with BC, Al, Fe, Cu, and Mn.
- Mean Se concentrations were highest at East Lansing and Irvine (20–21 ng/m³), intermediate at Manhattan and Tuxedo (9–10 ng/m³), and lowest at Seattle (6 ng/m³), except for a very high day at Seattle in March and a very high day at Manhattan in October. Se tended to vary greatly from day to day at both Manhattan and Tuxedo, and its concentrations at the two sites rose and fell together. Se did not correlate with any other components at Seattle and Irvine. Se is considered to be a marker of the Coal Combustion source category, but there are no coal combustion point sources in Southern California, so its high concentrations at Irvine indicated the influence of other sources.
- Mean S concentrations were highest at Tuxedo (17 µg/m³), followed by Manhattan (11.3 µg/m³), Irvine (7.6 µg/m³), East Lansing (6.5 µg/m³), and Seattle (3.8 µg/m³).
- Mean Pb concentrations were much higher at Manhattan than at any other site. However, they did not correlate with the concentrations of any of the other components studied.
- Mean P concentrations were much higher at Manhattan and Tuxedo than at Seattle, and the concentrations at the two sites rose and fell together. However, the peaks at Manhattan occurred on different days than those at Tuxedo, suggesting the influence of different sources. P correlated with S and Br at Manhattan, Tuxedo, and East Lansing and did not correlate with other components at Seattle and Irvine.
- Mean Na and Mg concentrations were much higher at Seattle than at Manhattan or Tuxedo and were somewhat higher at Manhattan than at Tuxedo, consistent with the wind directions and distances from large bodies of saltwater.
- Mean K concentrations were in the range of 298–479 ng/m³ at all five sites, except for a pronounced peak at Seattle in January and one at both Manhattan and Tuxedo in July. It did not correlate with other components except at East Lansing with Si and at Irvine with Br, Ca, and Si.
- Mean Cu concentrations were highest at Irvine (87 ng/m³) and lowest at East Lansing (5 ng/m³). At Manhattan, Tuxedo, and East Lansing, Cu did not correlate with other components. But at Seattle and Irvine, it correlated with BC (or EC), Ca, Fe, Mn, Si and Ti.
- Mean Fe concentrations were highly variable from day to day and were highest at Manhattan (1879 ng/m³) and Irvine (1610 ng/m³), intermediate at Seattle (921 ng/m³), and lowest at Tuxedo (466 ng/m³) and East Lansing (302 ng/m³).
- Mean Si concentrations were highest at Manhattan (1911 ng/m³) and Irvine (1658 ng/m³) and lower at East Lansing, Tuxedo, and Seattle (797–1399 ng/m³).

ECG CHANGES ASSOCIATED WITH CAPS

We first considered models with CAPs concentrations as the covariate for estimating acute effects. Quality assurance procedures, described previously (Hwang et al. 2005), were applied, and ECG data outside a preset normal range were eliminated.

The acute effects of CAPs on various ECG measures are shown in Table 2 for each of the five sites. (For simplicity's sake, we have limited our discussion to HR, SDNN, and RMSSD.) Frequency domain results are shown in Appendix D, which is available on the HEI Web site.

At Manhattan, CAPs concentrations with a 0-day lag (i.e., on the same day) were strongly associated negatively with HR and positively with SDNN and RMSSD at all four daily time periods. CAPs concentrations with a 1-day lag (i.e., on the previous day) were also highly significantly associated with the ECG measures at all four daily time

periods (except for SDNN in the 1:00 AM–3:59 AM time period). With a 2-day lag (i.e., 2 days earlier), there were few significant associations.

At Tuxedo, CAPs concentrations with a 0-day lag were significantly associated positively with HR and negatively with the two HRV time-domain measures (SDNN and RMSSD) at all four daily time periods (9:00 am–1:59 PM, 7:00 PM–9:59 PM, 10:00 PM–12:59 AM, and 1:00 AM–3:59 AM). There were many fewer significant associations for a 1-day lag and fewer still for a 2-day lag.

There were fewer acute effects of CAPs on the ECG measures at East Lansing, Seattle, and Irvine. At East Lansing and Irvine (but not at Seattle), CAPs concentrations with a 0-day lag were negatively associated with HR. At all three sites, CAPs concentrations were negatively associated with SDNN (except at Irvine with a 1-day lag) and with RMSSD (except at Irvine with a 0-day lag).

Acute changes (dots) and estimated chronic changes (fitted curves) in the HR and HRV measures are shown in Figures 5 through 10. At Manhattan, chronic changes included significant increases in HR during the 7:00 PM–9:59 PM time period for the first 50 days; these gradually decreased over the balance of the study. At Tuxedo, the exposures caused HR to decrease after a few days, reaching significance for the 10:00 PM–12:59 AM time period after 75 days of exposure. At the same time, HRV measures increased within a few days and remained elevated to the end. No significant patterns of chronic changes in HRV measures were identified. At East Lansing, Seattle, and Irvine, minimal changes were found in HR for all daily time periods until toward the end of the series of exposures, when HR in the exposed mice was slightly higher than in control mice during the 1:00 AM–3:59 AM time period.

Mouse Inhalation Exposure to CAPs from Five Airsheds

Table 2. Acute Effects of CAPs on HR and HRV Measures by Site^a (Table columns continue across next page)

Site	Lag	Time Period	HR (beats/min/[$\mu\text{g}/\text{m}^3$ CAPs])		Ln(SDNN)		Ln(RMSSD)		
			Value (β)	SE	Value (β)	SE	Value (β)	SE	
Manhattan	0	9:00 AM–1:59 PM	-0.103977	0.017023 **	0.000192	0.000091 **	0.000444	0.000101 **	
		7:00 PM–9:59 PM	-0.105056	0.014894 **	0.000208	0.000083 **	0.000508	0.000107 **	
		10:00 PM–12:59 AM	-0.089742	0.015636 **	0.000193	0.000080 **	0.000438	0.000104 **	
	1	1:00 AM–3:59 AM	-0.095897	0.014943 **	0.000182	0.000081 **	0.000440	0.000100 **	
		9:00 AM–1:59 PM	-0.083270	0.017147 **	0.000367	0.000091 **	0.000448	0.000101 **	
		7:00 PM–9:59 PM	-0.061434	0.015378 **	0.000261	0.000086 **	0.000525	0.000110 **	
	2	10:00 PM–12:59 AM	-0.079846	0.017683 **	0.000217	0.000091 **	0.000426	0.000118 **	
		1:00 AM–3:59 AM	-0.056716	0.016888 **	0.000107	0.000092	0.000358	0.000113 **	
		9:00 AM–1:59 PM	-0.043780	0.019134 **	0.000287	0.000102 **	0.000349	0.000114 **	
	Tuxedo	0	7:00 PM–9:59 PM	-0.022112	0.016220	0.000074	0.000091	0.000089	0.000116
			10:00 PM–12:59 AM	-0.006257	0.017306	0.000175	0.000089*	0.000155	0.000116
			1:00 AM–3:59 AM	-0.008877	0.016571	0.000241	0.000090**	0.000241	0.000111**
1		9:00 AM–1:59 PM	0.041439	0.011756 **	-0.000244	0.000065**	-0.000406	0.000079**	
		7:00 PM–9:59 PM	0.032763	0.012375 **	-0.000170	0.000065**	-0.000189	0.000068**	
		10:00 PM–12:59 AM	0.039494	0.011555 **	-0.000155	0.000059**	-0.000243	0.000070**	
2		1:00 AM–3:59 AM	0.064486	0.010852 **	-0.000194	0.000055**	-0.000294	0.000065**	
		9:00 AM–1:59 PM	0.041206	0.011242 **	-0.000297	0.000062**	-0.000292	0.000075**	
		7:00 PM–9:59 PM	0.026345	0.012300 **	-0.000190	0.000065**	-0.000196	0.000067**	
East Lansing		0	10:00 PM–12:59 AM	0.003916	0.011504	-0.000089	0.000059	-0.000117	0.000070*
			1:00 AM–3:59 AM	0.016091	0.010776	-0.000173	0.000055**	-0.000187	0.000065**
			9:00 AM–1:59 PM	-0.003336	0.011396	-0.000181	0.000063**	-0.000145	0.000076*
	1	7:00 PM–9:59 PM	0.017233	0.012374	-0.000085	0.000065	-0.000137	0.000068**	
		10:00 PM–12:59 AM	0.004233	0.011880	-0.000101	0.000061*	-0.000129	0.000072*	
		1:00 AM–3:59 AM	-0.016089	0.011305	-0.000026	0.000057	-0.000019	0.000068	
	2	9:00 AM–1:59 PM	-0.044625	0.026330 *	-0.000624	0.000335*	-0.000811	0.000385**	
		7:00 PM–9:59 PM	-0.014642	0.023587	-0.000030	0.000172	0.000006	0.000186	
		10:00 PM–12:59 AM	0.046999	0.029150	-0.000224	0.000190	-0.000232	0.000206	
	Seattle	0	1:00 AM–3:59 AM	0.037105	0.027808	-0.000319	0.000174*	-0.000378	0.000183**
			9:00 AM–1:59 PM	0.029576	0.025492	0.000338	0.000299	0.000346	0.000351
			7:00 PM–9:59 PM	0.024789	0.023495	-0.000208	0.000172	-0.000221	0.000184
1		10:00 PM–12:59 AM	0.047033	0.029033	-0.000066	0.000188	-0.000100	0.000204	
		1:00 AM–3:59 AM	0.026775	0.027828	-0.000109	0.000174	-0.000188	0.000184	
		9:00 AM–1:59 PM	0.060067	0.025207 **	-0.000666	0.000267**	-0.000651	0.000328**	
2		7:00 PM–9:59 PM	0.011791	0.025587	-0.000002	0.000188	-0.000118	0.000203	
		10:00 PM–12:59 AM	0.001306	0.031619	-0.000145	0.000207	-0.000158	0.000225	
		1:00 AM–3:59 AM	0.053807	0.030295 *	-0.000238	0.000191	-0.000303	0.000201	
Irvine		0	9:00 AM–1:59 PM	-0.073111	0.031511 **	0.000211	0.000248	0.000445	0.000275
			7:00 PM–9:59 PM	-0.051299	0.025524 **	0.000010	0.000209	0.000109	0.000223
			10:00 PM–12:59 AM	-0.016598	0.029750	-0.000181	0.000207	-0.000024	0.000236
	1	1:00 AM–3:59 AM	0.047332	0.033086	-0.000273	0.000202	-0.000307	0.000244	
		9:00 AM–1:59 PM	-0.024234	0.031173	0.000099	0.000249	0.000015	0.000274	
		7:00 PM–9:59 PM	0.016005	0.025230	-0.000064	0.000207	-0.000044	0.000221	
	2	10:00 PM–12:59 AM	0.032927	0.029384	-0.000232	0.000206	-0.000290	0.000235	
		1:00 AM–3:59 AM	0.067824	0.033050 **	-0.000342	0.000205*	-0.000411	0.000245*	
		9:00 AM–1:59 PM	0.035549	0.030638	-0.000227	0.000241	-0.000332	0.000267	
	Irvine	0	7:00 PM–9:59 PM	0.020676	0.025257	-0.000111	0.000206	-0.000265	0.000221
			10:00 PM–12:59 AM	-0.001804	0.029503	0.000081	0.000205	-0.000114	0.000234
			1:00 AM–3:59 AM	-0.000973	0.033203	-0.000035	0.000203	-0.000216	0.000245
1		9:00 AM–1:59 PM	-0.045481	0.011516 **	0.000045	0.000076	0.000137	0.000099	
		7:00 PM–9:59 PM	0.006860	0.010445	-0.000134	0.000074*	-0.000089	0.000085	
		10:00 PM–12:59 AM	-0.010725	0.011397	0.000013	0.000071	0.000038	0.000089	
2		1:00 AM–3:59 AM	-0.004833	0.012091	-0.000018	0.000070	0.000023	0.000087	
		9:00 AM–1:59 PM	0.002253	0.011149	0.000104	0.000073	0.000086	0.000096	
		7:00 PM–9:59 PM	-0.010621	0.010424	0.000077	0.000074	0.000044	0.000087	
2		10:00 PM–12:59 AM	-0.016663	0.011354	0.000035	0.000071	0.000063	0.000089	
		1:00 AM–3:59 AM	-0.007034	0.012015	0.000119	0.000070*	0.000106	0.000087	
		9:00 AM–1:59 PM	-0.010734	0.011446	0.000112	0.000075	0.000083	0.000099	
2	7:00 PM–9:59 PM	0.015244	0.010602	0.000028	0.000075	-0.000046	0.000087		
	10:00 PM–12:59 AM	0.003868	0.011566	0.000027	0.000072	-0.000035	0.000090		
	1:00 AM–3:59 AM	-0.002794	0.012268	0.000038	0.000071	0.000033	0.000088		

^a Ln indicates the natural log of the measure; * indicates $P < 0.05$, and ** indicates $P < 0.01$.

Table 2 (Continued). Acute Effects of CAPs on HR and HRV Measures by Site^a (Table columns continue from previous page)

Site	Lag	Time Period	Ln(LF)		Ln(HF)		LF/HF		
			Value (β)	SE	Value (β)	SE	Value (β)	SE	
Manhattan	0	9:00 AM–1:59 PM	0.000302	0.000200	0.000564	0.000214**	-0.00061	0.00013**	
		7:00 PM–9:59 PM	0.000447	0.000167**	0.000909	0.000204**	-0.00092	0.00015**	
		10:00 PM–12:59 AM	0.000345	0.000163**	0.000710	0.000198**	-0.00077	0.00016**	
	1	1:00 AM–3:59 AM	0.000372	0.000160**	0.000690	0.000188**	-0.00071	0.00014**	
		9:00 AM–1:59 PM	0.000428	0.000202**	0.000601	0.000216**	-0.00030	0.00014**	
		7:00 PM–9:59 PM	0.000692	0.000173**	0.001009	0.000211**	-0.00070	0.00016**	
	2	10:00 PM–12:59 AM	0.000473	0.000184**	0.000779	0.000225**	-0.00065	0.00018**	
		1:00 AM–3:59 AM	0.000226	0.000181	0.000649	0.000212**	-0.00078	0.00016**	
		9:00 AM–1:59 PM	0.000667	0.000226**	0.000688	0.000241**	-0.00021	0.00015	
	Tuxedo	0	7:00 PM–9:59 PM	0.000189	0.000182	0.000162	0.000222	-0.00010	0.00017
			10:00 PM–12:59 AM	0.000366	0.000181**	0.000437	0.000221**	-0.00018	0.00017
			1:00 AM–3:59 AM	0.000505	0.000178**	0.000587	0.000209**	-0.00014	0.00016
1		9:00 AM–1:59 PM	-0.000655	0.000145**	-0.000793	0.000164**	0.00030	0.00010**	
		7:00 PM–9:59 PM	-0.000398	0.000121**	-0.000407	0.000131**	0.00005	0.00008	
		10:00 PM–12:59 AM	-0.000330	0.000121**	-0.000449	0.000140**	0.00027	0.00009**	
2		1:00 AM–3:59 AM	-0.000431	0.000116**	-0.000547	0.000133**	0.00025	0.00009**	
		9:00 AM–1:59 PM	-0.000447	0.000142**	-0.000422	0.000159**	-0.00006	0.00009	
		7:00 PM–9:59 PM	-0.000281	0.000120**	-0.000225	0.000130*	-0.00012	0.00007	
East Lansing		0	10:00 PM–12:59 AM	-0.000096	0.000121	-0.000114	0.000139	0.00005	0.00009
			1:00 AM–3:59 AM	-0.000231	0.000115**	-0.000254	0.000132*	0.00004	0.00009
			9:00 AM–1:59 PM	-0.000173	0.000140	-0.000142	0.000159	-0.00002	0.00009
	1	7:00 PM–9:59 PM	-0.000214	0.000121*	-0.000273	0.000131**	0.00012	0.00008	
		10:00 PM–12:59 AM	-0.000234	0.000124*	-0.000233	0.000144	0.00004	0.00009	
		1:00 AM–3:59 AM	-0.000029	0.000120	-0.000053	0.000138	0.00004	0.00009	
	2	9:00 AM–1:59 PM	-0.000658	0.000707**	-0.002304	0.001057**	0.00088	0.00059	
		7:00 PM–9:59 PM	-0.000061	0.000347	0.000074	0.000383	-0.00009	0.00021	
		10:00 PM–12:59 AM	-0.000281	0.000364	-0.000431	0.000418	0.00021	0.00023	
	Seattle	0	1:00 AM–3:59 AM	-0.000616	0.000340*	-0.000637	0.000384*	0.00010	0.00022
			9:00 AM–1:59 PM	0.000416	0.000644	0.000442	0.000964	0.00004	0.00053
			7:00 PM–9:59 PM	-0.000426	0.000347	-0.000424	0.000385	0.00012	0.00020
1		10:00 PM–12:59 AM	-0.000090	0.000364	-0.000098	0.000418	0.00015	0.00022	
		1:00 AM–3:59 AM	-0.000239	0.000343	-0.000304	0.000388	0.00015	0.00022	
		9:00 AM–1:59 PM	-0.000595	0.000590	-0.000789	0.000880	0.00030	0.00049	
2		7:00 PM–9:59 PM	-0.000117	0.000379	-0.000086	0.000418	0.00023	0.00023	
		10:00 PM–12:59 AM	-0.000386	0.000398	-0.000235	0.000456	0.00007	0.00025	
		1:00 AM–3:59 AM	-0.000330	0.000373	-0.000371	0.000422	0.00036	0.00024	
Irvine		0	9:00 AM–1:59 PM	0.000717	0.000439	0.000984	0.000530*	-0.00052	0.00029*
			7:00 PM–9:59 PM	-0.000005	0.000370	0.000007	0.000433	0.00001	0.00026
			10:00 PM–12:59 AM	-0.000093	0.000383	0.000004	0.000453	-0.00020	0.00027
	1	1:00 AM–3:59 AM	-0.000625	0.000390	-0.000734	0.000464	0.00020	0.00028	
		9:00 AM–1:59 PM	0.000070	0.000437	0.000030	0.000525	0.00000	0.00029	
		7:00 PM–9:59 PM	-0.000102	0.000365	-0.000096	0.000428	-0.00001	0.00026	
	2	10:00 PM–12:59 AM	-0.000570	0.000380	-0.000596	0.000450	0.00008	0.00027	
		9:00 AM–1:59 PM	-0.000633	0.000393	-0.000631	0.000466	0.00001	0.00028	
		7:00 PM–9:59 PM	-0.000581	0.000427	-0.000692	0.000515	0.00019	0.00028	
	Irvine	0	10:00 PM–12:59 AM	-0.000397	0.000366	-0.000533	0.000429	0.00029	0.00025
			1:00 AM–3:59 AM	-0.000014	0.000380	-0.000217	0.000449	0.00029	0.00026
			9:00 AM–1:59 PM	-0.000097	0.000392	-0.000333	0.000466	0.00036	0.00028
1		9:00 AM–1:59 PM	0.000105	0.000166	0.000208	0.000218	-0.00022	0.00013*	
		7:00 PM–9:59 PM	-0.000272	0.000141*	-0.000233	0.000161	-0.00005	0.00009	
		10:00 PM–12:59 AM	-0.000037	0.000146	0.000073	0.000174	-0.00008	0.00010	
2		1:00 AM–3:59 AM	-0.000039	0.000140	0.000052	0.000166	-0.00009	0.00010	
		9:00 AM–1:59 PM	0.000133	0.000161	0.000069	0.000211	0.00013	0.00013	
		7:00 PM–9:59 PM	0.000227	0.000143	0.000289	0.000165*	-0.00004	0.00009	
2		10:00 PM–12:59 AM	0.000209	0.000146	0.000188	0.000175	-0.00009	0.00010	
		1:00 AM–3:59 AM	0.000229	0.000141	0.000205	0.000168	-0.00002	0.00010	
		9:00 AM–1:59 PM	0.000246	0.000165	0.000140	0.000217	0.00017	0.00013	
2	7:00 PM–9:59 PM	-0.000024	0.000143	-0.000037	0.000164	0.00000	0.00009		
	10:00 PM–12:59 AM	0.000082	0.000147	-0.000024	0.000177	0.00013	0.00010		
	1:00 AM–3:59 AM	0.000032	0.000141	0.000036	0.000169	0.00003	0.00011		

^a Ln indicates the natural log of the measure; * indicates $P < 0.05$, and ** indicates $P < 0.01$.

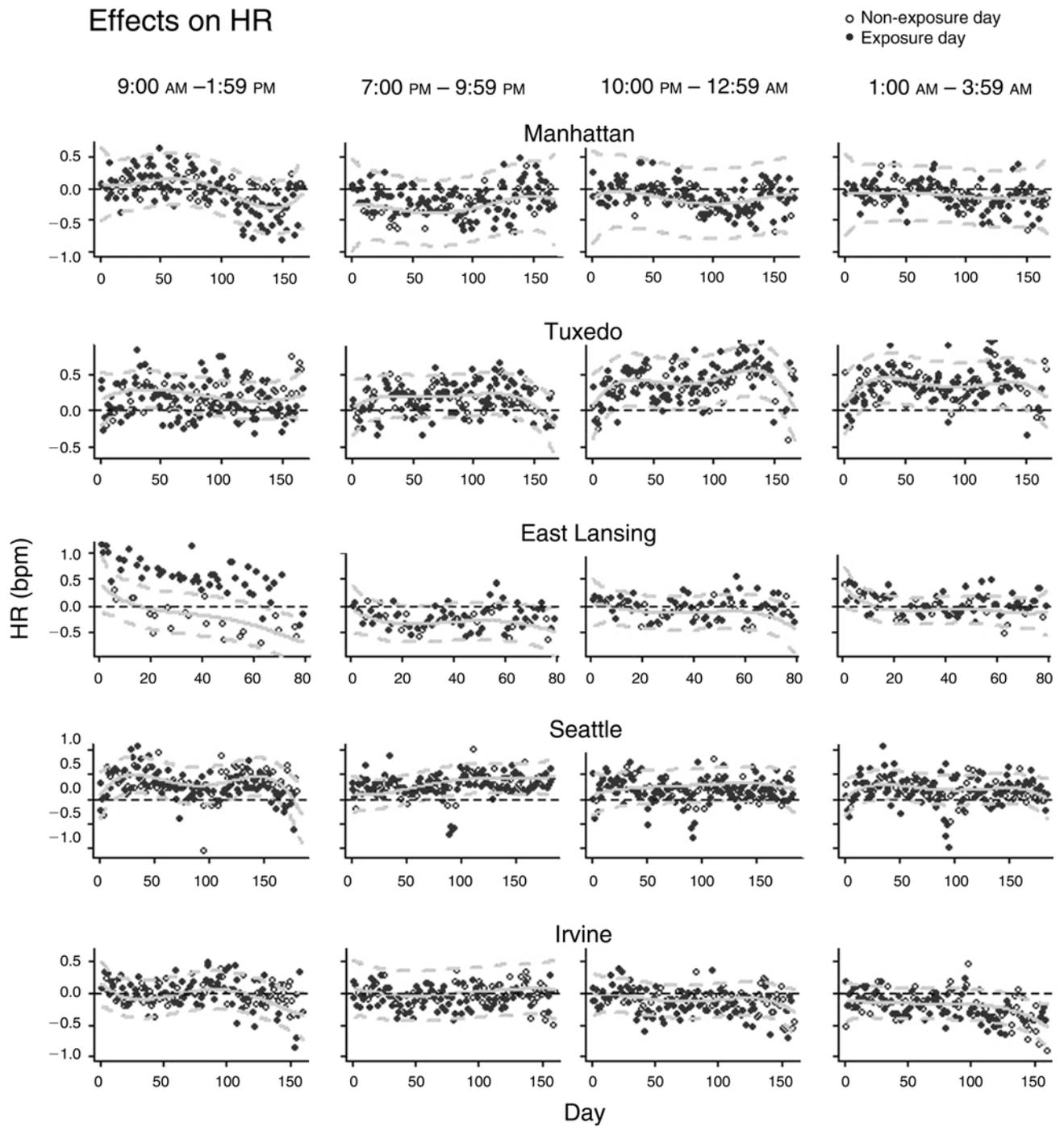


Figure 5. Acute changes (dots) and estimated chronic changes (solid lines) (with 95% confidence intervals [dashed lines]) in HR (bpm/ $\mu\text{g}/\text{m}^3$ CAPs) at selected times by site. Acute changes were calculated as differences between mean baseline-subtracted measures in the exposure and control groups. Note that the y-axis scales vary.

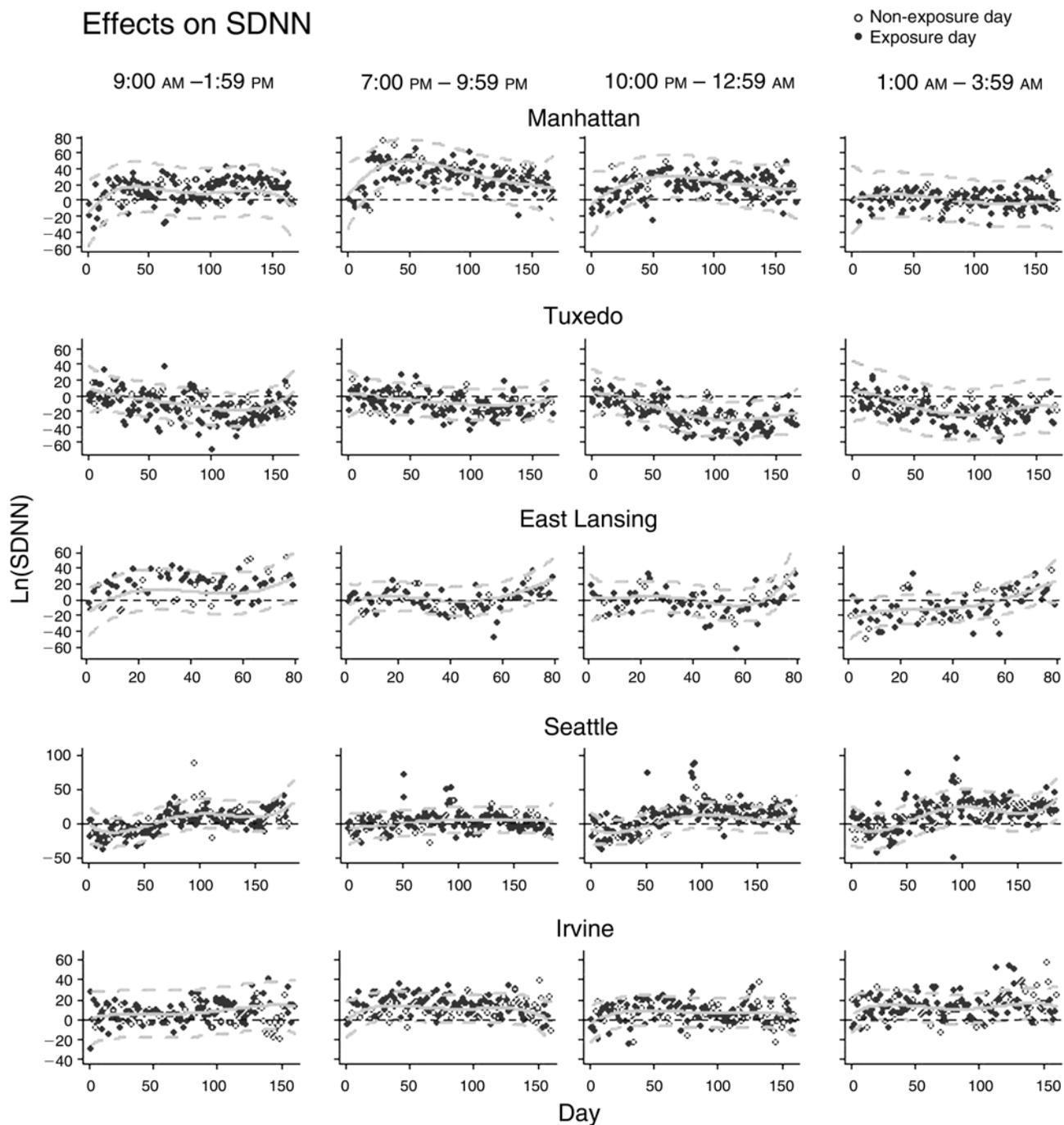


Figure 6. Acute changes (dots) and estimated chronic changes (solid lines) (with 95% confidence intervals [dashed lines]) in the natural log of the SDNN at selected times by site. Acute changes were calculated as differences between mean baseline-subtracted measures in the exposure and control groups. Note that the y-axis scales vary.

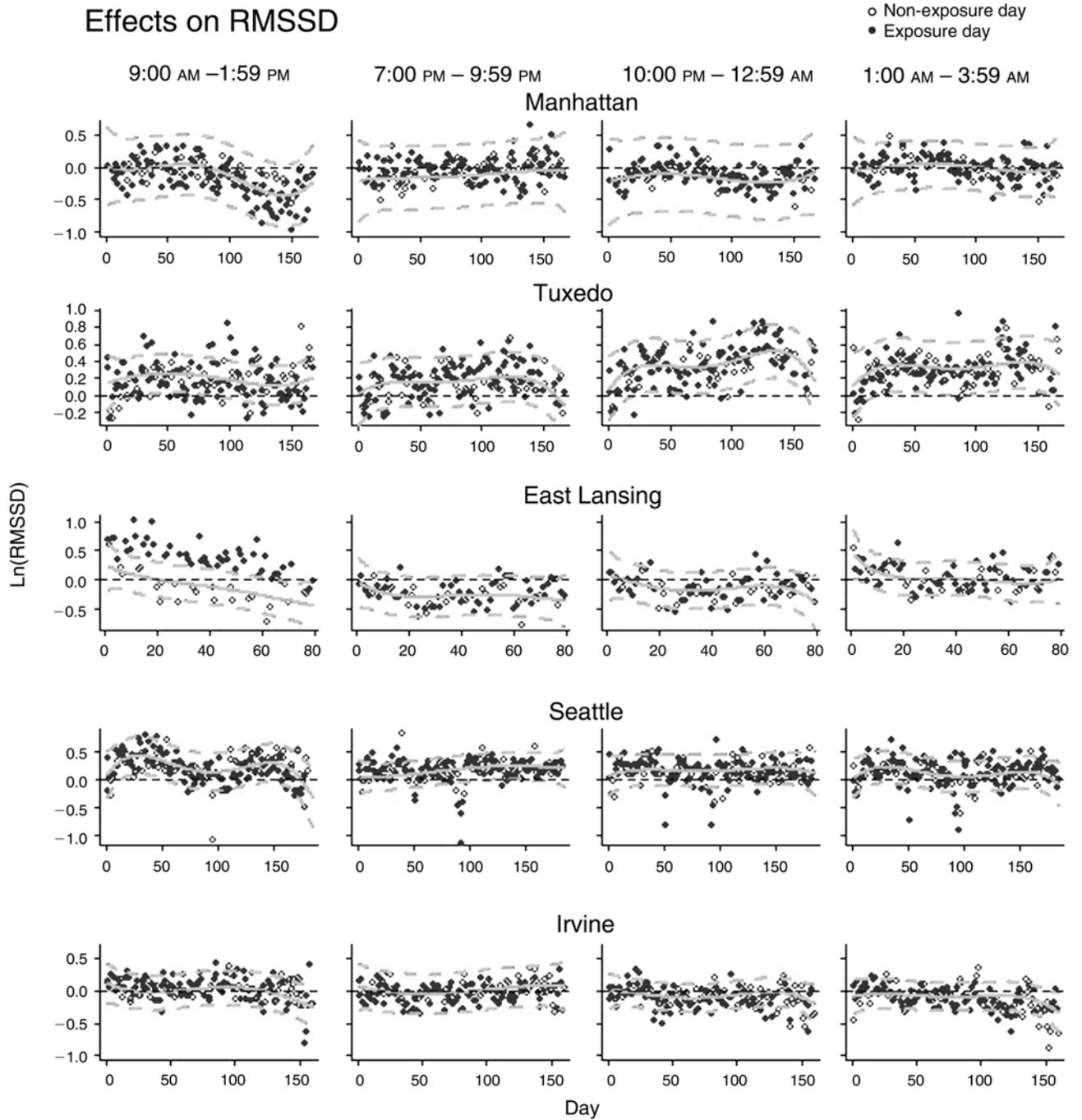


Figure 7. Acute changes (dots) and estimated chronic changes (solid lines) (with 95% confidence intervals [dashed lines]) in the natural log of the RMSSD at selected times by site. Acute changes were calculated as differences between mean baseline-subtracted measures in the exposure and control groups. Note that the y-axis scales vary.

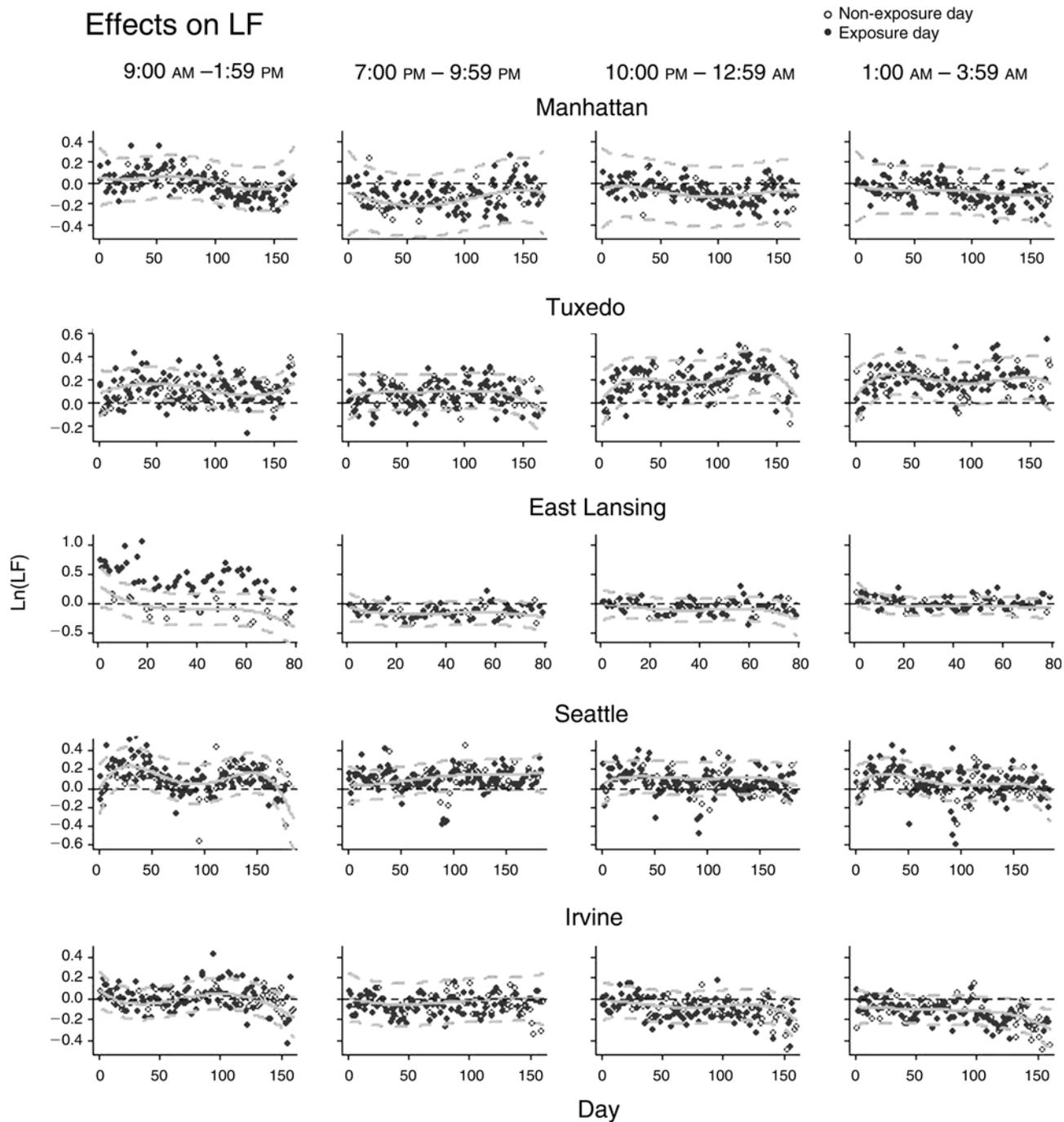


Figure 8. Acute changes (dots) and estimated chronic changes (solid lines) (with 95% confidence intervals [dashed lines]) in the natural log of the LF domain at selected times by site. Acute changes were calculated as differences between mean baseline-subtracted measures in the exposure and control groups. Note that the y-axis scales vary.

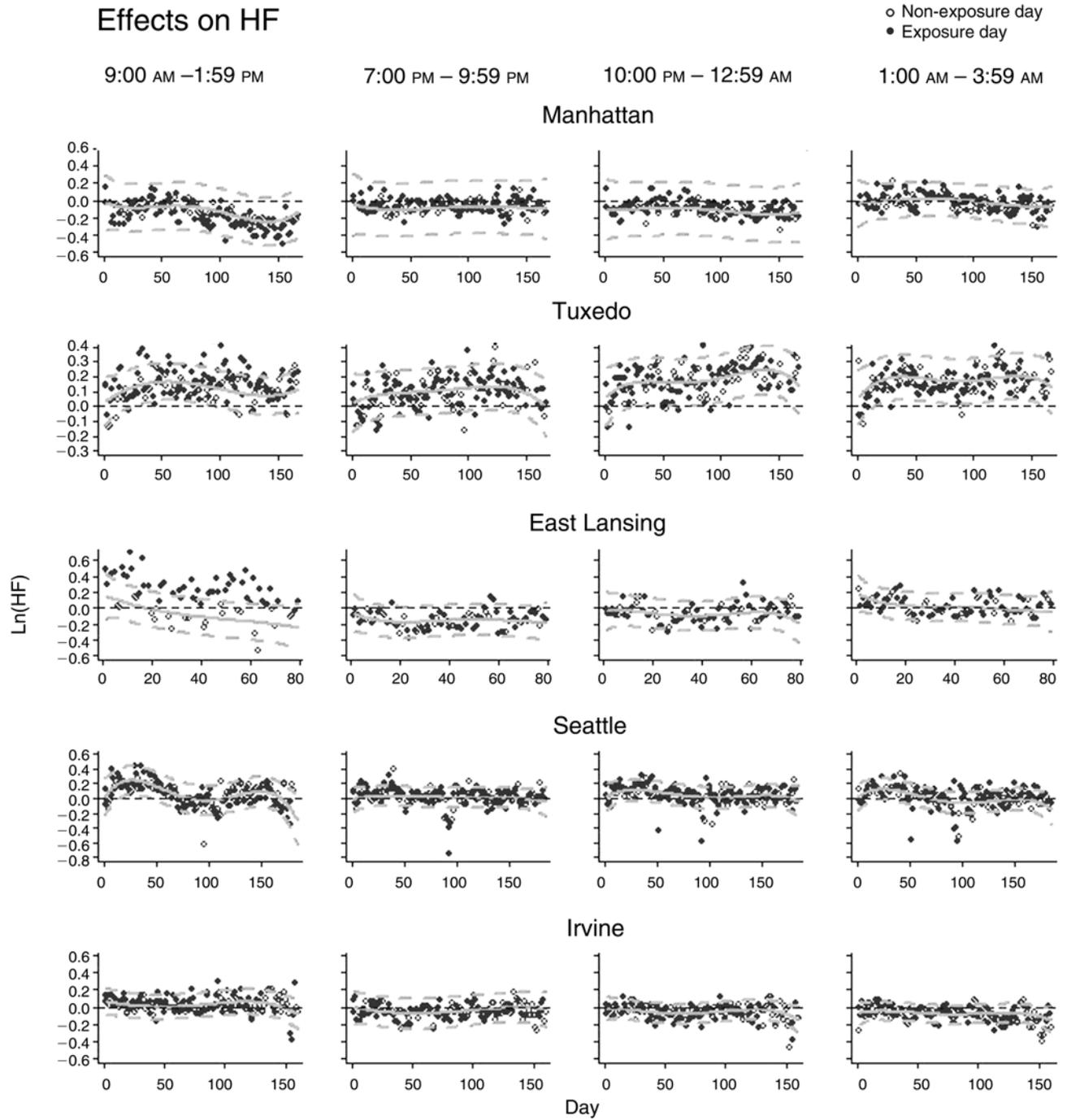


Figure 9. Acute changes (dots) and estimated chronic changes (solid lines) (with 95% confidence intervals [dashed lines]) in the natural log of the HF domain at selected times by site. Acute changes were calculated as differences between mean baseline-subtracted measures in the exposure and control groups. Note that the y-axis scales differ.

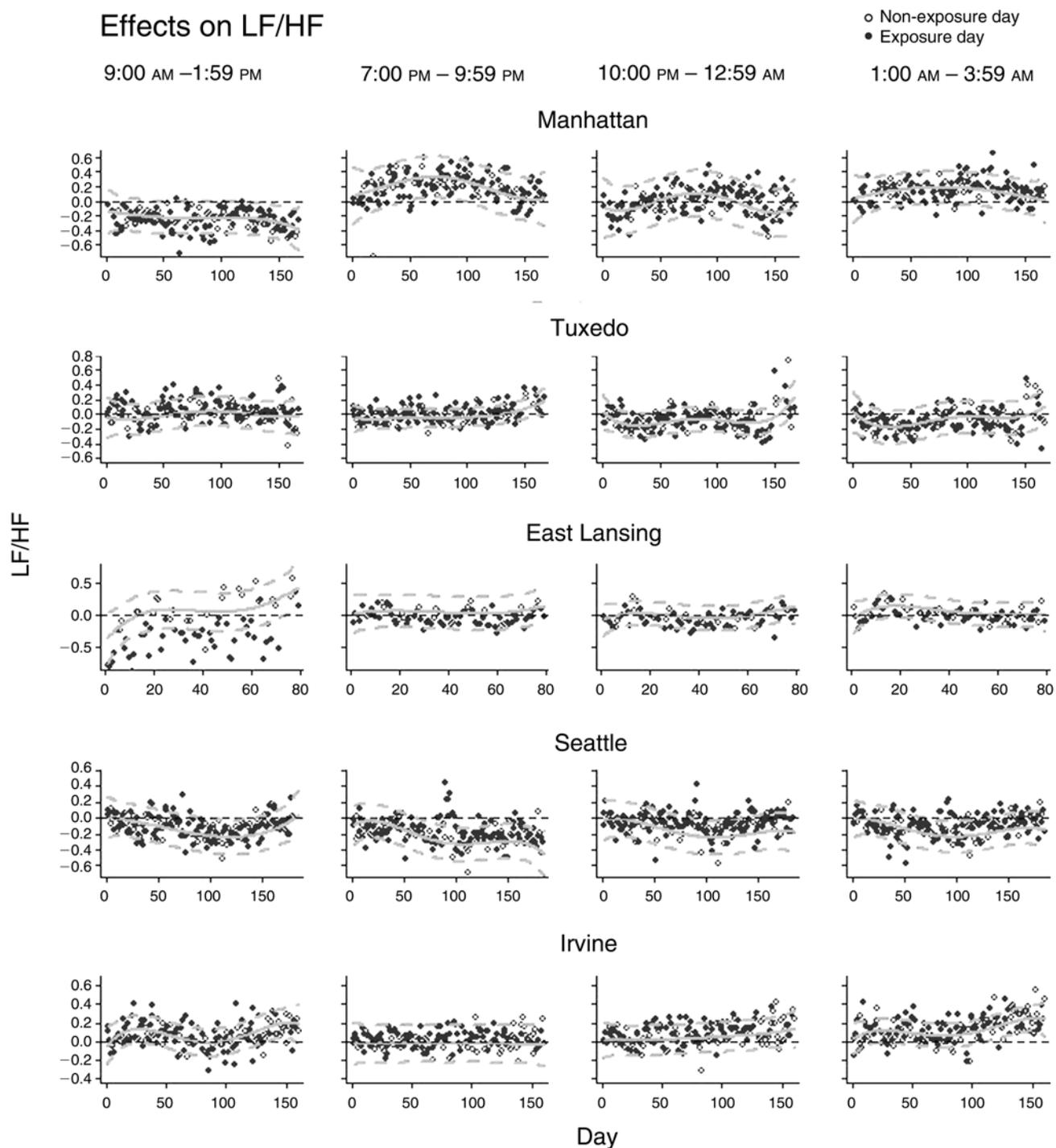


Figure 10. Acute changes (dots) and estimated chronic changes (solid lines) (with 95% confidence intervals [dashed lines]) in the ratio of the LF and HF domains at selected times by site. Acute changes were calculated as differences between mean baseline-subtracted measures in the exposure and control groups. Note that the y-axis scales differ.

IDENTIFYING SOURCE CATEGORIES AT EACH SITE

For Manhattan (Table 3), nine source categories were identified. We assigned PM_{2.5} that was rich in Ni and V to a Residual Oil Combustion category; rich in Na, Mg, and Cl to a Salt category; rich in EC and gaseous NO₂ to a Traffic category; rich in S and Se to a Sulfur–Coal category; rich in S and OC to a Secondary Aerosols category; rich in Fe and Mn to a Steel category (presumably from local construction or demolition); rich in Al, Si, and Ca to a Soil category; rich in K, Ba, and Cr to a Fireworks category (associated principally with July 4th fireworks); and rich in Pb and Zn to an Incineration category.

For Tuxedo (Table 4), four source categories were identified. We assigned PM_{2.5} rich in Se, S, P, and Br to a Sulfur–Coal category; rich in Si, Ti, Al, and Ca to a Soil category (not surprising as a source, given that Tuxedo is in a rural area); rich in Na and Cl to a Salt category; and rich in Fe, Ni, Zn, Ca, Mn, and V to a Ni Refinery category (because of the presence of both Fe and Ni). The Ni Refinery category also contained other PM components (Zn, Mn, and V) that were probably transported from residential heating in the New York City area (which produces PM high in Ni but not in other metals). Unlike the Ni at the other sites, the Ni at Tuxedo was not well correlated with V ($r = 0.32$ at Tuxedo

Table 3. Factor Loadings at Manhattan^a

	Incineration	Steel	Soil	Residual Oil Combustion	Sulfur–Coal ^b	Fireworks	Salt	Traffic	Secondary Aerosols ^b
Na	0.13						0.71		
Mg	-0.17		0.14	0.12			0.37		
Al		-0.11	0.71	0.18					0.22
Si		0.17	0.59	0.11				0.26	0.20
S			0.25	0.22	0.46				0.31
Cl	0.72		-0.11				0.31		-0.16
K		0.22		0.11	0.16	0.70	-0.13	-0.25	0.11
Ca	0.17	0.39	0.36	0.16			0.22		
V				0.74				0.12	0.20
Mn		0.95							
Fe	0.31	0.62	0.13					0.26	
Ni				0.71	0.11		0.23		-0.12
Cu	0.42	0.18	0.27	0.22	0.11	0.28	-0.12	0.22	-0.24
Zn	0.90								
Se			-0.22		0.57			0.10	0.22
Br			0.19		0.74		0.19	0.18	
Ba						0.77	0.12	0.19	
Pb	0.61		0.26		0.21				-0.20
OC			0.12		0.12			0.12	0.70
EC		0.21		0.26	0.19		0.12	0.48	0.20
NO ₂ ^c	-0.13			0.11	0.13		-0.18	0.61	0.12

^a Source categories are listed by strength of the factor analysis. Data shown are factor loadings (i.e., correlation coefficients between the chemical components and source categories). Values smaller than 0.01 are omitted.

^b Sulfate–Coal and Secondary Aerosols include long-range transported PM_{2.5}.

^c NO₂ data were obtained from the EPA database and are shown only for the Manhattan factor analysis.

versus 0.74 at Manhattan, 0.62 at Seattle, 0.59 at East Lansing, and 0.78 at Irvine) and was consistent with the plume of a distant upwind Ni refinery at Sudbury, Ontario, described in our previous study (Lippmann et al. 2006).

For East Lansing (Table 5), five source categories were identified. We assigned PM_{2.5} rich in Si, Ca, Al, and Fe to a Soil category; rich in S to a Sulfur–Coal category; rich in V and Ni to a Residual Oil Combustion category; rich in Zn and Cl to a Zn–Cl category; and rich in EC and OC to an EC–OC category.

For Seattle (Table 6), six source categories were identified. We assigned PM_{2.5} rich in Na, Mg, and Cl to a Salt category; rich in Al, Si, Ca, and Fe to a Soil category; rich in Ca, Mn, Cu, Fe, Zn, and EC to a Traffic and Road Dust category;

rich in K, Cu, and EC to a Biomass Combustion category; rich in V and Ni to a Residual Oil Combustion category; and rich in S and Br to a Sulfates category.

For Irvine (Table 7), six source categories were identified. We assigned PM_{2.5} rich in V and Ni to a Residual Oil Combustion category; rich in Si and Al to a Soil category; rich in Mn, Cu, Ca, and EC to a Traffic category; rich in K and EC to a Biomass Combustion category; rich in Cl and K to a Salt category; and rich in Pb and Zn to a Metals category.

For the source characterization model, the daily factor scores based on trace elements were considered to be interpretable as being associated with PM_{2.5} components (such as Se, which is largely derived from Coal Combustion) as covariates for estimating acute effects.

Table 4. Factor Loadings at Tuxedo^a

	Soil	Sulfur–Coal ^b	Ni Refinery	Salt
Na	–0.04	0.11	–0.05	0.75
Mg	0.15	–0.01	0.04	0.54
Al	1.01	–0.18	0.05	0.06
Si	0.68	0.10	0.27	0.14
S	0.46	0.57	–0.04	0.11
Cl	–0.15	–0.13		0.73
K	0.34	0.33	0.03	
Ca	0.31	0.20	0.59	0.11
V	–0.17	0.28	0.42	0.12
Mn	0.04	0.40	0.47	–0.02
Fe	0.18	0.34	0.69	0.09
Ni	0.13	–0.24	0.64	–0.03
Cu	0.39	0.17	0.36	–0.08
Zn	–0.04	0.12	0.65	–0.02
Se	0.05	0.76	0.06	0.05
Br	0.24	0.80	0.09	0.19
Pb	0.44	0.06	0.07	–0.09
OC	0.27	0.22	–0.10	0.01
EC	–0.03	0.32	0.11	–0.06

^a Source categories are listed by strength of the factor analysis. Data shown are factor loadings (i.e., correlation coefficients between the chemical components and source categories). Values smaller than 0.01 are omitted.

^b Sulfur–Coal and Ni Refinery include long-range transported PM_{2.5}.

Table 5. Factor Loadings at East Lansing^a

	Soil	Sulfur–Coal ^b	Residual Oil Combustion	Zn–Cl	OC–EC
Na	0.43	0.10	–0.19	0.29	
Mg	0.43	0.21	0.19		
Al	0.70	0.21	0.34		
Si	0.91				
S	0.11	0.77	–0.13		
Cl		–0.20		0.84	
K	0.42	0.38	–0.21	0.11	0.26
Ca	0.91	–0.25		0.12	0.16
V		0.19	0.66	0.28	
Mn	0.31	0.51	0.17	0.17	
Fe	0.63	0.16	–0.21	0.28	0.20
Ni	0.18	0.20	0.56	0.20	–0.12
Cu			–0.45	0.19	
Zn		0.38	–0.48	0.50	0.21
As	–0.14	0.32	0.34	0.28	0.15
Se		0.59	0.41	0.24	
Br	0.14	0.53	0.18	0.36	0.20
Ba	0.21	0.42	0.60		
Pb			–0.30		
OC		–0.14	0.17	0.11	0.77
EC			–0.10		0.70

^a Source categories are listed by strength of the factor analysis. Data shown are factor loadings (i.e., correlation coefficients between the chemical components and source categories). Values smaller than 0.01 are omitted.

^b Sulfur–Coal includes long-range transported PM_{2.5}.

Correlations among the CAPs components we examined and time-series plots for the source categories are shown by site in Appendix C.

ECG CHANGES ASSOCIATED WITH SOURCE CATEGORIES

Our tabulation of the significant associations between the source categories and ECG changes (measured as HR, SDNN, and RMSSD without distinction as to the direction of change) was based on a model that used the non-negative values of the source-based concentrations. (Bi-directional changes are attributable to stimuli that elicit adaptive functional changes, and we recognized that such changes were not necessarily adverse.) Table 8 shows the total number of significant associations found for each source category defined by our factor analyses and HR, SDNN, and RMSSD by site, with a breakdown by lag day and daily time period. It is notable that there were strong associations between at

least one source category and ECG changes (expressed as the sum of the number of significant changes in HR, SDNN, and RMSSD for all lag days) at all five sites.

At Manhattan, as shown in Table 8, the Residual Oil Combustion category with 0-, 1- and 2-day lags was more closely associated with ECG changes (a total of 54 significant change events) than was any other single source category. However, when the factor scores for the Secondary Aerosols category (high in S and OC) were combined with those for the Sulfur–Coal category (high in S, Se, and Br possibly from a Coal Combustion source category), these forms of long-range transported PM_{2.5} were even more closely associated with ECG changes (a total of 59 events of significant change). Other significant associations were those for the Salt (48) and Traffic (44) categories. Steel with a 0-day lag had a larger number of associations than the Sulfur–Coal category did and a smaller number than the Traffic category, but the differences did not last into the 1- and 2-day lags.

Table 6. Factor Loadings at Seattle^a

	Salt	Soil	Traffic and Road Dust	Biomass Combustion	Residual Oil Combustion	Sulfates ^b
Na	0.85	-0.17	0.02	-0.08	-0.05	0.25
Mg	0.71	0.14	0.09	-0.11	-0.01	0.19
Al	-0.10	0.82	0.02	0.11	0.03	0.07
Si	0.02	0.83	0.03	0.14	0.03	0.14
S	0.03	-0.06	-0.11	0.24	0.29	0.69
Cl	0.85	-0.05	-0.07	0.13	-0.04	-0.23
K	0.05	0.12	-0.08	0.63	-0.01	0.19
Ca	0.19	0.39	0.53	-0.08	0.01	0.19
V	-0.01	-0.02	0.08	-0.05	0.76	0.01
Mn	0.05	0.03	0.83	0.10	0.14	-0.02
Fe	-0.02	0.40	0.42	0.34	0.16	-0.01
Ni	-0.03	0.04	-0.06	0.03	0.79	0.08
Cu	-0.06	0.14	0.53	0.47	0.03	0.05
Zn	-0.04	0.20	0.43	0.30	0.03	-0.04
Br	0.05	0.18	0.07	0.04	0.02	0.67
Pb	-0.10	0.23	0.37	0.05	0.00	0.16
EC	-0.12	0.04	0.46	0.58	0.06	0.06

^a Source categories are listed by strength of the factor analysis. Data shown are factor loadings (i.e., correlation coefficients between the chemical components and source categories). Values smaller than 0.01 are omitted.

^b Sulfates includes long-range transported PM_{2.5}.

Table 7. Factor Loadings at Irvine^a

	Residual Oil Combustion	Soil	Traffic	Biomass Combustion	Salt	Metals
Na			-0.21			
Al		0.72	0.18		-0.32	
Si		0.75		0.24	-0.12	
S	0.64		-0.41	0.27		
Cl		-0.12		-0.13	0.62	0.20
K		0.51		0.44	0.43	
Ca		0.68	0.15	0.21	0.18	
V	0.89					
Mn	0.22	0.19	0.67	0.18		
Fe	-0.10	0.40	0.48	0.24	-0.10	0.23
Ni	0.86		0.13			
Cu	0.15	0.32	0.38	0.27		0.44
Zn		0.28	0.26	0.21	-0.14	0.58
Se	0.60	0.10	0.20		0.21	-0.21
Br	0.48	0.18		0.45		0.22
Ba		0.41	0.67			
Pb						0.59

^a Source categories are listed by strength of the factor analysis. Data shown are factor loadings (i.e., correlation coefficients between the chemical components and source categories). Values smaller than 0.01 are omitted.

Mouse Inhalation Exposure to CAPs from Five Airsheds

Table 8. Significant Associations Found Between Source Categories and HR and HRV Changes by Site

Source Category ^a	Sum	Lag 0	Lag 1	Lag 2	9:00 AM– 1:59 PM	7:00 PM– 9:59 PM	10:00 PM– 12:59 AM	1:00 AM– 3:59 AM
Manhattan								
Residual Oil Combustion	54	17	21	16	13	13	13	15
Salt	48	14	19	15	6	14	14	14
Traffic	44	13	21	10	9	12	11	12
Secondary Aerosols	44	14	22	8	10	6	13	15
Steel	17	14	1	2	2	4	4	7
Sulfur–Coal	15	5	0	10	5	4	3	3
Soil	13	3	8	2	8	2	1	2
Fireworks	8	1	3	4	3	0	3	2
Incineration	5	4	1	0	4	0	1	0
Tuxedo								
Sulfur–Coal	27	12	11	4	10	5	5	7
Soil	24	1	18	5	3	9	6	6
Salt	14	13	0	1	3	4	5	2
Ni Refinery	9	3	2	4	0	3	5	1
East Lansing								
Soil	6	1	0	5	0	1	5	0
Zn–Cl	6	6	0	0	0	0	0	6
Sulfur–Coal	5	4	0	1	0	0	4	1
OC–EC	2	1	1	0	0	2	0	0
Residual Oil Combustion	1	1	0	0	0	1	0	0
Seattle								
Soil	31	6	10	15	13	7	6	5
Residual Oil Combustion	13	1	5	7	1	3	1	8
Salt	8	2	1	5	2	2	0	4
Biomass Combustion	6	6	0	0	0	0	0	6
Sulfates	5	0	0	5	0	0	0	5
Traffic and Road Dust	3	1	2	0	1	2	0	0
Irvine								
Soil	20	1	5	14	1	7	7	5
Biomass Combustion	11	2	3	6	4	1	3	3
Residual Oil Combustion	10	3	3	4	5	4	0	1
Salt	5	1	4	0	3	1	1	0
Metals	4	3	1	0	3	1	0	0
Traffic	3	1	1	1	1	0	1	1

^a Sulfates, Sulfur–Coal, and Secondary Aerosols include long-range transported PM_{2.5}.

Similarly, the Soil category with a 0-day lag was associated with HR and some HRV measures but not for the 1- or 2-day lags; and Incineration with a 0-day lag had a strong effect but not for the 1- or 2-day lags.

Surprisingly, we detected source category contributions at Tuxedo (Table 8) — the Sulfur–Coal category (which has components typically associated with the Coal Combustion category) with 0-, 1-, and 2-day lags that were more closely associated with ECG changes (a total of 27 significant change events) than were any of the other source categories; the effect decreased for the 1- and 2-day lags. The second strongest association (24 events) was for the Soil category, but only with the 1-day lag. Because the factor score for the Soil category was higher in late spring than it was in summer (Appendix C), the Soil category at Tuxedo might have included PM attributable to residual oil combustion that was transported from New York City. Given the inland location of Tuxedo, it was surprising that the Salt category had the next highest number of significant associations (14) with ECG changes, though only for the 0-day lag. The Ni Refinery category affected ECG measures for all three lag days. Overall, in terms of the numbers of significant associations at Tuxedo, from highest to lowest, Sulfur–Coal > Soil > Salt > Ni Refinery.

At Seattle (Table 8), the Soil category with 0-, 1-, and 2-day lags was more closely associated with ECG changes (a total of 31 significant change events) than was any other

source category. It produced its effects during the actual exposure period (9:00 AM–1:59 PM), which wasn't seen at the other sites, except at Manhattan. The other source categories were only weakly associated with the ECG measures.

At East Lansing and Irvine (Table 8), the source categories were much more weakly associated with ECG changes than were those at the other sites. For Irvine, because the directions of the effect changes associated with the Residual Oil Combustion and Salt categories were mirror images of each other, we split the Residual Oil Combustion category into high and low Salt days. The results, shown in Table 9, indicated that on high Salt days (when the prevailing winds originated offshore) the Residual Oil Combustion category became a major source of significant associations; and that on low Salt days (when the prevailing winds originated inland, bringing traffic pollution to Irvine), the Traffic category had the most significant associations.

ECG CHANGES ASSOCIATED WITH INDIVIDUAL PM COMPONENTS

We also performed single-element analyses for 16 individual elements and ECG changes with 0-day lags. In order to keep the number of counts to a more manageable level, we only used the *P* values that were below 0.05 in estimating the strength of each element's associations with HR

Table 9. Significant Associations Found Between Source Categories and HR and HRV Changes at Irvine

Source Category	Sum	Lag 0	Lag 1	Lag 2	9:00 AM– 1:59 PM	7:00 PM– 9:59 PM	10:00 PM– 12:59 AM	1:00 AM– 3:59 AM
On High Salt Days								
Soil	23	2	8	13	3	9	6	5
Biomass Combustion	16	4	4	8	2	3	6	5
Residual Oil Combustion ^a	12	3	9	0	4	4	4	0
Traffic	11	4	2	5	4	5	0	2
Metals	11	7	1	3	3	3	5	0
Salt	6	0	6	0	0	0	6	0
On Low Salt Days								
Traffic	20	0	7	13	0	9	7	4
Residual Oil Combustion ^a	15	0	6	9	1	3	9	2
Biomass Combustion	9	3	5	1	2	1	5	1
Salt	7	0	5	2	0	1	4	2
Metals	7	1	0	6	0	3	4	0
Soil	4	3	1	0	3	1	0	0

^a Residual Oil Combustion on high and low Salt days.

and with the five HRV measures. The results by site are shown in Table 10. It can be seen that the largest numbers of ECG changes associated with individual elements were observed at Manhattan, followed by Tuxedo; the numbers decreased dramatically at (in descending order) Irvine, East Lansing, and Seattle.

For total CAPs and each CAPs component, we calculated mean concentrations by site and counted the number of significant differences ($P < 0.05$) in HR, SDNN, and RMSSD revealed in our 72 models (i.e., six outcomes for each of four daily time periods over three lag structures) for each site. For example, the average CAPs concentrations (Y) at the five sites were 122.9, 135.8, 60.5, 67.8, and 138.3 $\mu\text{g}/\text{m}^3$, and the number of significant differences (X) revealed in the models was 56, 38, 7, 3, and 1 for Manhattan, Tuxedo, East Lansing, Seattle, and Irvine, respectively. Table 11

shows the slope, r , and r^2 from the simple linear model $Y = a + bX$. As shown in the table, Ni had the highest concentration–response relationship ($r^2 = 0.96$), followed by Al ($r^2 = 0.81$), EC ($r^2 = 0.79$), P ($r^2 = 0.77$), S ($r^2 = 0.65$), V ($r^2 = 0.35$), Mg ($r^2 = 0.30$), Zn ($r^2 = 0.27$), and Se ($r^2 = 0.19$). The components with the lowest r^2 values were Cu ($r^2 = 0.00$), Cr ($r^2 = 0.02$), Si ($r^2 = 0.02$), Fe ($r^2 = 0.02$), Br ($r^2 = 0.03$), Na ($r^2 = 0.06$), Ti ($r^2 = 0.08$), and OC ($r^2 = 0.08$).

SUMMARY OF ASSOCIATIONS BETWEEN SOURCE CATEGORIES AND ECG CHANGES

Our tabulations of significant associations between source categories and ECG changes were based on a model that used the non-negative values of the source category–based concentrations. Again, as shown in Table 8, it is

Table 10. Significant Associations Found Between CAPs Components and HR and HRV Changes by Site

Pollutant	Manhattan	Tuxedo	East Lansing	Seattle ^a	Irvine	Total for Each Pollutant	Rank of Total for Each Pollutant
EC ^b	50	13	7		9	79	17
OC	18	35	7		31	91	13
Al	46	32	10	16	24	128	4
Br	49	49	10	16	20	144	2
Ca	40	34	16	3	22	115	7
Cr	8	16	1	1	5	31	21
Cu	10	20	7	8	12	57	19
Fe	26	32	18	20	16	112	8
K	13	42	24	3	23	105	10
Mg	30	12	3	15	27	87	14
Mn	7	27	2	11	12	59	18
Na	65	16	22	27	19	149	1
Ni	68	17	1	4	11	101	11
P	68	46	3	8	1	126	5
Pb	5	11	6	5	13	40	20
S	54	43	11	2	7	117	6
Se	38	40	4	0	2	84	15
Si	46	39	17	14	22	138	3
Ti	37	37	12	13	11	110	9
V	59	9	3	7	13	91	13
Zn	7	34	12	17	13	83	16
Total for each site	744	604	196	190	313	2047	

^a EC and OC were not measured in Seattle.

^b EC was used instead of BC.

notable that strong associations were found between at least one source category and ECG changes (expressed as the sum of the number of significant changes in HR, SDNN, and RMSSD for all lag days) at all five sites.

1. The largest number of significant associations found was 54 for the Residual Oil Combustion category at Manhattan. This category also had significant associations at Seattle and Irvine but not at East Lansing. (A Residual Oil Combustion category was not identified at Tuxedo.)
2. The Soil category had significant associations at all five sites.
3. The sulfur-related categories with a 0-day lag had a large number of associations at Manhattan (Secondary Aerosols), and a smaller number at Tuxedo (Sulfur-Coal), Seattle (Sulfates), and East Lansing (Sulfur-Coal). (No sulfur-related category was identified at Irvine.)
4. The Biomass Combustion category, with notable contributions to $PM_{2.5}$ concentrations at Seattle and Irvine, had its only effects at Seattle with a 0-day lag and was most prominent at Irvine with a 2-day lag. (A Biomass Combustion category was not identified at the other three sites.)
5. The Salt category, with the highest contribution to $PM_{2.5}$ concentrations at Seattle, had its largest number of associations at Manhattan; followed by Tuxedo, Seattle, and Irvine. (A Salt category was not identified at East Lansing.)
6. The Traffic category had a large number of associations at Manhattan and a smaller number at Seattle (Traffic and Road Dust) and Irvine. (A Traffic category was not identified at Tuxedo or East Lansing.)

Table 11. Combined Concentration–Response Relationships for CAPs, CAPs Components, and HR and HRV Changes

Pollutant	Slope	Correlation (r)	r^2	Correlation Rank
CAPs	0.742	0.483		
Ni	0.912	0.980	0.960	1
Al	7.183	0.898	0.807	2
EC ^a	18.600	0.889	0.791	3
P	2.175	0.878	0.771	4
S	183.352	0.804	0.646	5
V	0.337	0.590	0.348	6
Mg	13.537	0.549	0.302	7
Zn	-15.088	-0.519	0.269	8
Se	-0.147	-0.435	0.189	9
Pb	-6.105	-0.423	0.179	10
Ca	10.620	0.420	0.176	11
K	-1.855	-0.361	0.130	12
Mn	-1.357	-0.334	0.112	13
OC	-48.853	-0.288	0.083	14
Ti	0.509	0.280	0.079	15
Na	-23.570	-0.242	0.058	16
Br	-0.160	-0.164	0.027	17
Fe	-15.244	-0.144	0.021	18
Si	4.521	0.139	0.019	19
Cr	0.123	0.124	0.015	20
Cu	-0.139	-0.022	0.000	21

^a EC was used instead of BC.

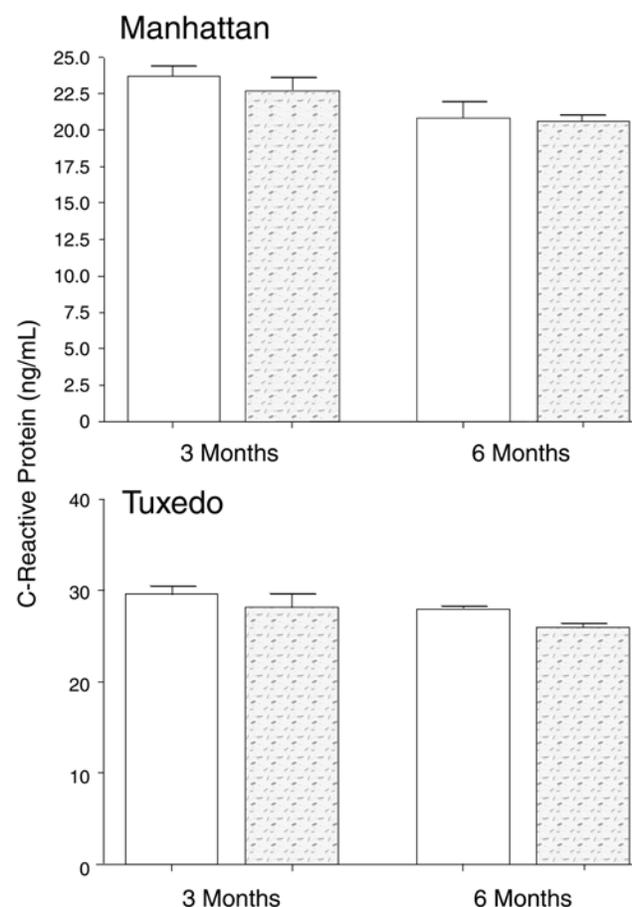


Figure 11. Effects of CAPs (patterned bars) and filtered air (empty bars) on serum C-reactive protein concentrations at Manhattan and Tuxedo (as measured by ELISA) after 3 and 6 months of exposures. Note that the y-axis scales vary.

CHANGES IN SERUM BIOMARKERS

We measured changes in serum biomarkers after 3 and 6 months of CAPs exposures at Manhattan and Tuxedo and after 2, 4, and 6 months of CAPs exposures at East Lansing, Seattle, and Irvine. No changes were observed in C-reactive protein (Figure 11). Serum IL-6 concentrations (Figure 12) were down-regulated at Irvine in mice after 6 months of CAPs exposure compared with the controls. Serum IL-10 (Figure 13) was up-regulated at Manhattan by CAPs at 3 months and down-regulated at Irvine at 6 months. A general

trend toward increased serum IL-12 concentrations (Figure 14) was found at Manhattan and Tuxedo at both 3 and 6 months. Serum GM-CSF (Figure 15) at Irvine was up-regulated by CAPs at 6 months. No changes were observed in IL-13 (Figure 16), monocyte chemoattractant protein 1 (Figure 17), TNF- α (Figure 18) or VEGF-A (Figure 19). These small, inconsistent changes were probably random events, with little or no biologic significance, indicating that there was no significant systemic inflammation at the time the animals were killed.

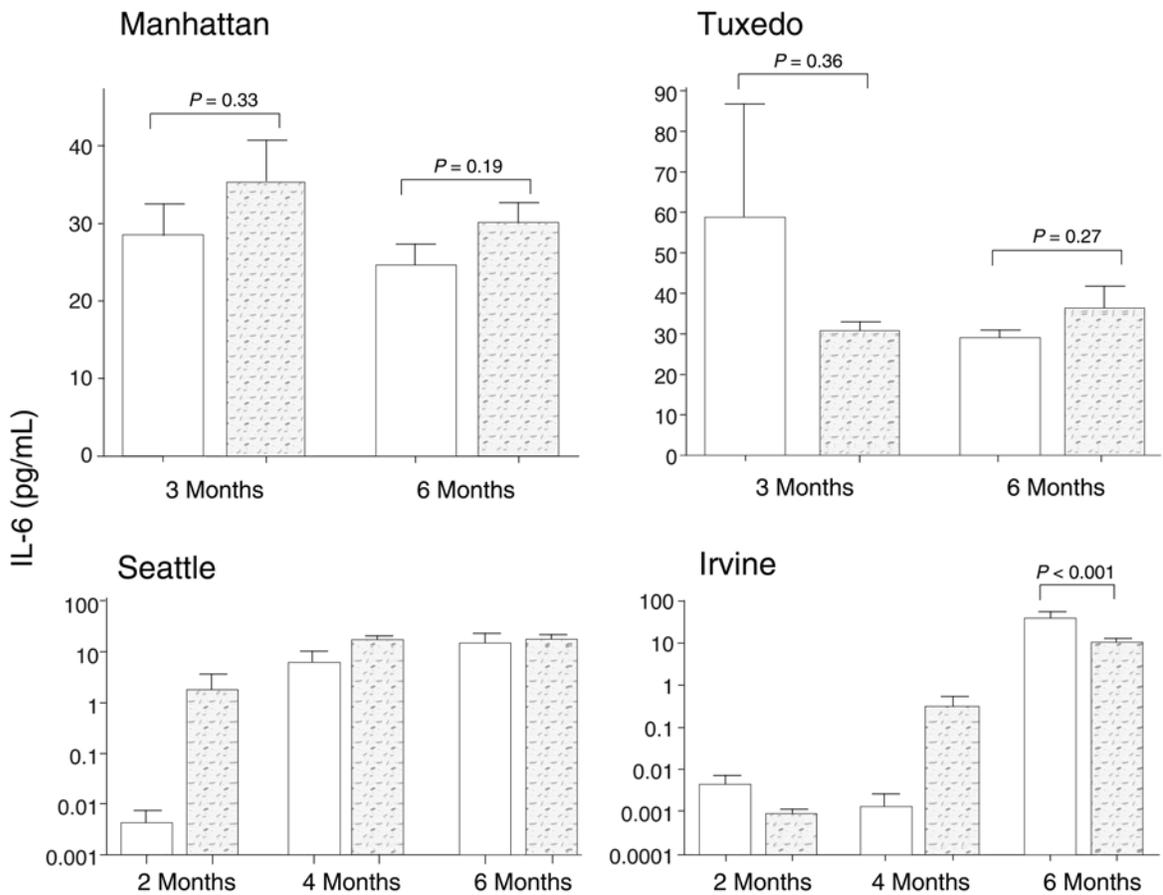


Figure 12. Effects of CAPs (patterned bars) and filtered air (empty bars) on serum IL-6 concentrations at Manhattan and Tuxedo (as measured by ELISA) and at Seattle and Irvine (as measured by Meso Scale Discovery) after 2, 3, 4, and 6 months of exposures. Note that the y-axis scales vary and are in some cases logarithmic.

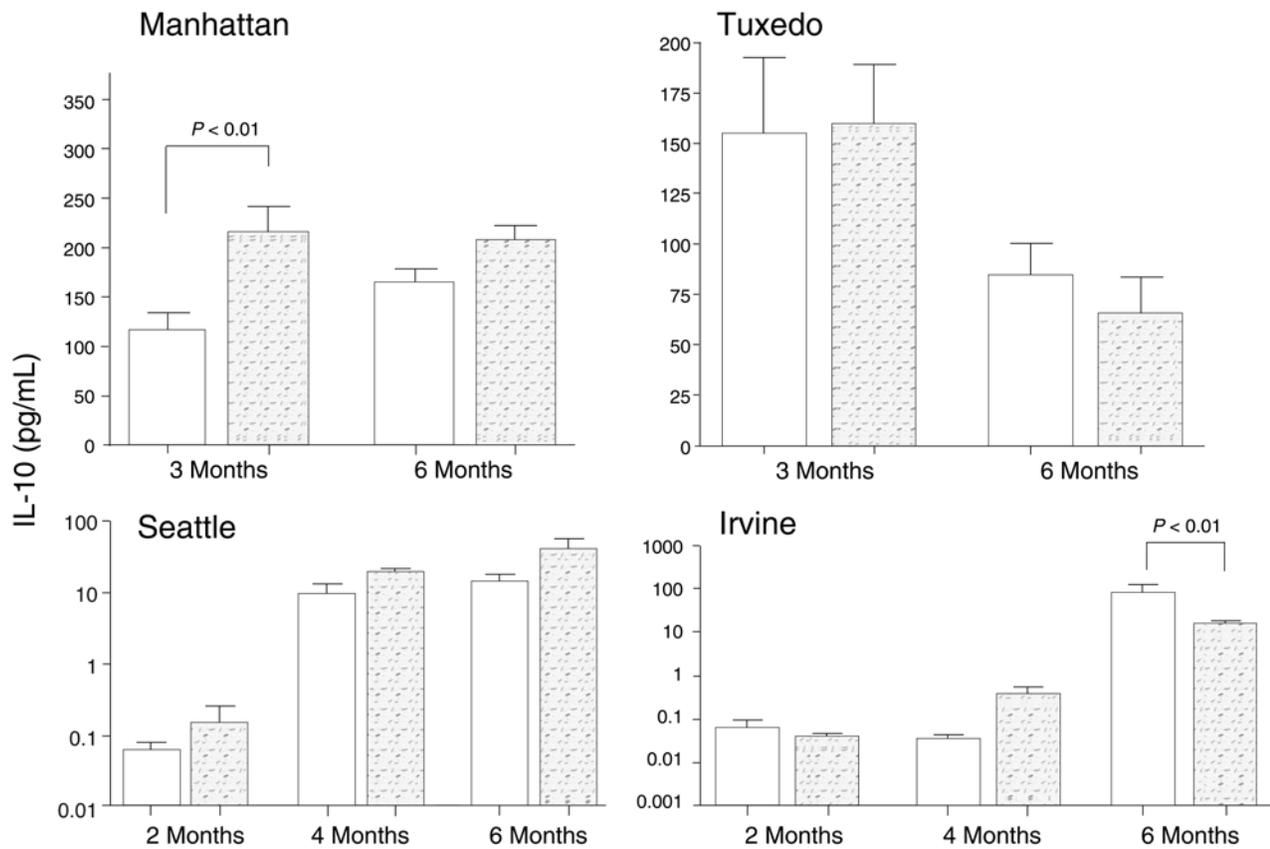


Figure 13. Effects of CAPs (patterned bars) and filtered air (empty bars) on serum IL-10 concentrations at Manhattan and Tuxedo (as measured by ELISA) and at Seattle and Irvine (as measured by Meso Scale Discovery) after 2, 3, 4, and 6 months of exposures. Note that the y-axis scales vary and are in some cases logarithmic.

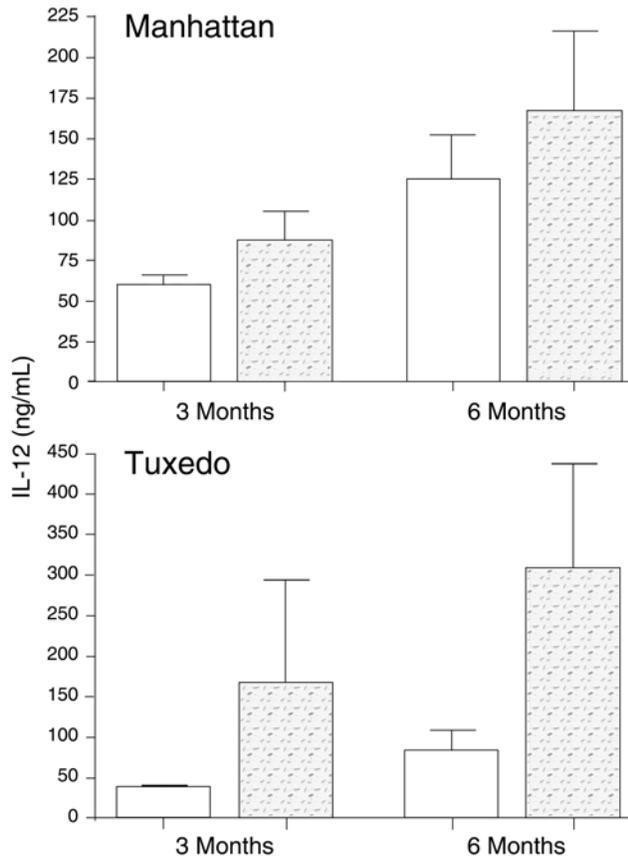


Figure 14. Effects of CAPs (patterned bars) and filtered air (empty bars) on serum IL-12 concentrations at Manhattan and Tuxedo (as measured by ELISA) after 3 and 6 months of exposures. Note that the y-axis scales vary.

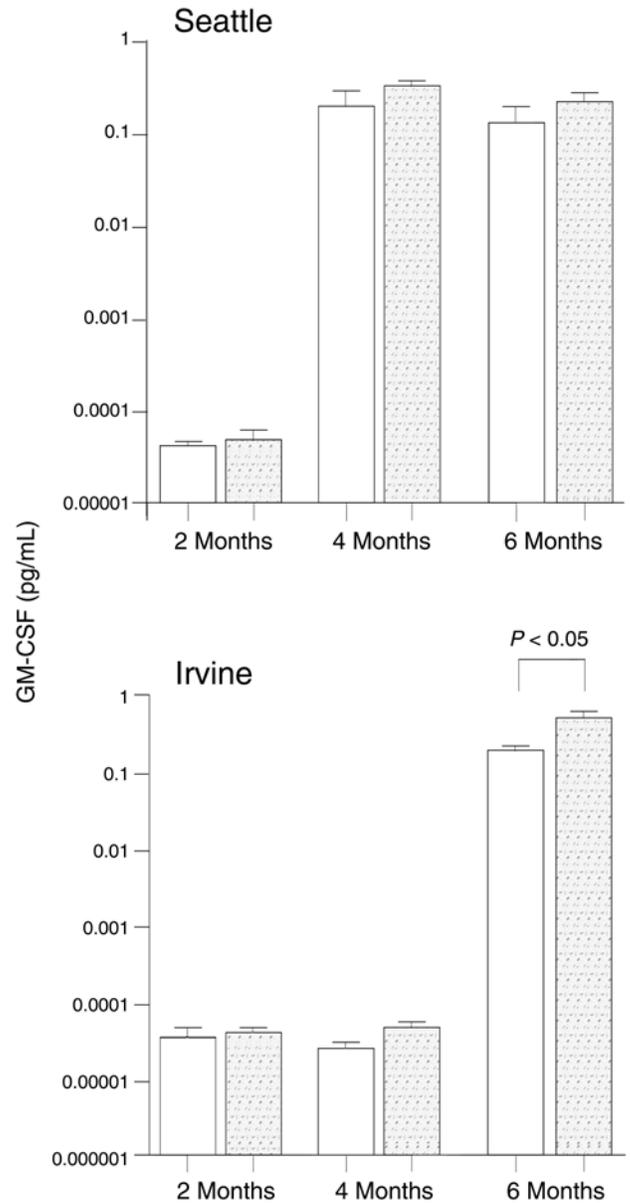


Figure 15. Effects of CAPs (patterned bars) and filtered air (empty bars) on serum GM-CSF concentrations at Seattle and Irvine (as measured by Meso Scale Discovery) after 2, 4, and 6 months of exposures. Note that the y-axis scales vary and are logarithmic.

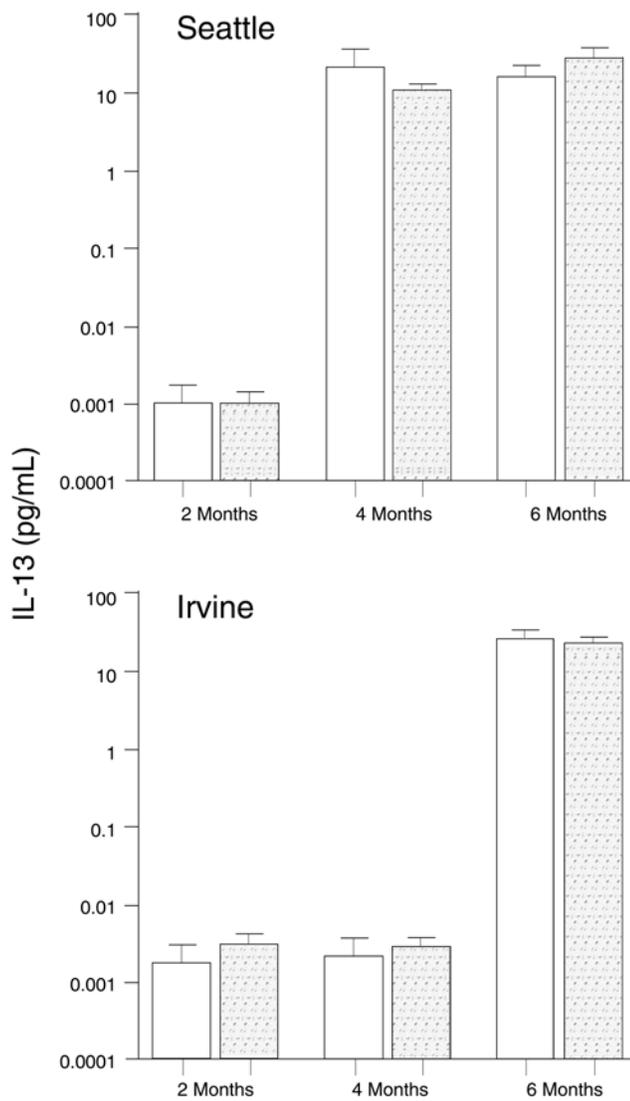


Figure 16. Effects of CAPs (patterned bars) and filtered air (empty bars) on serum IL-13 concentrations at Seattle and Irvine (as measured by Meso Scale Discovery) after 2, 4, and 6 months of exposures. Note that the y-axis scales are logarithmic.

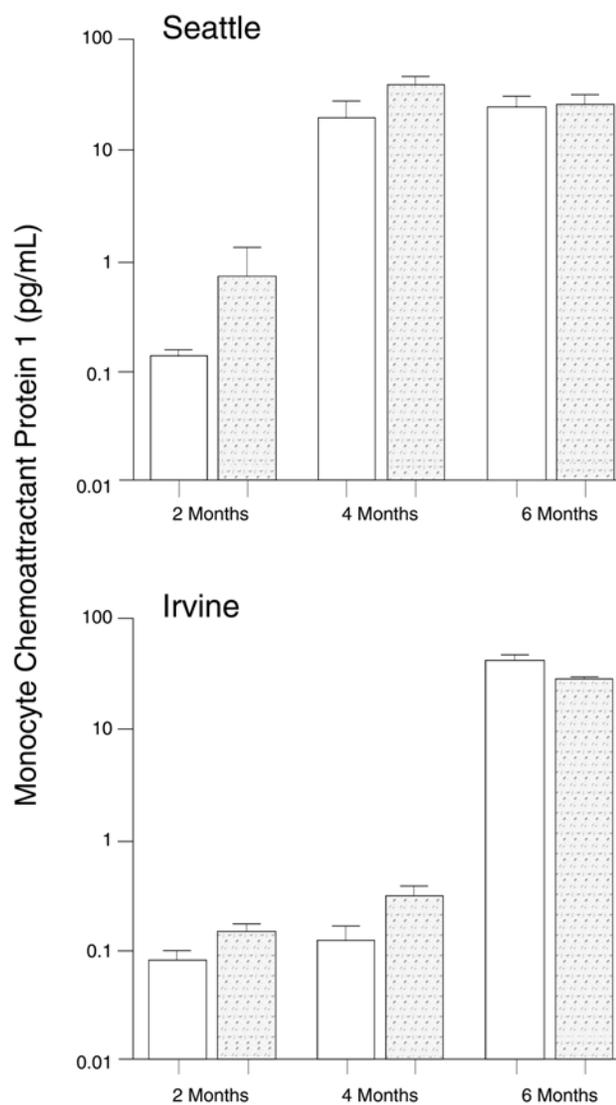


Figure 17. Effects of CAPs (patterned bars) and filtered air (empty bars) on monocyte chemoattractant protein 1 concentrations at Seattle and Irvine (as measured by Meso Scale Discovery) after 2, 4, and 6 months of exposures. Note that the y-axis scales are logarithmic.

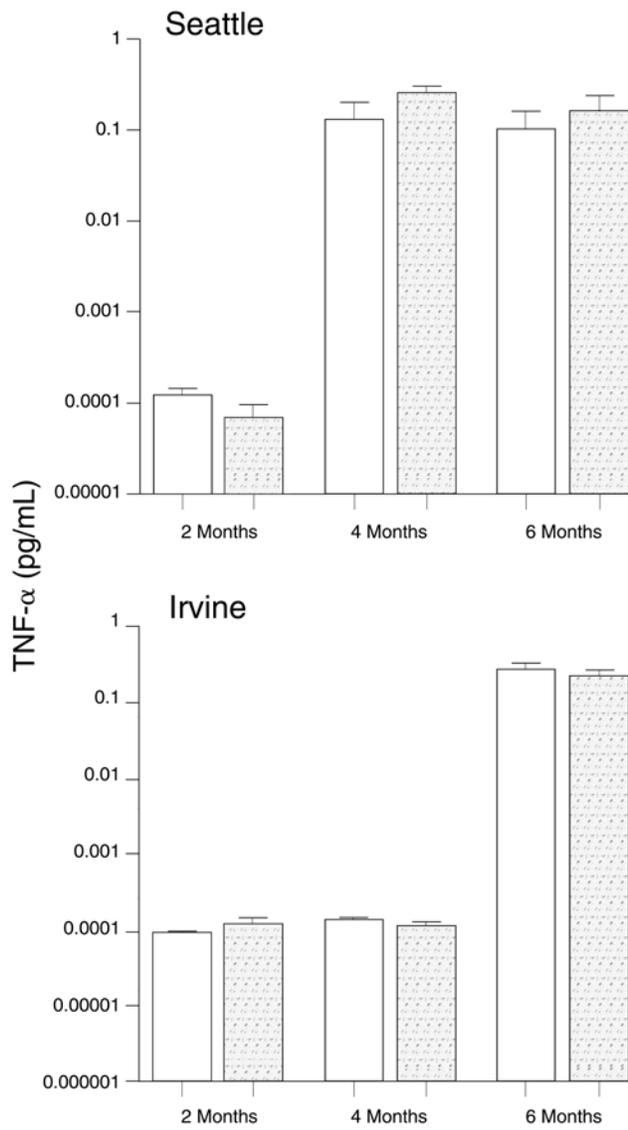


Figure 18. Effects of CAPs (patterned bars) and filtered air (empty bars) on serum TNF- α concentrations at Seattle and Irvine (as measured by Meso Scale Discovery) after 2, 4, and 6 months of exposures. Note that the y-axis scales vary and are logarithmic.

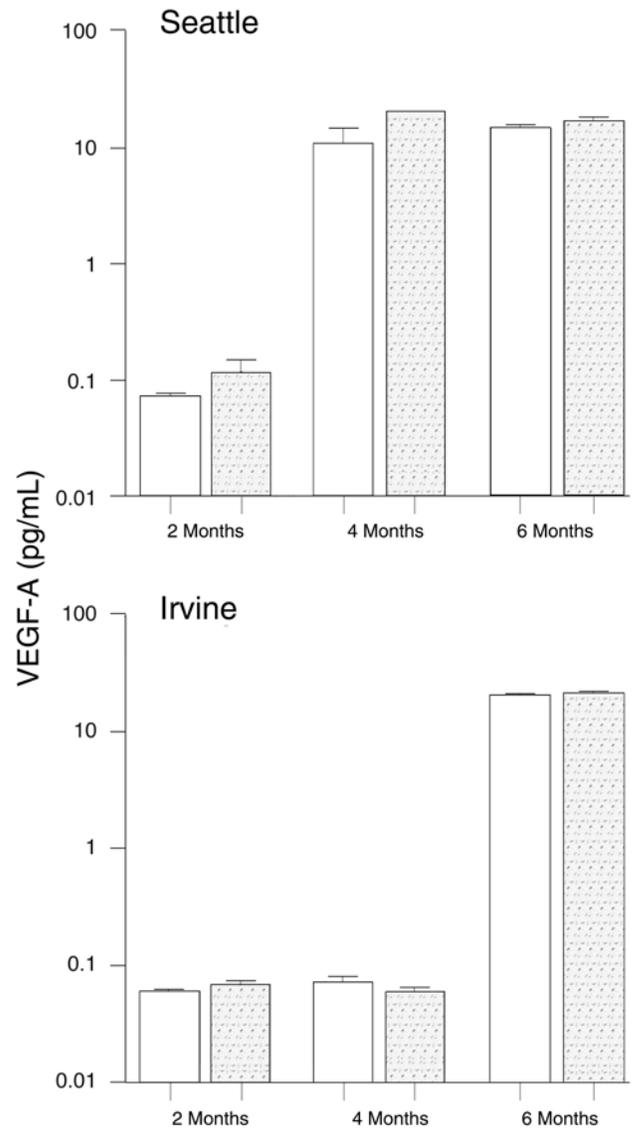


Figure 19. Effects of CAPs (patterned bars) and filtered air (empty bars) on VEGF-A concentrations at Seattle and Irvine (as measured by Meso Scale Discovery) after 2, 4, and 6 months of exposures. Note that the y-axis scales are logarithmic.

ATHEROSCLEROTIC PLAQUE PROGRESSION

Table 12 shows the results of our in vitro measurements of atherosclerotic plaque progression in the BA and LCCA of the mice at all five sites. Plaque in the BA after 6 months of exposure varied by site, with more progression for CAPs-exposed than for control mice at Manhattan (40% occlusion versus 32%) and Tuxedo (34% occlusion versus 23%), where sulfur concentrations were much higher than at the other three sites, and at East Lansing (28% occlusion versus 23%). There was no significant plaque progression at Seattle (46% occlusion versus 47%), where the concentrations of sulfur and CAPs were lowest, or at Irvine (29% occlusion for both groups), where sulfur concentrations were the second lowest but CAPs concentrations were second highest. The CAPs concentration at East Lansing was only about half that at Irvine, and the contribution of the Traffic source category to local PM_{2.5} was much smaller at East Lansing than at Irvine, but East Lansing ranked high together with Manhattan and Tuxedo in the magnitude of the effect on plaque progression. It therefore appears that the PM_{2.5} attributable to the Coal Combustion category (which is what East Lansing, Manhattan, and Tuxedo have in common in terms of source categories associated with this outcome) was more

influential on plaque progression in the mice than that from any other source category, including the Traffic and Residual Oil Combustion categories.

The spontaneous development of plaque (i.e., the plaque found in the controls [a common finding in ApoE^{-/-} mice]) was greater in the LCCA compared with that in the BA, and the increase in plaque progression caused by CAPs exposure in the LCCA was therefore not as apparent; significant increases in the LCCA were seen only at Tuxedo at 6 months (52% occlusion versus 43%) and at Irvine at 2 months (34% occlusion versus 32%).

Because the start of the experiments was delayed for various reasons by up to 4 months at Seattle and up to 2 months at Irvine, the mice at these sites were slightly older at the start of exposures than the mice at the other sites. It is therefore possible that the smaller increases in plaque progression in the CAPs-exposed animals compared with those in the controls after 6 months of exposure at Seattle and Irvine could have been caused by the presence of higher baselines at the beginning of the CAPs exposures. However, as shown in Figure 20, plaque progression did occur over time in both arteries for all control groups at all sites, with the exception of the LCCA at Irvine. In these

Table 12. Plaque Size Progression During Subchronic CAPs Inhalation Exposures by Site, as Measured by Ultrasound Biomicroscopy^a

Site (Months)	Average Plaque Area (% of Total Cross-Section Area)									
	BA					LCCA				
	Filtered-Air Control	<i>n</i>	CAPs	<i>n</i>	<i>P</i>	Filtered-Air Control	<i>n</i>	CAPs	<i>n</i>	<i>P</i>
Manhattan (3)	23.7 ± 4.8	20	22.1 ± 4.0	21	0.26	33.7 ± 5.3	20	34.9 ± 6.2	21	0.52
Manhattan (6)	32.2 ± 5.9	10	40.2 ± 9.0	10	0.03	54.3 ± 2.6	10	53.6 ± 5.4	10	0.73
Tuxedo (3)	20.7 ± 4.5	15	25.2 ± 6.9	15	0.04	36.1 ± 5.0	15	33.7 ± 6.9	15	0.28
Tuxedo (6)	23.3 ± 8.8	11	34.2 ± 8.8	13	0.02	42.5 ± 6.6	11	52.2 ± 10.7	13	0.02
East Lansing (2)	19.8 ± 3.1	12	21.5 ± 3.0	12	0.17	24.9 ± 2.5	12	26.9 ± 4.0	12	0.15
East Lansing (4)	22.0 ± 2.4	11	23.5 ± 2.8	12	0.18	28.8 ± 3.4	11	29.6 ± 3.6	12	0.61
East Lansing (6)	23.4 ± 4.5	11	27.8 ± 4.5	12	0.03	29.8 ± 4.3	11	32.9 ± 4.1	12	0.09
Seattle (2)	40.2 ± 4.7	11	35.8 ± 7.3	13	0.10	21.9 ± 9.6	11	29.3 ± 11.0	13	0.10
Seattle (4)	44.0 ± 5.0	6	38.5 ± 7.2	6	0.19	50.8 ± 7.2	6	57.7 ± 6.2	6	0.11
Seattle (6)	47.0 ± 6.6	11	46.4 ± 4.7	11	0.80	54.1 ± 4.4	11	57.3 ± 5.7	11	0.16
Irvine (2)	26.7 ± 2.5	12	27.2 ± 3.2	12	0.67	31.9 ± 2.5	12	34.4 ± 2.7	12	0.03
Irvine (4)	27.4 ± 4.3	12	30.2 ± 4.5	12	0.13	30.7 ± 4.5	12	32.5 ± 4.1	12	0.33
Irvine (6)	29.0 ± 3.1	12	29.4 ± 4.4	12	0.77	31.5 ± 3.7	12	30.5 ± 3.0	12	0.47

^a *P* values in **boldface** are statistically significant. *n* = animals.

control animals, plaque progression over time in the BA appeared to be similar, albeit starting from different baselines (at 2 or 4 months, when the first scans took place, no plaque was detectable when the animals arrived at the study sites). In the LCCA, the Manhattan and Seattle control mice appeared to have greater plaque progression over time than the control mice at Tuxedo and East Lansing; no plaque progression was seen in control mice at Irvine. Because the progression in the BA in the control mice at all five sites appeared to be similar, we do not believe that the differing baselines affected the progression in the CAPs-exposed mice. The lack of progression in the CAPs-exposed mice — compared with the control mice — at Seattle and Irvine, we believe, was caused by differences in the composition of the

CAPs at the two sites (not by the possibility, for example, that the plaques could no longer grow larger).

To support our findings using ultrasound biomicroscopy, we performed additional quantitative evaluation of plaque progression imaging longitudinal sections of aortas in mice exposed to filtered air or CAPs for the Seattle and Irvine sites.

Figure 21 shows representative images of atherosclerotic plaques of ApoE^{-/-} mice exposed to filtered air or CAPs for 6 months. (One of the CAPs-exposed mice at Seattle and three of the control mice at Irvine were not included in the analysis, because the aortas were broken during tissue harvesting.) Because the mice in Seattle were older by 4 months when the exposures started, there were

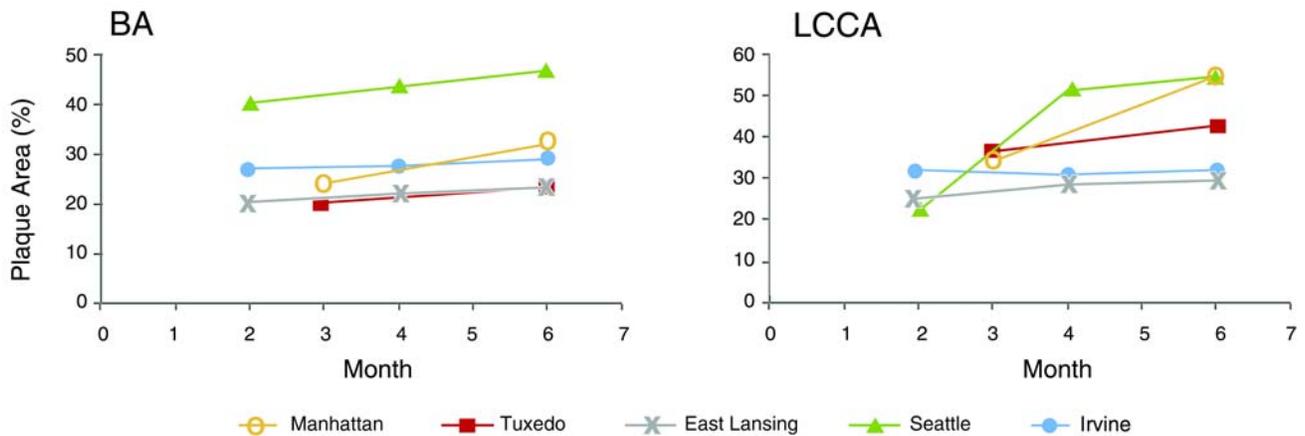


Figure 20. Plaque progression by site in the BA and LCCA of control mice exposed to filtered air.

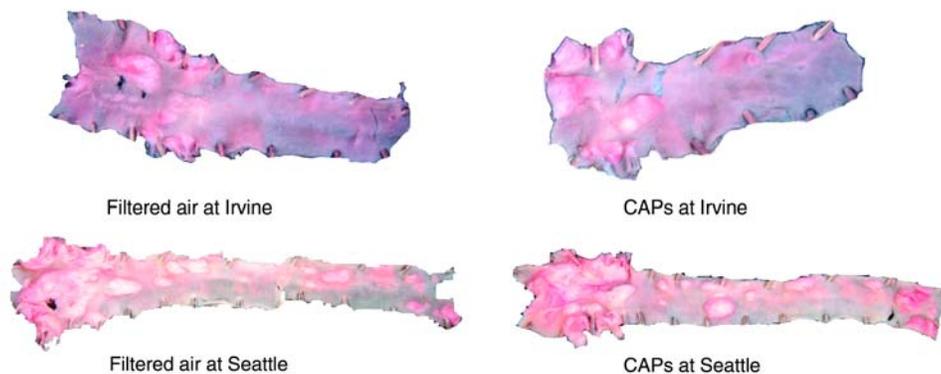


Figure 21. Representative images of atherosclerotic lesions on the aortas of ApoE^{-/-} mice exposed to filtered air or CAPs for 6 months at Irvine and Seattle. The percent plaque areas for these animals were similar to their corresponding group means.

greater lesion areas in both the control and CAPs-exposed mice at Seattle compared with those exposed at Irvine. Because only thoracic aortas were available for the mice at Irvine, we also compared the plaque areas of thoracic aorta for the Seattle mice.

As shown in Figure 22, we found no significant differences between results for the control and CAPs-exposed mice at Seattle or Irvine (P values shown are for the t test), although there was a nonsignificant increase in plaque area for CAPs exposures at Irvine, where the mass concentration was twice as high as that at Seattle. Plaque areas of the thoracic aorta in Seattle mice were larger than those of the entire aorta in both the control and CAPs-exposed mice. This observation is consistent with the literature reporting that more plaque lesions were found in the thoracic aorta, especially in the aorta arch, than in the abdominal aorta of ApoE^{-/-} mice. CAPs exposure did not affect the lesions in this aorta segment. Together with our visual maps of the extent of occlusion in the East Lansing mice at 6 months, these supplemental analyses (1) confirmed the validity of our previously tabulated results, (2) found evidence of plaque progression at Irvine mice exposed to filtered air and of greater progression in those exposed to CAPs; and (3) confirmed that plaque formation at East Lansing was somewhat greater than at Irvine, given that plaques were observed in both the BA and the LCCA.

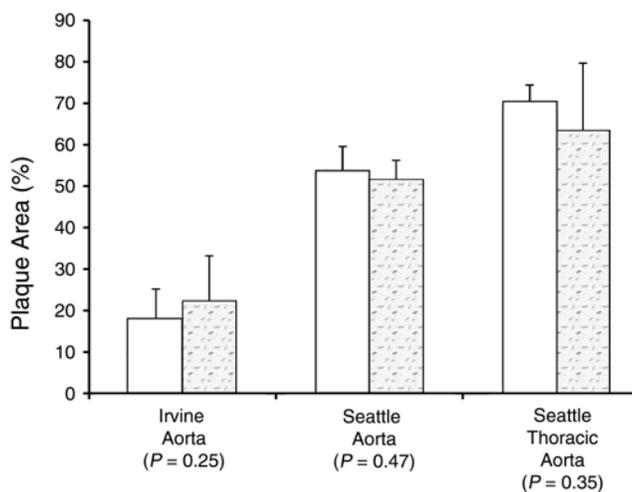


Figure 22. Effects of CAPs (patterned bars) and filtered air (empty bars) on plaque areas of the aortas of mice exposed at Irvine and of the aortas and thoracic aortas of mice exposed at Seattle. Animals were evaluated after 6 months of exposure. Data are based on results from 17 and 12 mice exposed to filtered air and CAPs at Irvine, respectively, and 7 each for filtered air and CAPs at Seattle.

SUMMARY OF KEY FINDINGS

The exposure-chamber concentrations of CAPs and most CAPs components — especially S — were much lower at Seattle and East Lansing than at Manhattan, Tuxedo, and Irvine. In Seattle, S is mostly of local origin (i.e., from traffic and marine transport), in contrast with the other four sites, where most of the S is from Coal Combustion or Residual Oil Combustion in the region and more distant areas.

The average CAPs concentration in the exposure chamber for 30 hr/week at Seattle was 61.0 $\mu\text{g}/\text{m}^3$, which, when averaged over the 168 hours of a week, was 10.9 $\mu\text{g}/\text{m}^3$ per hour. This was not only lower than the previous annual average PM_{2.5} NAAQS of 15 $\mu\text{g}/\text{m}^3$, but also lower than the new annual average PM_{2.5} NAAQS of 12 $\mu\text{g}/\text{m}^3$. It is thus notable that, despite the low concentrations of the CAPs exposures used in our study (as averaged over the entire study duration, including nonexposure periods), statistically significant exposure-related ECG changes were found in the mice for some of the source-related CAPs components.

The source categories most significantly associated with changes in HR and HRV at the five sites were the sulfur-related categories (96), Soil (81), Residual Oil Combustion (78), and Salt (75) categories. HR and HRV changes were not consistently associated with total CAPs, BC, or the Traffic (and Road Dust) source categories. The strongest associations of all were for the Soil category with 2-day lags (39), followed by the sulfur-related categories with 0-day lags (35).

At Seattle the correlation between the Residual Oil Combustion and Sulfates categories was high ($r = 0.57$), as would be expected if the secondary sulfates in the CAPs were attributable, to a substantial degree, to the combustion of residual oil (which generally has a high S content).

The correlation between the source categories for Residual Oil Combustion and Salt at Seattle was 0.84, suggesting that the Salt source at the Seattle site (i.e., at the University of Washington School of Public Health laboratory) is located on or near Puget Sound to the west, which is where long-range transport ships are docked. This is consistent with the negative correlation (-0.74) between the source categories for Residual Oil Combustion and Biomass Combustion, the smoke from which is emitted mostly from residential areas to the northeast and south of the city; it is also consistent with a negative correlation (-0.59) between the Biomass Combustion and Sulfates categories.

At Irvine, as noted earlier, the changes in HR and HRV for the Residual Oil Combustion and Salt categories were mirror images of each other. When we split the Residual

Oil Combustion category into high and low Salt days, the results (Table 9) indicated that Traffic became the dominant source category of significant associations on low Salt days (when the prevailing winds originated inland, bringing traffic pollution to Irvine). By contrast, on high Salt days (when prevailing winds originated offshore), Residual Oil Combustion became a major source of significant associations.

SHORT-TERM ASSOCIATIONS BETWEEN SOURCE CATEGORIES AND ECG CHANGES

As expected, the concentrations of CAPs and all of its source-related components other than those elements associated with the Biomass Combustion and Salt source categories were much lower at Seattle than at the other four sites.

Despite the much lower levels of exposure to Residual Oil Combustion emissions (Ni and V) at Seattle than at Manhattan, the Residual Oil Combustion source category still had significant, albeit fewer, associations with ECG changes at Seattle than at Manhattan, and the number of associations with these transition metals at Seattle was considerably larger than that for any source category at Seattle other than the Soil category, indicating an apparent lack of a response threshold.

It was not surprising that the sulfur-related categories, which showed significant associations with ECG changes at both Manhattan and Tuxedo, had few such associations at Seattle, where exposures to Sulfates were much smaller. Nor was it surprising that the Traffic (and Road Dust) source category, which had the third strongest influence in traffic-choked Manhattan, had few, if any, associations — or was not identified as a source category — at sites that were located away from traffic: at Tuxedo, where our research facility was located in a large state park in rural-suburban New York State; at Seattle, where our research facility was located on a large college campus in a suburban area on Lake Washington, near Puget Sound in Washington State; or at East Lansing, where our research facility was located on a large college campus in south-central Michigan.

LONG-TERM ASSOCIATIONS BETWEEN SOURCE CATEGORIES AND PLAQUE PROGRESSION

Because very little temporal resolution was possible in terms of plaque progression over the 6-month exposure periods, our ability to determine which elements, other components, or source category-related mixtures in the PM_{2.5} were most influential on this important measure was severely limited. As noted earlier, changes in plaque area in the BA after 6 months of exposure varied by site. Significantly larger increases in plaque area were found in the BA of the CAPs-exposed mice compared with the

controls at Manhattan (40% occlusion versus 32%) and at Tuxedo (34% occlusion versus 23%), where mean S and Ni concentrations were much higher than at the other three sites, and at East Lansing (28% occlusion versus 23%), where mean Ni concentrations were the lowest of the five sites. No significant differences in plaque progression were found between the exposure and control groups at Seattle (where mean S and CAPs concentrations were the lowest) or at Irvine (where mean S concentrations were third lowest and CAPs concentrations were highest). Because the CAPs concentrations at East Lansing were only about half those at Irvine and the Traffic source category was not identified at East Lansing, it appears that the PM_{2.5} attributable to the Coal Combustion category (which is what East Lansing, Manhattan, and Tuxedo have in common in terms of source categories associated with this outcome) was more influential on plaque volume progression in the ApoE^{-/-} mice than the contributions from all other PM_{2.5} sources, including the source categories for Traffic and Residual Oil Combustion.

We measured plaque lesion areas on the aortas of CAPs-exposed mice and of controls at Irvine and at East Lansing after 6 months of exposure. These results confirmed the lack of significant progression of the plaque lesion area related to CAPs exposure at Seattle and Irvine previously demonstrated using ultrasound biomicroscopy.

DISCUSSION

Our first hypothesis, in terms of in vivo responses in an animal model, was that PM_{2.5} is capable of producing acute health effects of public concern but that the effects might differ according to the chemical composition of the PM_{2.5}. The results of our time-series analyses of HR and HRV in ApoE^{-/-} mice over 6 months of daily CAPs exposures at five sites whose airsheds varied substantially in terms of the composition of their PM_{2.5} were consistent with this hypothesis. These results clearly showed that transition metals were generally more influential in producing HR and HRV changes than were soil-derived elements or OC and that a Residual Oil Combustion source category was generally more influential than any of the other source categories. There were often significant associations between the sulfur-related categories and EC and ECG changes, but these associations might have been caused by the pollutants' origins in the same combustion sources as the transition metals.

Our second hypothesis was that long-term PM_{2.5} exposures are closely associated with chronic health effects. The changes that we found in baseline HR and HRV in the

ApoE^{-/-} mice over 6 months of daily CAPs exposures at the five sites (whose airsheds, again, varied substantially in PM_{2.5} composition) were consistent with this hypothesis, as was the significant progression of aortic plaque at the three sites with long-term exposures to Coal Combustion emissions.

Our third hypothesis was that the source-apportionment techniques that have been developed and refined in recent years would provide a useful basis for identifying the principal PM_{2.5} air pollution source categories and specific chemical components of PM_{2.5} that have the greatest impacts on a variety of acute and chronic health issues. As noted above, our findings clearly supported this hypothesis.

Our fourth hypothesis was that the health effects caused by ambient PM_{2.5} exposures are more likely to be observed in animal models that represent sensitive subgroups within overall human populations. The effects of short-term CAPs exposures on HR and HRV were clearly evident at our research sites at Manhattan and Tuxedo, and to a lesser extent at Seattle and Irvine, demonstrating that the assay technology employed could detect such effects in a dose-dependent manner in this sensitive animal model.

SHORT-TERM MORTALITY AND MORBIDITY RESPONSES TO EXPOSURE TO TRANSITION METALS

The results reported here indicated statistically significant associations between ECG changes and Ni (one of the transition metals found in Residual Oil Combustion emissions and other sources) over a wide range of concentrations — with averages of ~70 ng/m³ found at Manhattan, 16 ng/m³ at Tuxedo, and < 7 ng/m³ at East Lansing, with no evidence of a threshold. These results add to the growing body of evidence that short-term exposure to transition metals might pose public health risks. In a recent critical review (Lippmann and Chen 2009), we summarized evidence from both epidemiologic and toxicologic studies that utilized chemical-speciation data to identify PM_{2.5} components that had significant associations with adverse health effects, including excess daily mortality in humans, hospital admissions for cardiac causes in humans, and ECG changes in mice.

OTHER SHORT-TERM CARDIOVASCULAR RESPONSES TO EXPOSURE TO TRANSITION METALS

The primary rationale for this study was to determine the associations between PM_{2.5} and its components and various measures of cardiovascular outcomes over the course of 6 months of daily (weekday) 6-hr inhalation

exposures to CAPs. Other cardiovascular outcomes that might have been of equal or greater interest, such as blood pressure, myocardial tissue oxygenation, and contractility, could not routinely be made and warrant further study. In collaborative studies, we have in fact already examined the effect of CAPs inhalation on blood pressure (Sun et al. 2008), insulin resistance (Sun et al. 2009; Xu X et al. 2011), cardiac remodeling (Ying et al. 2009), and oxidative stress in adipose tissues (Xu Z et al. 2011). Future studies are needed, if and when technical means are developed, on the effects of CAPs on myocardial tissue oxygenation and on contractility.

We cannot conclude that the cardiovascular changes measured in this study led to pathogenesis of the heart or were just adaptive responses. However, we do know, based on our prior and parallel studies of the progression of atherosclerotic plaque in ApoE^{-/-} mice chronically exposed to CAPs, that such exposures are significantly associated with pathogenesis of the cardiovascular system. In the current study, we could not identify specific PM_{2.5} components that were significantly associated with plaque progression but were able to show that significant plaque progression occurred in the arteries of CAPs-exposed ApoE^{-/-} mice at Manhattan, Tuxedo, and East Lansing and not in those exposed at Seattle or Irvine. The Coal Combustion source category accounted for a substantial fraction of the PM_{2.5} mass at Manhattan, Tuxedo, and East Lansing but not at Seattle or Irvine, suggesting, but not proving, that it has a causal role in plaque progression.

CONCLUSIONS

The variety of particle-size distributions, PM_{2.5} compositions, and component concentrations of the PM_{2.5} at Manhattan, Tuxedo, East Lansing, Seattle, and Irvine were all likely contributors to the substantial differences found in the acute and longer-term cardiovascular responses to sub-chronic CAPs inhalation exposures at the five sites. At this point it is not possible to specify the roles of each of these variables adequately. Our findings suggest that EC and the sulfur-related source categories could not account for the acute HR and HRV effects associated with PM_{2.5}. They also point to a Residual Oil Combustion source category as having the largest acute effects at Manhattan and lesser effects at Seattle, Irvine, and East Lansing. A strong influence of Ni on HR and HRV was seen in a previous sub-chronic CAPs inhalation study in ApoE^{-/-} mice at Tuxedo, when there were 14 specific days with high Ni concentrations but extremely low V concentrations (Lippmann et al. 2006). Together, these findings suggest that responses to Ni

exposure do not have a threshold for measurable functional effects. The results of the exposures at East Lansing, Seattle, and Irvine, where the composition of the PM_{2.5} was substantially different from that at Manhattan and Tuxedo, enabled us to do a more thorough analysis of the roles of PM_{2.5} components in eliciting ECG changes. When taking the concentrations of the various elements into consideration at all five sites, Ni had the highest concentration–response relationship ($r^2 = 0.96$), followed in decreasing order of r^2 by Al ($r^2 = 0.81$), EC ($r^2 = 0.79$), P ($r^2 = 0.77$), S ($r^2 = 0.65$), and V ($r^2 = 0.35$). The Residual Oil Combustion source category is characterized by high levels of Ni, V, S, and EC, but on a regional scale much more S is contributed by the Coal Combustion source category.

In terms of the associations of source categories with HR and HRV changes, we found the following:

- Ni sources (i.e., the Residual Oil Combustion or Ni Refinery categories) were present at all sites.
- The sulfur-related categories had large numbers of associations with a 0-day lag at Manhattan and Tuxedo, but not at the other three sites, where concentrations were much lower.
- The Soil category had its largest numbers of association with 1-day lags at Manhattan and Tuxedo and with 2-day lags at Seattle and Irvine. This could have been caused by differences in soil composition.
- The Biomass Combustion category, with notable contributions to PM_{2.5} concentrations only at Seattle and Irvine, had its only associations with a 0-day lag at Seattle and a 2-day lag at Irvine.
- The Salt category, with notable contributions to PM_{2.5} concentrations at all sites except East Lansing, had its most prominent associations with a 1-day lag at Manhattan, Tuxedo, and Irvine and a 2-day lag at Seattle.
- The Traffic (and Road Dust) categories had the third highest number of associations at Manhattan, and fewer associations at Seattle and Irvine. The Traffic source category was not identified at Tuxedo or East Lansing.

In terms of atherosclerotic plaque progression, we made *in vivo* measurements of plaque area in the BA and LCCA of the mice undergoing subchronic CAPs exposure at all five sites. Plaque progression in the LCCA varied by site, from substantial at Manhattan (25%) and Tuxedo (47%),

where sulfates and Ni concentrations were much higher than at the other three sites, to East Lansing (19%), where Ni concentrations were the lowest of the five sites. There was no plaque area progression at all at Seattle, where sulfates and CAPs concentrations were lowest, or at Irvine, where the sulfates concentration was the third lowest but the CAPs concentration was highest. Because the CAPs concentrations at East Lansing were only about half those at Irvine and a Traffic source category was not identified at East Lansing, it appears that the PM_{2.5} attributable to the Coal Combustion category (which is what East Lansing, Manhattan, and Tuxedo have in common in terms of source categories associated with this outcome) was more influential on plaque progression in the mice than that attributable to any of the other source categories, including Traffic and Residual Oil Combustion.

We thus conclude that the changes we observed in short-term ECG measures were most closely associated with sources of Ni (the Residual Oil Combustion source category at four of the sites and a distant upwind Ni refinery at Tuxedo) and that the aortic plaque progression we observed over 6 months, a long-term effect, was most closely associated with the Coal Combustion source category. The association of various components of ambient air pollution with various short- and long-term health effects has been previously established in connection with pulmonary function in children; for example, peak ozone has frequently been associated with short-term functional deficits; and Gauderman and colleagues (2004) have demonstrated significant associations between lung function growth in children and long-term average concentrations of PM_{2.5}, NO₂, and acid vapors (but not ozone). This is analogous to our demonstration, in this NPACT project, that the Coal Combustion source category for PM_{2.5} was associated, in both mice and humans, with chronic cardiovascular effects but not with acute effects such as transient cardiac function in mice or with cardiovascular hospital admissions in humans. By contrast, we have also shown that transition metals in ambient PM_{2.5} were associated with transient cardiac function in mice and with cardiovascular hospital admissions in humans, while the Coal Combustion source category was not associated with these short-term responses.

NPACT Study 2. In Vitro and in Vivo Toxicity of Exposure to Coarse, Fine, and Ultrafine PM from Five Airsheds

Terry Gordon, Morton Lippmann, Arthur Nádas, and Christina Hickey

ABSTRACT

BACKGROUND

A number of recent multi-city studies have reported associations between exposure to various ambient PM mass concentrations and human health effects. Because the toxicity of PM* varies with particle size, season, and location, PM mass concentration might not be the best indicator of PM-induced health effects. The overall aim of the current study was to investigate the role of PM composition on in vitro and in vivo acute toxicity. In coordination with Chen Study 1, we selected five U.S. airsheds — Manhattan, in New York City, New York; Sterling Forest State Park in Tuxedo, New York; East Lansing (for Study 1) and Ann Arbor (for Study 2) in Michigan; Seattle in Washington; and Anaheim (for Study 2) and the University of California–Irvine (for Study 1 and the 100-day study in Study 2) in the Los Angeles (LA) area of California — on the basis of expected differences in the source categories and chemical composition of the PM in these airsheds (as well as the presence of local collaborators with appropriate toxicologic expertise).

This Investigators' Report is one part of Health Effects Institute Research Report 177, which includes Investigators' Reports of three other studies, a Commentary by the NPACT Review Panel, an HEI Statement about the research project, and a Synthesis of the NPACT Initiative relating this report to Research Report 178. Correspondence concerning this Investigators' Report may be addressed to Dr. Terry Gordon, New York University Medical Center, 57 Old Forge Road, Tuxedo, NY 10987. Terry.Gordon@nyumc.org.

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* A list of abbreviations and other terms appears at the end of the Investigators' Report.

METHODS

To examine the comparative toxicity of PM source categories and components, in vitro and in vivo studies were conducted with PM from the five airsheds. PM was collected at each site and evaluated in vitro in primary cells or cell lines derived from mouse airway epithelium, vascular endothelium, and cardiomyocytes. The epithelial and endothelial cells were used to study oxidative stress, and the cardiomyocytes were used to study the cellular mechanisms that underlie observed PM-induced changes in cardiac beat frequency. The in vitro experiments were conducted with PM extracts from 12-day sampling periods at the five sites over two seasons and in three particle-size ranges (target $n = 360$ samples). The in vivo experiments were conducted with PM from the 12-day sampling periods and from extended 100-day sampling periods at two sites — Manhattan and the LA area — in three particle-size ranges; target $n = 600$. To assess the role of source categories and specific trace elements in ambient PM, all PM samples were analyzed for composition by inductively coupled plasma mass spectroscopy (ICP–MS). For the in vivo studies, we treated FVB/N mice by aspiration with 50 μg coarse PM ($\text{PM}_{10-2.5}$), fine PM ($\text{PM}_{2.5-0.2}$), and ultrafine PM ($\text{PM}_{0.2}$) collected in both summer and winter at all five sites for the 12-day study and at the two sites for the 100-day study.

RESULTS

Reactive oxygen species (ROS) formation differed by site, season, and particle size. After treating the mice for 6 and 24 hours with PM collected in the 12-day study, messenger ribonucleic acid (mRNA) expression levels showed differences that also depended on site, season, and particle size, with the greatest changes observed for heme oxygenase-1 (HO-1) and interleukin 8 (IL-8) mRNA. In terms of in vivo comparative toxicity of the size-fractionated PM, polymorphonuclear leukocyte (PMN) levels measured in lavage fluid were significantly higher in mice treated with the coarse and fine PM than with the ultrafine PM. In vivo data

did not correlate with the data for *in vitro* ROS production, suggesting that *in vitro* ROS measures might not be a good indicator of *in vivo* PM toxicity. Furthermore, analysis of PM trace elements supported the hypothesis that certain elemental components of PM drive PM-induced health effects. The most important PM contributions were attributed to the Traffic/Brake Wear and Residual Oil Combustion source categories. In the 100-day *in vivo* study, pulmonary inflammation was greatest in animals treated with the coarse PM; fine and ultrafine PM produced smaller yet still significant changes.

CONCLUSIONS

The design of this study allowed a toxicologic comparison of coarse, fine, and ultrafine fractions of ambient PM. Although similar experiments in cells *in vitro* and in rodents *in vivo* have been reported with PM collected over longer time periods (Hetland et al. 2001; Gilmour et al. 2007), the current study was unique in its collection of size-fractionated ambient PM on a daily basis in two seasons in the 12-day study and at two sites in the 100-day study. As expected, the results suggested that, in addition to PM mass, PM source categories and components drive the adverse health effects of inhaled ambient PM. In some of the *in vitro* experiments, a daily sampling effect appeared to drive the variability in response at some sites but not others. Overall, however, the study's data demonstrate that the toxicity of PM is driven by a complex interaction of particle sampling site, season of the year, and particle size. These findings suggest that, as hypothesized in the overall NPACT study, PM components — as dictated by PM source categories — are responsible for the adverse health effects of ambient PM. Importantly, as demonstrated here and in the overall NPACT study, the responsible source categories and components can vary in turn with the cardiovascular or pulmonary endpoint being assessed.

INTRODUCTION

Although there are associations between PM exposure and adverse health effects, the magnitude of the associations differs from place to place, and there are inconsistencies among studies as to the variables (e.g., the specific size fraction) responsible for the observed effects (Laden et al. 2000; Pope et al. 2002; Delfino et al. 2004). Hetland and associates (2001) have suggested that, although coarse PM ($> 2.5 \mu\text{m}$) typically makes up a large portion of total PM mass, fine PM ($\leq 2.5 \mu\text{m}$) and ultrafine PM ($\leq 0.10 \mu\text{m}$) might be more toxic because of higher toxic-metal content or greater surface-to-mass ratio, respectively. Studies have

shown that, on an equal-mass basis, smaller particles can induce a larger biologic response. For particles of varying compositions, however, this does not always hold true (Ovrevik et al. 2005; Warheit et al. 2007), suggesting that for such particles the effects of surface area and size can in some cases be less important than their composition.

The general population is exposed to a myriad of local and regional pollution sources that can each produce different mixtures of PM components. It has become clear that PM mass concentration alone, the current regulatory metric, might not be the best indicator of PM-induced health effects. The overall aim of the current study was to investigate the role of PM composition on *in vitro* and *in vivo* toxicity.

In parallel with Chen Study 1, we selected five U.S. sites on the basis of expected differences in the chemical composition of their airsheds (Manhattan, in New York City, New York; Sterling Forest State Park in Tuxedo, New York; East Lansing [for Study 1] and Ann Arbor [for Study 2] in Michigan; Seattle, Washington; and Anaheim and the University of California–Irvine in the LA area of California) and the presence of willing local collaborators. Ambient PM was collected at each site.

Our *in vitro* experiments were conducted in primary cells or cell lines derived from human airway epithelium and vascular endothelium, and mouse cardiomyocytes. The epithelial and endothelial cells were used to study oxidative stress; the cardiomyocytes were used to study the cellular mechanisms that underlie observed PM-induced changes in cardiac beat frequency.

The *in vitro* experiments were conducted with PM extracts from 12-day collection periods at the five sites over two seasons of the year and three particle-size ranges (target $n = 360$). The *in vivo* experiments were conducted using the PM samples from the 12-day sampling period and extended 100-day sampling periods at Manhattan and the LA area (i.e., two sites and three particle-size ranges; target $n = 600$).

FVB/N mice were treated with $50 \mu\text{g}$ PM suspensions by oropharyngeal aspiration and were examined for pulmonary inflammation and injury 24 hours later. (*In vitro* experiments were not performed for the 100-day study.) To assess the role of specific PM source categories and trace elements in ambient PM, the PM samples were analyzed for composition by ICP–MS. (Although the 100-day PM samples were extracted and digested, the ICP–MS analysis of them had not been completed when this report was published.)

This study addressed three of the overall NPACT study's four initial hypotheses. They are restated here more specifically in terms of the current study's *in vitro* and *in vivo* experiments on acute responses to short-term exposures to PM:

1. Coarse PM, fine PM, and ultrafine PM are each capable of producing acute health effects of public health concern, but the effects might differ according to particle-size range and particle composition within each size range.
2. The source-apportionment techniques that we have developed and refined in recent years provide a useful basis for identifying major PM air pollution source categories as well as specific chemical components having the greatest impacts on a variety of acute and chronic health effects.
3. The acute health effects caused by short-term exposures to PM samples in various particle-size ranges collected at multiple sites can usefully be studied in cells *in vitro* and in animal models treated *in vivo* by aspiration.

RATIONALE

We have studied the short-term health-related responses to treatment with PM samples that differed in composition. In our *in vivo* studies we aspirated, into mouse lungs, extractions of size-segregated PM that we collected at five U.S. sites located in different geographic areas. Each of these PM fractions was also tested *in vitro* using pulmonary, cardiac, and vascular cells. Although CAPs inhalation studies using coarse and ultrafine PM could supplement existing CAPs studies using fine PM and provide valuable information to help policymakers create regulations targeted to the most influential pollutant sources, such broader studies, if conducted throughout the United States and in multiple seasons, would be prohibitive in cost. In addition, inhalation studies in rodents would not be able to evaluate the relative lung toxicity of inhaled coarse PM, because of its inability to get past the rodents' upper respiratory tracts, where larger particles are filtered out. Our *in vivo* aspiration and *in vitro* studies were therefore proposed to efficiently gather critical comparative information on acute cardiopulmonary responses to PM fractions from ambient air that would otherwise not be available.

METHODS

PM SAMPLES

Collection Sites

For the 12-day study, 360 size-segregated ambient PM samples were collected from five different U.S. sites between August 2007 and August 2008. The sampling sites were selected in order to provide a large geographic diversity and diversity of PM source categories. The sites were at Manhattan's Mount Sinai School of Medicine in New York City, New York (an urban setting); Sterling Forest State Park in Tuxedo, New York (a rural northeastern setting); Ann Arbor, Michigan (a regional Midwestern setting); Seattle, Washington (a setting whose PM source categories included wood smoke in winter); and Anaheim, in the LA Basin of California (a setting whose PM source categories included aged traffic emissions). Twenty-four-hour samples were collected daily for 2 weeks to permit greater temporal resolution in identifying PM source categories and components (except for weekend collections, which were conducted over 48-hour periods). Because PM samples from different seasons of the year have been shown to cause different adverse health effects (Becker et al. 2005), the sampling at all five sites was conducted in both summer and winter.

For the 100-day study, size-segregated ambient PM samples were collected at Hunter College in Manhattan and at the University of California–Irvine between May and September 2010 and March and July 2010, respectively. The sampling sites in Manhattan were different for the 12-day samples (collected at Mount Sinai at 10 East 101st Street) and the 100-day samples (collected at Hunter College at 1st Avenue and 26th Street, 5 miles south of Mount Sinai) because the Mount Sinai building was closed for demolition between the 12- and 100-day sample collections, necessitating a switch. The California sampling site was also moved for the 100-day sample collection from Anaheim to Irvine, which is 18 miles southeast of Anaheim. Irvine was the site of a 6-month CAPs exposure study (described in Chen Study 1) that was conducted at the time on the campus of the University of California–Irvine.

Sample Collection

Ambient PM samples were collected using Harvard high-volume cascade impactors (ChemVol model 2400, Thermo Electron, East Greenbush, NY), which allowed for efficient and simultaneous collection of large amounts of multiple size-segregated PM samples. Particles larger than 10 μm were captured in the uppermost stage on a substrate

holder coated with silicone grease. $PM_{10-2.5}$ and $PM_{2.5-0.2}$ were collected on polyurethane foam (PUF) impaction substrates (Thermo Electron, East Greenbush); $PM_{0.2}$ was collected on a final 17-cm polypropylene substrate (G5300, Monadnock Non-Wovens, Mount Pocono, PA). Note that although the cut-off size for the ultrafine stage was 0.15 μm , it is referred to as $PM_{0.2}$ throughout this report. The flow rate of the ChemVol samplers was calibrated with a hot-wire anemometer before being deployed in the field. Adjustments to maintain the flow rate were made according to changes in pressure across the system. After sampling, all substrates were weighed according to a standard weighing protocol (adapted from EPA weighing protocols) in a weighing facility maintained at $22^\circ \pm 0.5^\circ\text{C}$ and 35% to 45% relative humidity and stored at 4°C until extraction. One field blank for each size fraction was taken at each site in both summer and winter, for a total of 30 field blanks (i.e., > 8% of the total sample number [360] in the 12-day study). Field blanks for each substrate were handled in the same manner as the PM samples. Fireworks, rich in metals, were utilized as a positive control in the *in vitro* oxidative stress assay. Pyrotechnic display fireworks were set off inside a 1- m^3 stainless steel chamber, and the PM_{10} size fraction was collected with a high-volume sampler using the G5300 polypropylene substrate used in the ultrafine stage of the ChemVol sampler.

Before each sampling regimen, all stages of the impactor were cleaned with mild soap and water, wiped with 0.1 N sodium hydroxide, and rinsed thoroughly with distilled water. At the change of each set of substrates (daily except for weekend samples), the equipment was cleaned with 70% ethanol, and Teflon forceps were used in an attempt to eliminate cross-contamination among samples and keep the system as sterile as practical.

Extraction of PM from Collection Substrates

PUF Substrate PM was removed from collection substrates by sonication, concentrated by lyophilization, and stored following a protocol modified from Becker and colleagues (2005). Handling of substrates was performed in a laminar-flow hood to maintain sterility as much as possible. PUF substrates from the upper two impactor stages were transferred to sterile 50-mL glass tubes, pre-wet with 5 mL 70% ethanol, and submerged in approximately 24 mL sterile Milli-Q water. Capped tubes were placed in a rack for 1 hour in an ultrasonic water bath (FS110 Ultrasonic Cleaner, Fisher Scientific, Waltham, MA) with a maintained temperature of $< 28^\circ\text{C}$. After sonication, the tubes were gently shaken and then returned to the sonicator for 1 additional hour. After the second sonication, the tubes were removed from the water bath, again gently

shaken, and agitated with a sterile serologic pipette to increase the amount of PM recovered without causing the substrate to abrade. The extracted material was then transferred into a labeled, pre-weighed, sterile 50-mL polypropylene tube. The PUF substrates were rinsed with 10 mL sterile Milli-Q water, which was then added to the same tube. The tubes were placed in a -20°C freezer overnight and transferred for storage to a -70°C freezer.

Polypropylene Substrate The polypropylene final-stage filters were placed in a sterile 4-L glass beaker with the PM-exposed side down. The filter was pre-wet with 25 mL 70% ethanol, followed by enough sterile Milli-Q water to completely submerge the filter. A sterile 1-L flask was placed on top of the filter to prevent it from floating. The beaker was covered with plastic paraffin film and placed in an ultrasonic water bath for 1 hour with a maintained temperature of $< 28^\circ\text{C}$. The filter was then agitated by swirling and placed back in the sonicator for 1 additional hour. Following sonication, the PM extract was transferred to a sterile 250-mL bottle, the filter was rinsed with 10 mL sterile Milli-Q water, and this final 10 mL was added to the same bottle. The bottles were placed in a -20°C freezer overnight and transferred for at least 4 hours of storage to a -70°C freezer.

Sample Lyophilization

Extracted PM samples were lyophilized using a 4.5-L benchtop freeze-dryer (Labconco, Kansas City, MO). After lyophilization, the tubes and bottles were handled as follows:

50-mL tubes: When no liquid remained in the 50-mL tubes, they were removed from the lyophilizer, immediately capped, and transferred to a desiccator in a temperature- and humidity-controlled weighing room. After at least 24 hours of acclimation, the tubes were post-weighed.

250-mL bottles: When less than 5 mL remained in the 250-mL bottles, they were removed from the lyophilizer, immediately capped, and transferred to a laminar-flow hood. PM extracts were transferred to pre-weighed 50-mL tubes by adding a total volume up to 10 mL sterile Milli-Q water, followed by four rounds of 1 minute of sonication and 1 minute of vortexing. The lyophilization procedure used for the earlier 50-mL tubes was then repeated for these samples.

Weighing Protocol

The weights of the PUF and polypropylene substrates before and after each sample collection and of the polypropylene tubes before and after PM extractions were obtained using an analytic balance (XS105 DualRange Analytical Balance, Mettler-Toledo, Hightstown, NJ) in the weighing

room. Each filter was assigned a barcode with a unique identification number that was used throughout the study as the ID number for the corresponding sample. Before each weighing session, two standards (100 and 200 mg) and weighing-room blank substrates corresponding to the material being weighed during that session (i.e., the 1.3-cm PUF, 0.64-cm PUF, polypropylene substrate, or 50-mL polypropylene tube) were weighed to quality-assure the stability of the balance. All collection substrates and tubes were acclimated in the weighing room for at least 24 hours before pre- or post-weighing. Tare and gross weights were determined as the average of two consecutive weighings with a difference of less than 0.05 mg. These pre- and post-weights were recorded to determine extraction efficiencies and the net weight of the material collected for each sample. The mean (\pm SD) extraction efficiency was $80\% \pm 15\%$, $81\% \pm 16\%$, and $64\% \pm 20\%$ for the coarse, fine, and ultra-fine collection substrates, respectively. Because the extraction efficiency was not 100%, a limitation of the study was that all components (such as volatiles and semivolatiles) might not have been extracted from the substrates at equal efficiencies.

Resuspension and Storage

Once the net weight was determined, each PM extract was resuspended in a volume of sterile Milli-Q water at a final concentration of 5 mg/mL, 1 mg/mL, or 250 μ g/mL (depending on the total amount of PM recovered from each substrate and the amount needed for the in vitro studies [250 μ g/mL] and the in vivo studies [1 mg/mL]). Field blank samples generally had no or low net weights and were therefore resuspended at the equivalent median mass of the samples they were serving to control (e.g., the field blank PUFs for the fine PM stages were resuspended with a volume of water equal to that used to resuspend the median mass of all the fine PM samples). After the sterile Milli-Q water was added to the lyophilized PM, the samples were sonicated for 1 minute and vortexed for 1 minute; this process was repeated for a total of four rounds. Aliquots of the PM samples were then transferred into pre-labeled, sterile cryovials and stored at -70°C .

IN VITRO EXPERIMENTS IN THE 12-DAY STUDY

Cell Types, Cultures, and Viability

Bioassays were conducted using three cell types (bronchial epithelial cells, vascular endothelial cells, and cardiomyocytes) selected on the basis of their biologic plausibility for showing effects of PM treatment. We used both primary and immortalized cell cultures (see below). Multiple aliquots of the same passage for each immortalized cell type

were kept frozen in liquid nitrogen. The epithelial and endothelial cells were thawed and grown for 2 weeks before conducting experiments. Each bioassay with immortalized cell lines was conducted within 2 months of initial thawing in order to keep the cell passage between experiments relatively consistent. Additionally, subsets of the experiments were repeated using primary epithelial and primary endothelial cells.

Bronchial Epithelial Cells An immortalized human bronchial epithelial cell line (BEAS-2B, American Type Culture Collection, Rockville, MD) was maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% FBS (Gemini Bio Products, Calabasas, CA) and 1% penicillin–streptomycin (Gibco, Grand Island, NY).

Primary human bronchial epithelial cells (HBEPc, PromoCell, Heidelberg, Germany) were used for validation of the results in the BEAS-2B cell line. We screened various lots of donor cells for ROS production; to reduce variability we reserved a single lot (#5092901.5) for use in the experiments. The HBEPc cells were maintained in bronchial epithelial growth medium (BEGM, Lonza) containing 1% penicillin–streptomycin and supplemented with bovine pituitary extract (0.004 mL/mL), epidermal growth factor (10 ng/mL), insulin (5 μ g/mL), hydrocortisone (0.5 μ g/mL), epinephrine (0.5 μ g/mL), triiodo-L-thyronine (6.7 ng/mL), transferrin (10 ng/mL), and retinoic acid (0.1 ng/mL), as supplied by the manufacturer (PromoCell). All cultures were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide (CO_2).

Vascular Endothelial Cells A human pulmonary microvascular endothelial cell line (HPMEC) designated as HPMEC-ST1.6R was kindly provided by Dr. C. James Kirkpatrick and Dr. Vera Krump-Konvalinkova of Johannes Gutenberg University in Mainz, Germany. Clone HPMEC-ST1.6R cells have been shown to have characteristics similar to those of primary HPMEC cells in terms of an angiogenic response on Matrigel protein film and similar expression of various constitutively expressed endothelial markers (von Willebrand factor, platelet endothelial cell adhesion molecule, and vascular endothelial growth factor receptor 1, as well as the inducible cell adhesion molecules intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1, and E-selectin) (Unger et al. 2002).

Primary human lung blood microvascular endothelial cells (HMVEC-LBI, Lonza) were used for validation of the results in the HPMEC-ST1.6R cell line. Again, multiple lots were screened, and a single lot (#0000123873) was reserved for use. Because these cells senesce after 12 population doublings, all experiments were conducted between passages 2 and 3.

The HPMEC-ST1.6R and HMVEC-LBI cultures were both maintained at 37°C in a humidified atmosphere containing 5% CO₂ and grown in an endothelial cell growth medium (Endothelial Cell Growth Medium-2, Lonza) containing 1% penicillin–streptomycin and supplemented with growth factors and 2.5% FBS (EGM-2 BulletKit, Lonza).

Cardiomyocyte Cells Significant cardiac effects have been associated with exposure to inhaled PM. Various heart rate changes in addition to changes in heart rate variability have been reported. For these reasons, we conducted experiments studying beat frequency and gene expression using cardiomyocytes derived from transgenic mouse embryonic stem cells (Lonza). The cells were cultured in cardiomyocyte medium (Cor.At, Axiogenesis, Cologne, Germany) according to the manufacturer's technical manual. All cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂. The experiments were conducted using day 3–4 confluent monolayers of spontaneously beating myocytes; the baseline beat rate of the individual cultures used for the experiments was fairly consistent, at 128 ± 33 (mean ± SD) beats/min.

Primary cultures of neonatal rat cardiomyocytes were isolated for optical mapping experiments, as previously described by Rohr and colleagues (2003). (Voltage-sensitive dyes can be used to map activation and repolarization in cardiomyocytes. As the dyes are excited, an optical signal is produced that mimics an action potential, allowing visualization of both the activation and repolarization. This allows for evaluation of the propagation of an excitation wave for the visual measurement of its wavelength.) Ventricular cardiomyocytes were obtained by excising ventricles from groups of 9–16 neonatal Wistar Hannover rats (1–2 days old). Cells were seeded at a density of 2.3×10^3 viable cells/mm² on collagen-coated Petri dishes and maintained in a humidified atmosphere at 37°C with 5% CO₂; the media were changed daily. Treatment with PM was conducted on day 3 of culture. Because it would be unlikely for biologically significant amounts of inhaled particles, particularly those in the coarse or fine modes, to translocate in particulate form from the lung to the cardiovascular system, the experiments conducted with the cardiac myocytes utilized only the ultrafine, soluble fraction of PM.

Cell Viability Analysis Cell viability was measured in the epithelial and endothelial cells by two methods: (1) the release of the cytoplasmic enzyme LDH into the supernatant of the cell cultures, as described by Pozzi and colleagues (2005); and (2) a standard clonal survival assay. Cytotoxicity was not measured in the cardiomyocyte cells, because the cells maintained appropriate functioning (i.e., spontaneous beating), indicating that cell death did not

occur. For the LDH assay, cells were seeded in 96-well plates at a density of 8000 cells per well and treated with 10, 50, or 100 µg/mL PM for 24 hours. LDH release from the cells was determined using an enzymatic colorimetric assay (Cytotoxicity LDH Detection Kit, Takara Bio, Madison, WI). Results were calculated by expressing LDH activity in the culture supernatant as a percentage of total LDH activity, measured as LDH activity in the supernatant from cells incubated with a nonionic detergent (1% Triton X-100) for 20 minutes.

The standard clonal survival assay was used to validate the LDH assays by evaluating the survival and growth of cells treated with a subset of the PM samples (Mehta et al. 2008). BEAS-2B cells were seeded in triplicate at a density of 100 cells per well in six-well plates (Corning, Corning, NY). After an incubation period of 24 hours, the medium was changed to DMEM Nutrient Mixture F-12 (DMEM/F12; Gibco) containing 2% FBS (Gemini Bio Products) and no phenol red, and PM samples were added at 9, 45, 90, 225, 450, or 900 µg/mL (1, 5, 10, 25, 50, or 100 µg/cm², respectively). After 24 hours, the PM treatments were terminated by removing the PM medium, washing the cells two times with PBS (to remove residual PM), and feeding them with fresh culture medium (DMEM). After an incubation time of 9 to 10 days, the colonies were again washed with PBS, fixed with 50% methanol, and stained with 0.05% crystal violet, and the number of surviving colonies was scored. Colony-formation ability was calculated by comparing the plating efficiency of treated cells with the plating efficiency of untreated cells.

PM Treatments and Composition Analyses

Initially, dose–response experiments conducted with ambient PM and a positive control identified 50 µg/mL as a non-toxic dose appropriate for the bioassays (see section on cell viability analyses). Although this 50-µg/mL concentration of PM applied to the cells was the same for all assays (except as noted), the areas of the tissue-culture plates varied. The treatment doses for each assay, with the corresponding PM per surface area covered, are shown in Table 1.

To examine the relative contribution of PM mass as measured in ambient samples, experiments based on percent mass exposures were conducted on a subset of samples ($n = 12$, Table 2) from the Manhattan and LA Basin sites in summer and winter. The samples were prepared by calculating concentrations compared with a fixed PM_{2.5} concentration of 100 µg/mL; the other two size fractions were adjusted according to their mass ratio to fine PM for that sampling day.

To compare the roles of soluble versus insoluble PM, soluble PM samples were prepared by centrifuging the total samples for 10 minutes at $12,000 \times g$ and collecting the supernatant without disrupting the pellet.

All extracted PM samples were stored at -70°C until use. After thawing, the samples were placed in an ultrasonic water bath for at least 20 minutes with a maintained temperature of $< 28^{\circ}\text{C}$. Immediately before applying the PM to the cells, the samples were vortexed for 10 seconds

to fully suspend the particles. For the ROS, lactate dehydrogenase (LDH), quantitative real-time polymerase chain reaction (qPCR), and clonal survival assays, cells were washed with phosphate-buffered saline (PBS), and the medium was changed to DMEM/F12 (Gibco) containing no phenol red, 2% FBS, and 1% penicillin–streptomycin before PM treatment. Cells were grown in 10% FBS (as described in the section on cell types and cell culture), and we reduced the concentration of the serum to 2% for the final bioassays.

Table 1. PM Doses for in Vitro Assays

Assay	PM Concentration ($\mu\text{g}/\text{mL}$)	PM per Surface Area ($\mu\text{g}/\text{cm}^2$)
DCFH-DA (ROS production)	50	20
LDH (cell viability)	50	30
Clonal survival (cell viability)	9, 45, 90, 225, 450, and 900	1, 5, 10, 25, 50, and 100
qPCR (gene expression)		
BEAS-2B cells	50	8.5
HPMEC-ST1.6R cells	100	17
Cardiomyocytes	50	25
Spontaneous beat frequency	50	40

Endotoxin Analysis Endotoxin concentrations for a subset of the PM extractions ($n = 60$) were determined by the quantitative kinetic–chromogenic limulus amoebocyte lysate (LAL) protocol as recommended by the manufacturer (Lonza, Walkersville, MD). The amount of endotoxin present in each sample was calculated by comparing its reaction time against a standard curve and expressing the result in endotoxin units (EU)/mL. Duplicate samples were run for each PM sample analyzed.

Acid Digestion of PM Samples PM samples and field blanks were digested by way of a modified microwave protocol from the EPA’s analytic procedure Method 200.7 (U.S. EPA 1994), and the sample digestions were analyzed by ICP–MS. All materials used for the digestions were acid-cleaned to minimize the risk of contaminating the samples. After acid-cleaning, all materials were rinsed three times with Milli-Q water and air-dried in a HEPA-filtered clean

Table 2. Dose Calculations Used for Percent Mass ROS Experiments

Location (Season)	PM Size Fraction	Net Weight/Size (mg)	Total PM Mass (mg)	% of Total PM Mass	Final Concentration ($\mu\text{g}/\text{mL}$)
Manhattan (summer)	Coarse	16.49	40.20	41	95.6
	Fine	17.28		43	100.2
	Ultrafine	6.43		16	37.3
Manhattan (winter)	Coarse	14.20	37.54	38	91.5
	Fine	15.53		41	100.1
	Ultrafine	7.81		21	50.4
LA Basin (summer)	Coarse	47.34	103.53	46	101.5
	Fine	46.77		45	100.3
	Ultrafine	9.42		9	20.2
LA Basin (winter)	Coarse	42.12	104.97	40	87.5
	Fine	48.21		46	100.1
	Ultrafine	14.64		14	30.4

room. Perfluoroalkoxy (PFA) plastic digestion vials (Savillex, Minnetonka, MN) were washed in a chelating detergent (Micro-90, International Products, Burlington, NJ), rinsed, heated in 50% hydrochloric acid for 1 hour, and heated for 1 hour in 50% nitric acid (HNO₃), followed by a cleaning microwave digestion run using the digest solution (66% Optima-grade HNO₃). All vials were rinsed with Milli-Q water between each step. After the microwave digestion runs, the PFA digestion vials were cleaned by soaking in 10% HNO₃ for a minimum of 3 hours, followed by three rinses with Milli-Q water and air-dried in the clean room.

Thawed PM samples were sonicated in an ultrasonic water bath for 20 minutes, followed by vortexing immediately before adding the sample to cleaned PFA vials in a laminar-flow hood. For each sample, 100 µg PM was added to the vial, and 66% Optima-grade HNO₃ was used in the digestion reactions. For quality control, Milli-Q water blanks (the negative control) and additional PM samples consisting of National Institute of Standards and Technology standard reference material 1648 were included in each run. Sealed vessels were placed in the microwave digestion oven (MDS-200 Microwave Laboratory Digestion System, CEM Corp., Worcestershire, UK) and underwent a two-step digestion process. In the first step, pressure was ramped up to 50 pounds per square inch over a period of 30 minutes and then maintained at this pressure for 20 minutes. In the second step, the vessels were allowed to cool for 30 minutes. After each run, the vessels were opened in the hood, the vials were removed, and their exteriors were rinsed three times with Milli-Q water. Following air-drying, 5 mL Milli-Q water was added to bring the final acid concentration to 4%. Samples were then transferred into acid-cleaned and labeled hydrofluoropolyether plastic bottles (Fisher Scientific).

Characterization of Trace Elements The elemental composition of the PM samples was determined by ICP-MS in the laboratory of Dr. Steven Chillrud at Columbia University's Lamont-Doherty Earth Observatory in Palisades, New York, following a modified version of a protocol previously described by Ross and colleagues (2000). Samples were analyzed on a single-collector double-focusing magnetic sector ICP-MS (Axiom, VG Elemental, Winsford, UK). Indium-normalized sample data were quantified by comparing them against standard curves. The elements measured were beryllium (Be), Mg, P, S, K, Ca, scandium (Sc), Ti, V, Cr, Mn, Fe, cobalt (Co), Ni, Cu, Zn, As, Se, strontium (Sr), silver (Ag), cadmium (Cd), tin (Sn), antimony (Sb), cesium (Cs), lanthanum (La), Pb, and thallium (Tl). The blank filters, National Institute of Standards and Technology standard reference material 1648 PM samples, and water blanks

were incorporated for quality assurance, and the appropriate water-blank subtractions were made as described previously (Kinney and Thurston 1993) to determine trace-element concentrations.

Measuring ROS, Acellular Oxidative Capacity, and Markers of Inflammation

DCFH-DA Assay The generation of intracellular ROS was assessed using the 2',7'-dichlorofluorescein-diacetate (DCFH-DA) assay, which measures the ROS-mediated conversion of DCFH-DA into fluorescent 2',7'-dichlorofluorescein (DCF) (LeBel et al. 1992). The assay was conducted in the bronchial epithelial and vascular endothelial cells. Cells were seeded at 5000 cells per well in black 96-well plates (Corning), cultured for 2 days, and then loaded with 10 µM DCFH-DA (Invitrogen, Carlsbad, CA) for 30 minutes at 37°C. After incubation, the dye-loaded cells were washed two times with PBS, and 100 µL fresh medium (DMEM/F12 with no phenol red) was added to the cells. 25-µL PM samples were added in triplicate, and DCF fluorescence was quantified using a microplate reader (HTS 7000, Perkin Elmer, Waltham, MA) at excitation and emission wavelengths of 485 nm and 535 nm, respectively. ROS measurements were performed at 180 minutes for the epithelial cells and at 300 minutes for the endothelial cells. Each well's initial measurement was used as a baseline, and ROS production was calculated as the increase in fluorescence intensity and normalized by subtraction of the vehicle control (water). Cells in the vehicle control wells (in triplicate) received the same volume of sterile MilliQ water used in the wells treated with PM extracts. Media- and cell-only control wells were also run in triplicate, as were positive control metal-rich fireworks PM extracts.

Acellular Oxidative Capacity To assess the innate oxidative capacity of the extracted PM samples, a subset of 60 samples (the same 60 used in the gene expression and in vivo mouse assays described below) was shipped on dry ice to collaborators Dr. Flemming Cassee and Dr. Miriam Gerlofs-Nijland at the National Institute for Public Health and the Environment (RIVM) in the Netherlands and tested according to protocols established at the RIVM laboratory for the ability of the ROS generated by the PM samples in solution (i.e., not within cells) to deplete ascorbic acid and dithiothreitol.

Gene Expression of Markers of Inflammation

PM-induced effects on gene expression were evaluated by examining mRNA-transcript levels of specific genes selected for each cell type. The gene expression measured in the BEAS-2B cells was for colony stimulating factor 2

(CSF-2), HO-1, IL-6, IL-8, and vascular endothelial growth factor A (VEGF-A). The gene expression measured in the HPMEC-ST1.6R cells was for HO-1, ICAM-1, IL-8, thioredoxin reductase 1 (TXNRD1), and VEGF-A.

The BEAS-2B and HPMEC-ST1.6R cell lines were seeded in triplicate at a density of $8\text{--}10 \times 10^4$ cells in 12-well plates (Corning) and grown until they reached approximately 80% confluency. After 6- and 24-hour PM treatments, the cells were washed twice with PBS, and total RNA was isolated following a standard protocol, using a homogenizer and RNA purifier (QIAshredder and RNeasy Mini Kits, Qiagen, Valencia, CA). The quantity and quality of the RNA were determined using a spectrophotometer (NanoDrop 1000, Thermo Fisher Scientific, Wilmington, DE); the ratio of the absorbance at 260 and 280 nm was between 1.9 and 2.1 for all samples used in these studies.

Total RNA was stored at -80°C and subsequently reverse-transcribed into complementary DNA (cDNA) using a reverse-transcription kit (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Foster City, CA) as described by the manufacturer. The cDNA was stored at -20°C until use.

Relative mRNA levels of the genes were quantified by qPCR (TaqMan Gene Expression Assay and 7900 Real-Time PCR System, Applied Biosystems). As specified in the manufacturer's technical guide, PCR conditions consisted of a 10-minute hot start at 95°C , followed by 40 cycles of 30 seconds at 95°C and 1 minute at 61°C , and relative expression levels were identified using the comparative C_T method. All expression levels were normalized to the endogenous control gene glyceraldehyde 3-phosphate dehydrogenase.

Cardiomyocyte Function

Spontaneous Beat Frequency Beat frequencies were measured for individual wells of spontaneously beating mouse stem cell-derived cardiomyocytes (Lonza). Baseline beat rate measurements (beats/min) were obtained using a confocal microscope (DMI 6000B, Leica Microsystems, Mannheim, Germany) connected to a digital camera (DFC340 FX, Leica). The soluble fraction of the PM samples at a stock concentration of $250 \mu\text{g/mL}$ was sonicated for 20 minutes before being applied to the cells at a final concentration of $50 \mu\text{g/mL}$. The soluble samples, prepared as described above, were thawed, vortexed, and applied to the cells at equal volumes of the total PM. The effect on beat frequency was measured by way of real-time counting by eye at 0, 30, 60, 120, 180, 240, and 1440 minutes. For quality assurance, a confocal software platform (LAS-AF 2.0.2, Leica) was used to capture live images at 117-msec intervals, and a subset of the videos was counted twice; no

differences between the counts were observed. Three biological replicates for each sample were analyzed; results are represented as the average percent of baseline.

Conduction Velocity and Action Potential Duration

Cultures of neonatal rat cardiomyocytes were treated with soluble fractions of PM or the vehicle control on day 3 of culture. Primary cultures of the neonatal rat cardiomyocytes were isolated for optical mapping experiments, as described by Rohr and colleagues (2003). After 24 hours, the conduction velocity and the action potential duration at 70% and 50% repolarization were measured in the cultures using high-resolution optical mapping in Dr. Gregory Morley's laboratory at NYU Langone Medical Center. As previously described (Vasquez et al. 2010), cells were stained with the fluorescent dye di-8-ANEPPS ($135 \mu\text{mol/L}$; Invitrogen) and kept in a recording solution (1% FBS in Hanks buffered salt solution at 37°C , pH 7.4) for the duration of the experiment. Cells were stimulated at a basic cycle length of 400 msec with bipolar electrodes ($250\text{-}\mu\text{m}$ diameter, $800\text{-}\mu\text{m}$ separation; FHC, Bowdoin, ME), and mapping was performed on a fixed-staged upright microscope (BX51WI, Olympus) with a reflective light fluorescence attachment, a 100-watt mercury arc lamp, and a complementary metal-oxide-semiconductor camera (MiCAM ULTIMA, SciMedia). Measurements were taken at 250 frames/sec with 14-bit resolution from a 100×100 pixel array, giving a spatial resolution of $81.6 \mu\text{m}$ at $2.5\times$ magnification. Conduction velocity and action potential duration calculations for individual pixels were analyzed as described previously (Leaf et al. 2008; Morley et al. 1999).

IN VIVO EXPERIMENTS

In the first set of in vivo experiments, a subset of 60 PM samples from the 12-day study was used to evaluate the effects of PM treatment on markers of inflammation in the lungs of mice. The subset included samples of each of the three size fractions that produced the two highest and the two lowest ROS responses at each of the five sampling sites studied in the in vitro experiments. In the second set of in vivo experiments, daily samples collected at Manhattan and at Irvine in the 100-day study were used to treat the mice. Note that, because of equipment malfunctions, only 67 and 93 days' worth of PM sampling occurred at the two sites, respectively, out of the targeted 100 days.

In both studies, male and female FVB/N mice (ages 6 to 10 weeks) were treated with $50 \mu\text{L}$ of the vehicle control (water) or a 1 mg/mL PM resuspension (i.e., $50 \mu\text{g}/\text{mouse}$) by aspiration while under isoflurane anesthesia (Rao et al. 2003). At 24 hours after treatment, the mice were killed with pentobarbital and exsanguinated by cardiac puncture. The

lungs were lavaged twice with 1.2 mL PBS without Ca⁺ or Mg⁺, and cell counts, cell differentials (macrophages, neutrophils, eosinophils, and epithelial cells), and total protein concentrations were determined. The serum and remaining aliquots of cell-free lavage samples were archived at -70°C. Results of the in vivo experiments are expressed as the mean ± standard error. *N* = 6 per group (*n* = 3 males and *n* = 3 females) for the PM samples in the 12-day study, and *n* = 3 per group (sex varied randomly among the groups) for the PM samples in the 100-day study. The study protocol was approved by the Institutional Animal Care and Use Committee (Laboratory Animal Protocol #101002-02).

STATISTICS

For the toxicologic assays, all in vitro data were expressed as the mean of three biologic replicates, unless otherwise noted. Box plots of the ROS data were generated using SAS 9.2 (SAS Institute, Cary, NC). The statistical significance between data from different samples (i.e., different sites, seasons, or sizes) was determined by ANOVA followed by the Wilcoxon rank-sum test for the ROS experiments (using the SAS software) and the Dunnett post-hoc test for the cardiomyocyte beat frequency experiments (using graphic and statistics software [GraphPad Prism, GraphPad Software, La Jolla, CA]). Statistically significant differences are reported when *P* ≤ 0.05; they are indicated as such in the relevant figures. Heat maps for the qPCR results were generated using statistical and biologic-validation software (MultiExperiment Viewer 4.6, Dana-Farber Cancer Institute, Boston, MA). Linear regression analysis between elemental components and biologic endpoints was conducted with the assistance of Dr. Lall using statistical software (S-Plus 2000, TIBCO Software, Palo Alto, CA); the significance of the slope from the linear regressions was evaluated using spreadsheet software (Excel, Microsoft, Redmond, WA). Factor analysis was conducted by Dr. Kazuhiko Ito, also of NYU Langone Medical Center, and Dr. Lall to understand how the elemental components varied together and whether or not the differences in PM composition could account for the observed differences in the biologic responses. A varimax rotation was applied to the data, and factor scores were used to interpret associations between the chemical composition and toxicologic endpoints.

RESULTS

The results of the 12-day in vitro and in vivo studies are presented first, followed by the results of the 100-day in vivo study. For the 12-day study, bioassay results are presented first, followed by data on PM composition and then

statistical correlations between the bioassay results and the data on PM composition and source categories. Trace-element data for the 100-day study were not yet available when this report was published.

IN VITRO 12-DAY STUDY

PM Effects on ROS and Markers of Inflammation

In the in vitro ROS assay, 50 µg/mL was used as the test concentration for each extracted PM sample. This concentration was chosen on the basis of a pilot concentration-response ROS study with a small number of PM samples and the fact that it was not observed to cause overt toxicity in the LDH toxicity assay (only 11 of the 360 PM samples had LDH release levels greater than 15%) or in a clonal survival assay (data not shown). The DCFH-DA assay was used to measure intracellular ROS production in response to the 360 samples and to the extractions from the 30 field blanks (one for each site, season, and size) in the BEAS-2B airway epithelial and HPMEC-ST1.6R vascular endothelial cell lines and in primary cells. Data for the ROS experiments were expressed in average relative fluorescence units (RFUs) for all samples in each category, after subtraction of the mean control water value. Positive (fireworks PM₁₀ effluent) and negative (medium alone) controls were included on each 96-well plate used in these assays.

After treatment with the PM samples, both cell lines generated the same pattern of ROS production (Figure 1). The relative fluorescence (mean ± SD) generated by the field blanks was generally low, as expected; it was only slightly higher (43.9 ± 45.7 and 58.0 ± 38.5 RFUs) than that of the medium alone (27.5 ± 33.0 and 16.4 ± 16.1 RFUs) for the two cell lines, respectively. The ROS responses to PM (Figure 2) were significantly correlated between the two cell lines (*r* = 0.46, *P* < 0.0001). For brevity's sake, therefore, the remainder of this report presents results primarily for the BEAS-2B cells but includes results for both cell types where appropriate.

As shown in Figure 3 and Table 3, the amount of ROS induced in the BEAS-2B cells was significantly influenced by the three main PM variables: sampling site, season of the year, and particle size. Greater mean ROS production (incorporating all PM size fractions) was induced by the samples from Manhattan (179.3 ± 105.0 RFUs) and the LA Basin (166.0 ± 108.4 RFUs) than by those from Tuxedo (132.0 ± 56.8 RFUs), Seattle (100.2 ± 100.0 RFUs), and Ann Arbor (87.8 ± 121.1 RFUs) (top panel, Figure 3). Because the samples from each site were added to the cells at the same mass concentration (50 µg/mL), these results suggest that composition differences among the samples accounted for the influence of site as the effect modifier.

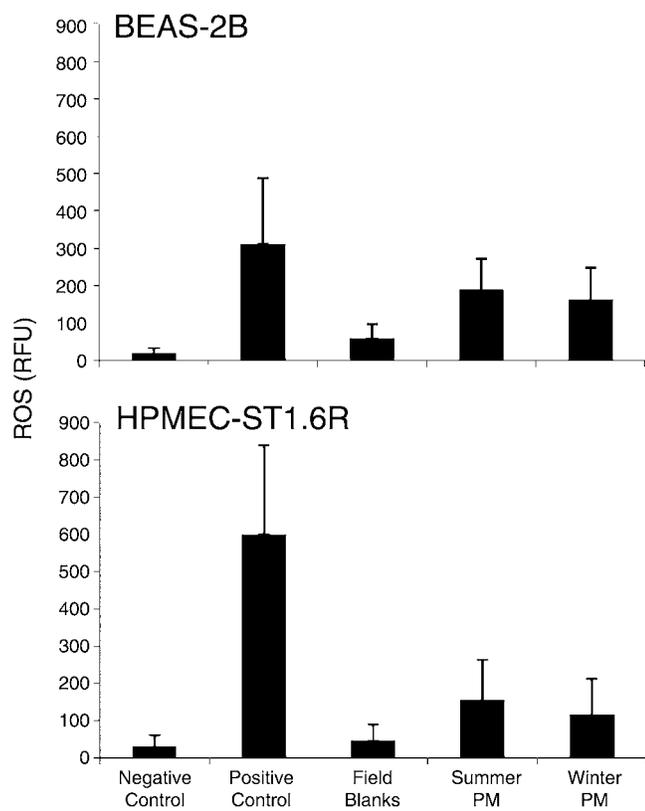


Figure 1. Average ROS production for negative (medium) and positive (metal-rich fireworks PM) controls, field blanks, summer PM, and winter PM in BEAS-2B (top) and HPMEC-ST1.6R (bottom) cells. Error bars indicate 1 SD. Data are expressed as relative fluorescence units (RFUs) in the DCFH-DA assay.

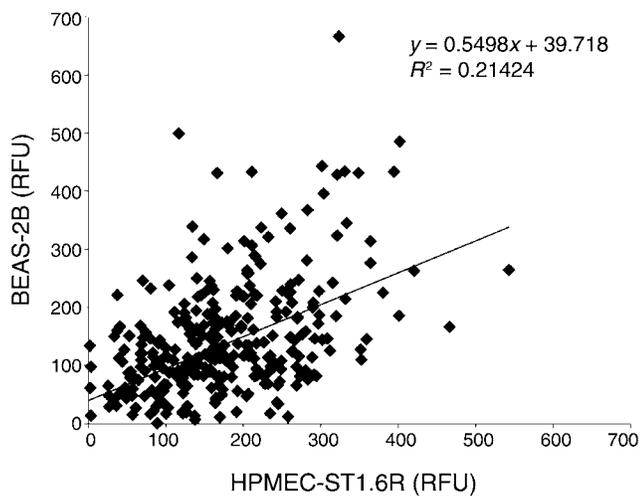


Figure 2. Correlation between ROS production, as measured in the DCFH-DA assay, in BEAS-2B and HPMEC-ST1.6R cells ($P < 0.0001$).

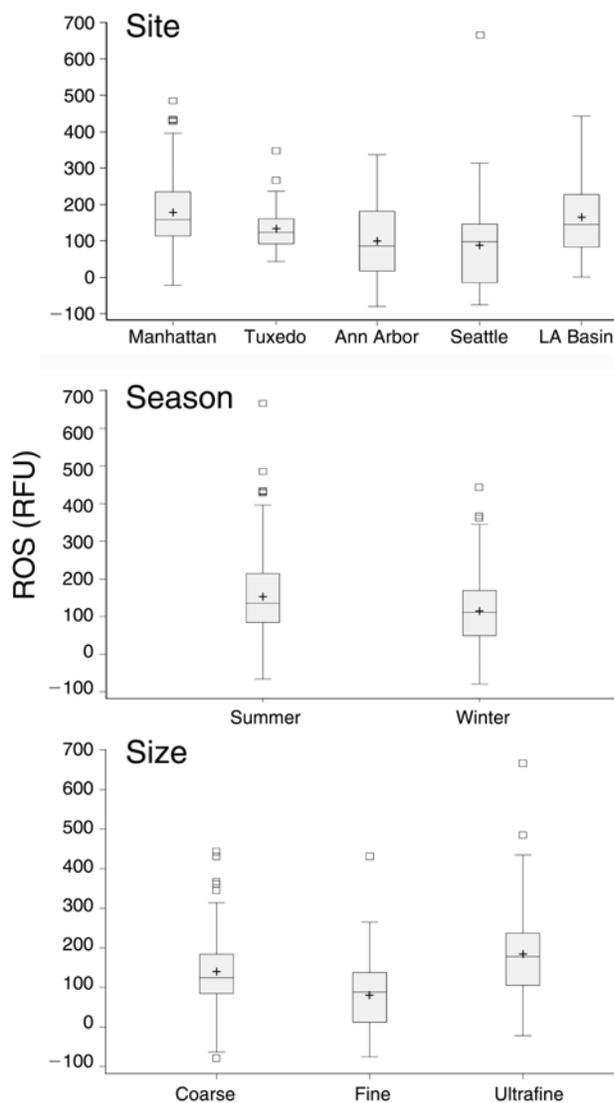


Figure 3. Effects of PM sampling site (top), sampling season (middle), and particle-size ranges (bottom) on ROS production in BEAS-2B cells. Box plots indicate 25th, 50th (median), and 75th percentiles as well as mean (+), minimum (lower fence), and maximum (upper fence) values. Minimum and maximum values were calculated as 1.5 times the interquartile range for the 25th and 75th percentiles, respectively. Small squares indicate outliers.

Season had a small but statistically significant effect on ROS production. Samples collected in the summer generated statistically greater mean ROS (153.6 ± 109.4 RFUs) than samples collected in winter (113.3 ± 98.6 RFUs) (middle panel, Figure 3). Similarly, the three PM size fractions generated small but statistically significant variations in ROS response (bottom panel, Figure 3, and Table 3), with the largest response from the ultrafine fraction and the smallest response from the fine fraction.

Table 3. Statistically Significant Associations Between PM Variables and ROS Production in BEAS-2B and HPMEC-ST1.6R Cells^a

Factor	ROS Production	
	BEAS-2B	HPMEC-ST1.6R
Sampling site	$P < 0.000001$	$P = 0.0000001$
Particle size	$P < 0.000001$	$P < 0.000001$
Season	$P = 0.0000108$	$P = 0.0010675$
Site–size interactions	$P < 0.000001$	$P = 0.0000003$
Site–season interactions	NS	NS
Size–season interactions	$P = 0.00011720$	$P = 0.0015110$

^a Significant associations (ANOVA) are listed for each cell type. NS = not significant.

In addition to these individual variable effects, statistical interactions were observed between some of the variables. There was a significant interaction between particle size and season, and particle size and site; however, there was no interaction between season and site (Table 3).

To illustrate interactions among the variables, Figure 4 shows important differences in patterns of effect (again, the results presented are for BEAS-2B cells, but our results for the HPMEC-ST1.6R cells yielded the same conclusions). For example, as described above, season as a single variable had a significant effect on ROS production induced by PM extracts (Table 3 and Figure 3). This effect was observed for all five sites (Figures 4) but was dependent on particle size, insofar as generally only fine and ultrafine summer PM extracts consistently produced greater ROS than fine and ultrafine winter PM extracts. Also, whereas PM samples from Tuxedo induced an ROS effect that had relatively small variability and was largely independent of particle size or season (Figure 4), size and season strongly influenced the ROS responses of cells treated with PM from Seattle and the LA Basin (Figure 4). These results demonstrate that the effects of size and season (and their interactions) on ROS response were dependent on the sampling site.

The principal variables — sampling site, season, and particle size — thus contributed significantly to the observed variations in ROS production, showing, as hypothesized, that in addition to PM mass the source categories (and therefore composition) of PM are important contributors to PM toxicity. Moreover, day-to-day variability in PM composition appeared to have an equal or even greater effect on ROS

production than the site, season, and size variables, though only at certain sites. For example, the ultrafine summer PM samples from the LA Basin showed considerable daily variation in ROS production; those from Tuxedo showed much less daily variation (Figure 5), suggesting that the day-to-day composition of the ultrafine summer PM samples varied to a greater extent at the LA Basin than at Tuxedo. In addition, there did not appear to be a significant effect for weekdays versus weekends on ROS production (data not shown). Although a breakdown by season and particle size showed some site-dependent differences in ROS production, the breakdown only included two weekend samples per site, and therefore no statistical analyses were performed.

These findings suggest that the daily variations in PM-induced ROS response might be attributable to rapid changes in the elemental composition of the air pollution mixture, which also suggests that knowing the chemical composition of all the samples might provide better insights into the mechanisms and source categories behind the variability.

Water-Soluble Extract Versus Total PM Extract

To understand whether PM-induced ROS production could be attributed directly to interactions between solid PM and cells or to water-soluble components in aqueous extracts of the PM samples (which contained both particles and soluble components), primary airway epithelial and endothelial cells were treated with total PM extracts (at 50 $\mu\text{g}/\text{mL}$) or the equivalent volumes of the soluble fraction for a subset of the PM samples. The primary endothelial cells did not respond to the total or soluble PM extracts, and therefore only the primary epithelial cell data are discussed here. The relative role of soluble PM varied among the samples (Figure 6). Overall, the soluble fraction accounted for approximately 55% of the ROS production by the total PM. This percentage was fairly consistent across the coarse, fine, and ultrafine size fractions (on average 53%, 60%, and 53%, respectively; Figure 6). The results from these cells also demonstrated, however, that for some selected samples the total PM and the soluble fraction generated similar amounts of ROS (as was the case, for example, for coarse PM collected in summer at Manhattan and Tuxedo [Figure 6]).

Equal Mass Versus Percent Mass Exposures The ROS experiments described above were conducted using equal mass concentrations of 50 $\mu\text{g}/\text{mL}$. Because actual mass concentrations of various PM size fractions exist in the ambient atmosphere in various ratios, we repeated a subset of the ROS experiment using cells treated with PM extracts from two sites in the relative proportions measured in ambient air. Table 2 shows the size-fraction ratios of individual PM samples

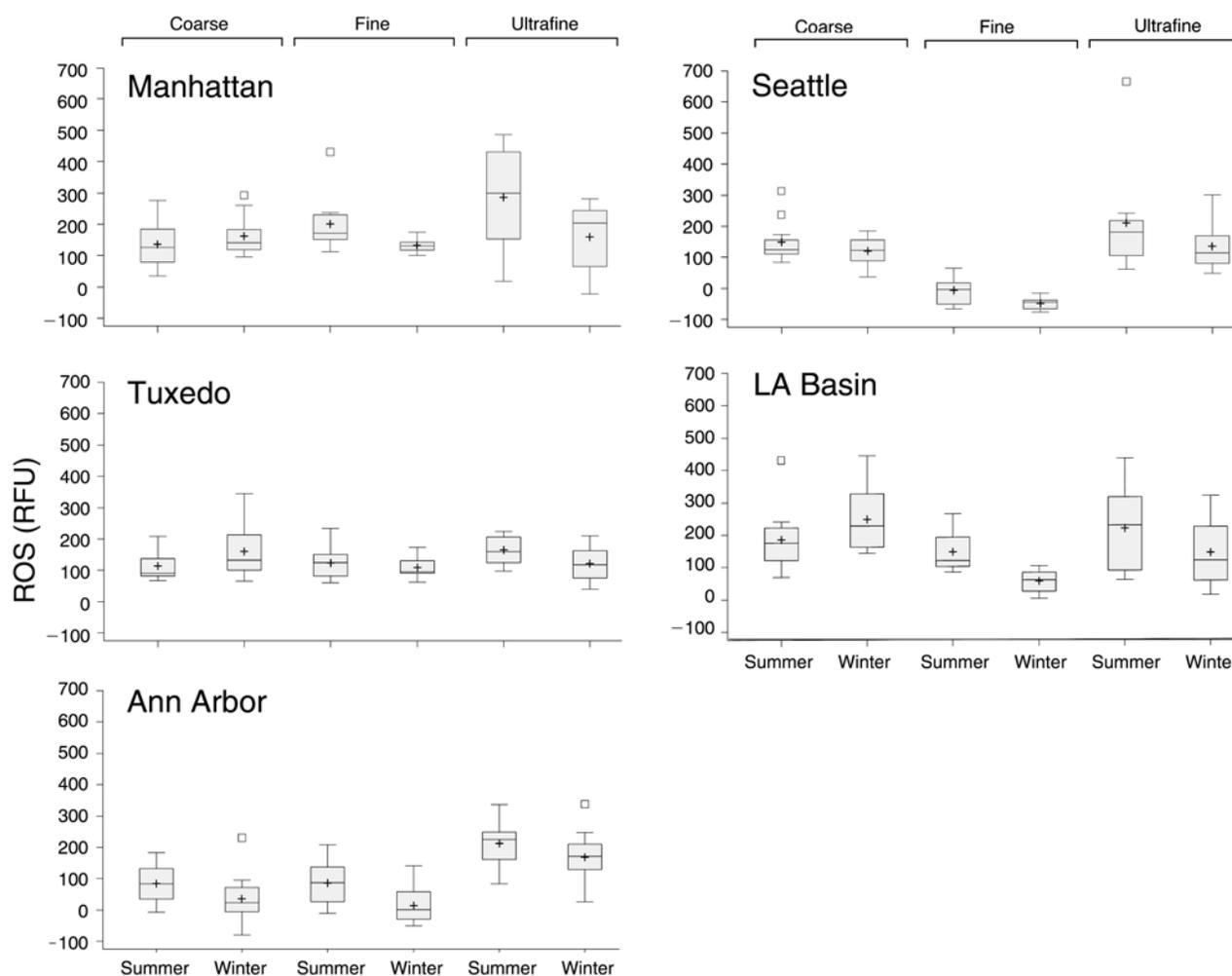


Figure 4. Effects of PM sampling season and particle size, by sampling site, on ROS production in BEAS-2B cells. Box plots indicate 25th, 50th (i.e., median), and 75th percentiles as well as mean (+), minimum (lower fence), and maximum (upper fence) values. Minimum and maximum values were calculated as 1.5 times the interquartile range for the 25th and 75th percentiles, respectively. Small squares indicate outliers.

collected at Manhattan and the LA Basin in summer and winter. Overall, the coarse and fine fractions made up approximately equal percentages of the ambient PM mixture (41% and 44%, respectively). The ultrafine fraction made up an average of 15% of the mixture, ranging from 21% in the Manhattan winter sample to 9% in the LA Basin summer sample.

Relative ROS results varied considerably depending on particle size (Figure 7). Coarse PM, for example, induced similar amounts of ROS in three out of four samples for both the relative percent mass dosing and the equal mass treatment (i.e., 50 $\mu\text{g}/\text{mL}$). Ultrafine PM, by contrast, induced significantly less ROS in three out of four cases for the relative percent dosing than for the equal mass treatment. Fine PM unexpectedly induced greater amounts of ROS in three

out of four samples for the relative percent dosing than for the equal mass treatment.

Acellular Oxidative Potential of PM Samples Sixty PM samples were assessed for the innate capacity of PM samples to produce ROS in solution (i.e., acellularly). Collaborators at RIVM in the Netherlands evaluated the samples for their ability to deplete ascorbic acid and dithiothreitol in the assay mixture. As shown in Table 4 and Table 5, acellular oxidative potential was significantly correlated with ROS production by vascular endothelial cells treated with the same PM samples. There was no correlation, however, between the acellular oxidative potential and the in vivo responses in mice treated with PM (data not shown).

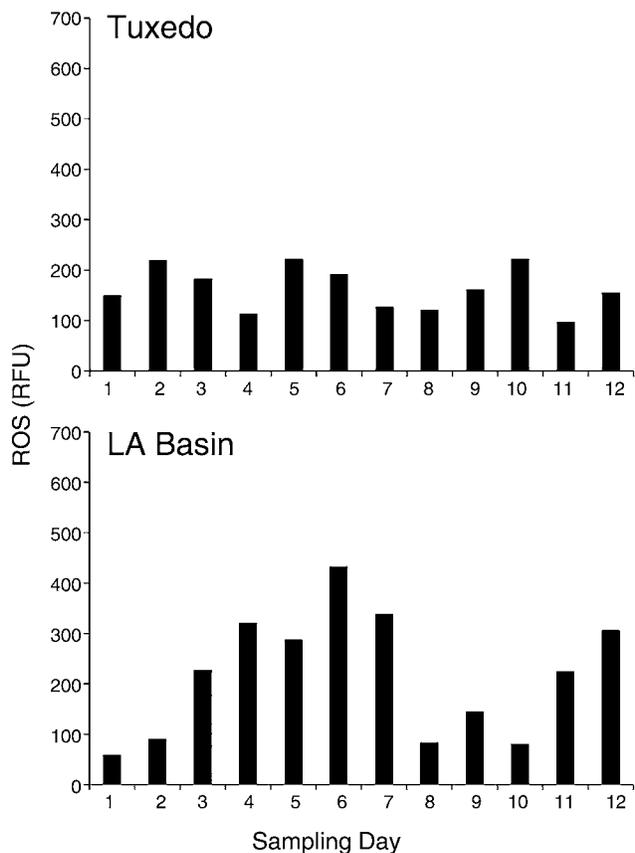


Figure 5. Daily variation in ROS production in BEAS-2B cells treated with ultrafine summer PM collected daily for 12 days in Tuxedo (top) and the LA Basin (bottom). Values are reported as the mean RFU of two biologic replicates.

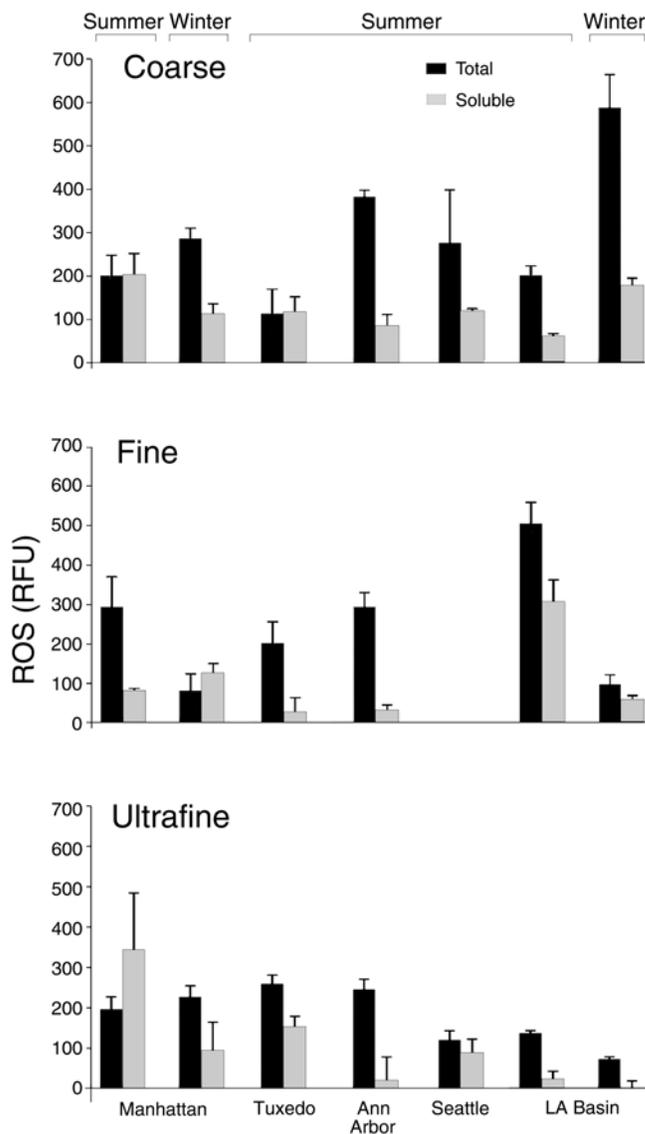


Figure 6. ROS production in primary bronchial epithelial HBEpC cells treated with total (black) and soluble (gray) fractions of coarse (top), fine (middle), and ultrafine (bottom) PM samples collected on the same day. Values are reported as the mean RFU (\pm SE) of three biologic replicates. No summer fine sample was available for Seattle.

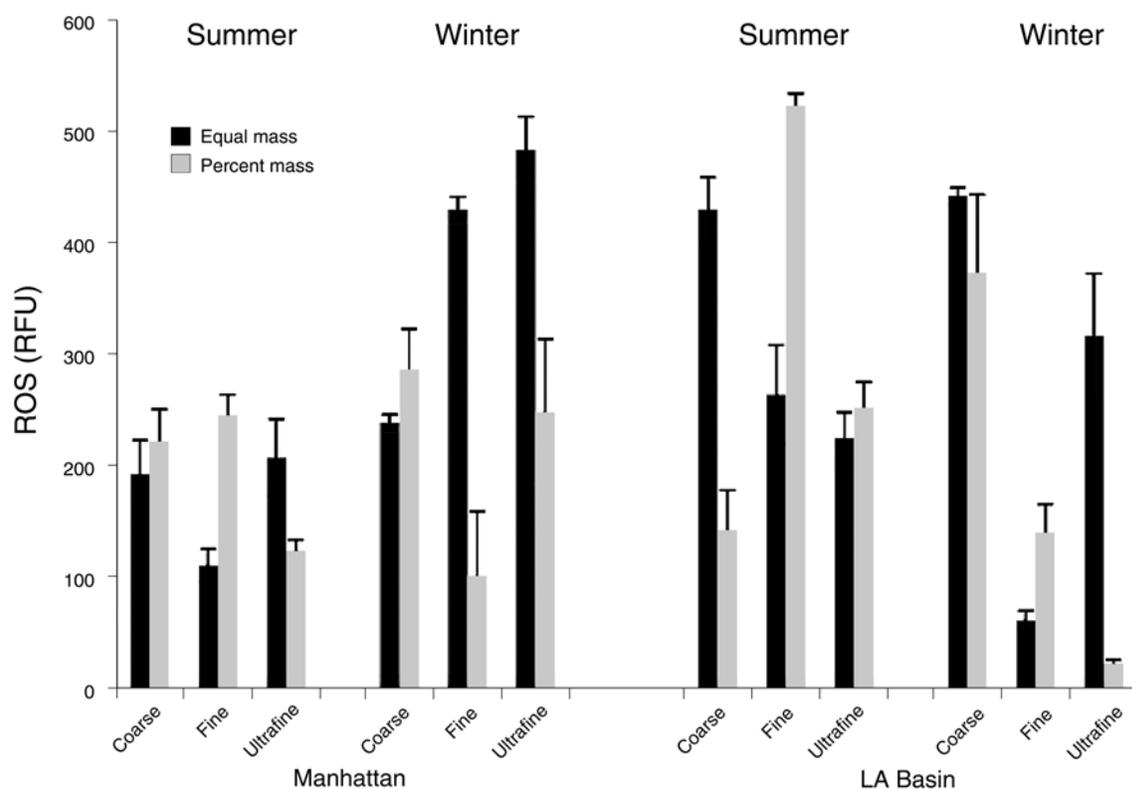


Figure 7. Comparison of ROS production in BEAS-2B cells treated with PM from Manhattan and the LA Basin in size fractions at equal mass concentrations (black) and at percent mass concentrations reflecting their proportions in ambient air (gray). Values are reported as the mean RFU (\pm SE) of three biologic replicates.

Table 4. Correlations Between Results for Ascorbic Acid and Dithiothreitol Assays and PM-Induced Protein Concentrations and PMNs in Mouse Lavage Fluid^a

	Ascorbic Acid Assay	Dithiothreitol Assay
Protein concentrations	-0.02	-0.01
PMNs (% of total cells)	-0.00	0.05

^a Pearson correlations (r) for the 60 PM samples used in the in vivo aspiration experiment.

Table 5. Correlations Between Results for Ascorbic Acid and Dithiothreitol Assays and PM-Induced ROS Production in HPMEC-ST1.6R and BEAS-2B Cells^a

	Dithiothreitol Assay	Ascorbic Acid Assay	HPMEC-ST1.6R Cell ROS	BEAS-2B Cell ROS
Dithiothreitol assay	1			
Ascorbic acid	0.33	1		
HPMEC-ST1.6R Cell ROS	0.37	0.63*	1	
BEAS-2B Cell ROS	0.19	0.44*	0.64*	1

^a Pearson correlations (r) for the 60 PM samples used in the in vivo aspiration experiment. * = $P < 0.01$.

mRNA Expression of ROS and Inflammation Genes

To identify transcriptional changes that might result from the treatment of cells with PM, we measured mRNA expression levels of selected markers of ROS and inflammation using qPCR in the BEAS-2B and HPMEC-ST1.6R cells at 6 and 24 hours post-treatment for a subset of PM samples ($n = 45$; $n = 15$ for each size fraction). For the mean responses of all samples together (i.e., regardless of site, season, and size), HO-1 mRNA was significantly induced at 24 hours in the BEAS-2B cells and at both 6 and 24 hours in the HPMEC-ST1.6R cells (Figure 8). In addition, IL-8 mRNA was significantly elevated compared with the control at 24 hours in the BEAS-2B cells (Figure 8). Although they were not statistically significant changes, increased expression of IL-8 and TXNRD1 mRNA was observed in the HPMEC-ST1.6R cells at 6 hours.

When considering gene expression induced by specific PM size fractions, all three size fractions were shown to significantly induce HO-1 at 6 hours in the HPMEC-ST1.6R cells (data not shown), but particle size did not significantly modulate any other gene at either time point in either cell line. More important, individual PM samples were sometimes found to alter mRNA expression to varying extents, thus suggesting that PM sample composition had a strong influence on these genetic markers of ROS and inflammatory responses. All alterations greater than threefold compared with controls are shown in Table 6 and Table 7. Although each value represents only one sample ($n = 3$ replicates) and thus has low statistical power for multiple comparisons, trends in the results are evident. For example, HO-1 mRNA expression showed the most changes at both time points and in both cell lines. The data also indicate that although an individual sample might have altered mRNA expression at 6 hours, the effect did not necessarily persist at 24 hours. IL-8 and VEGF-A also showed that several PM samples produced greater than threefold changes in mRNA expression, although VEGF-A was induced in airway epithelial cells and generally suppressed in vascular endothelial cells (Figure 8 and Tables 6 and 7). Additionally, certain samples altered the gene expression of all the markers of ROS and inflammation measured, including F-151 (an LA Basin summer fine sample) at 24 hours in the BEAS-2B cells as well as F-104 and UF-104 (Manhattan winter fine and Manhattan winter ultrafine samples collected on the same day) at 24 hours in the HPMEC-ST1.6R cells. The observations in the HPMEC-ST1.6R cells suggest a unique composition of fine and ultrafine samples for that collection day in Manhattan.

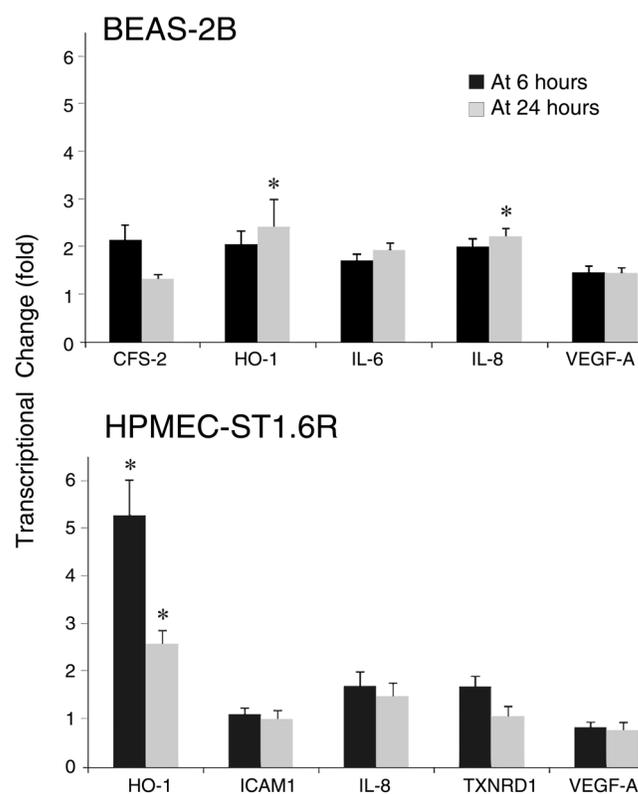


Figure 8. Relative abundance of mRNA in BEAS-2B cells (top) and HPMEC-ST1.6R cells (bottom) at 6 and 24 hours after exposure to 50- and 100- μ g/mL concentrations of PM, respectively. Values are reported as the mean fold change (\pm SE; $n = 35$ – 40 /group) for results from PM combined for sampling site, sampling season, and particle size. Data are expressed as the relative fold increase over control. * indicates statistically significant changes ($P \leq 0.05$) compared with the control.

As shown in the heat maps in Figure 9, hierarchical clustering patterns for site, season, or size were not apparent in the BEAS-2B cells at either 6 or 24 hours: the expression of mRNA (indicated by shades of red to green to represent increased and decreased expression, respectively, compared with control cell responses) did not produce a clear clustering of samples by site, season, or size. Similar results were observed for HPMEC-ST1.6R cells that were treated at a higher dose (100 μ g/mL; data not shown). Thus, the lack of hierarchical clustering also suggests that the composition of individual PM samples was the most important variable for gene expression changes.

Table 6. Significant Changes^a in mRNA Expression After PM Exposure in BEAS-2B Cells^b

Sample ID	Description of Sample	CSF-2	HO-1	IL-6	IL-8	VEGF-A
6-Hour Exposure						
C-104	MWC	3.23				
C-140	ASC				4.47	
C-51	LWC		5.01		3.96	
C-53	LWC		5.82		3.57	
F-25	MSF	8.01		3.98		
UF-25	MSU	4.78			3.83	3.57
UF-29	MSU	10.71		3.72	6.09	3.30
UF-37	SSU	4.88	11.01		3.80	4.66
24-Hour Exposure						
C-104	MWC			4.00	3.35	
C-140	ASC				3.58	
C-151	LSC		3.75			
C-45	SSC		3.28			
C-51	LWC		3.99	3.16		
C-53	LWC		6.19	3.89	5.23	
F-127	LSF		3.15			
F-151	LSF	3.07	4.55	3.09	3.27	3.21
F-38	SSF		5.29		5.45	
F-44	SSF		3.82			
UF-104	MWU		3.18			
UF-37	SSU		4.06			
UF-7	TSU				3.09	

^a Greater than threefold.

^b M = Manhattan, T = Tuxedo, A = Ann Arbor, S = Seattle, L = LA Basin, S = summer, W = winter, C = coarse, F = fine, and UF = ultrafine.

PM Effects on Cardiomyocyte Function

PM Effects on Spontaneous Beat Frequency To determine whether ambient PM can affect the spontaneous beat frequency of cardiomyocytes, we initially compared responses in primary rat and mouse cardiomyocytes and cardiomyocytes derived from mouse stem cells. The cardiomyocytes derived from the mouse stem cells were easier to use and had the most consistent beat frequency (data not shown). Confluent monolayers of these cardiomyocytes were therefore treated with 50 µg/mL of the water-soluble components of size-fractionated summer and winter PM samples from Manhattan. The spontaneous beat frequency of the cultures was

measured at various times after treatment (Figure 10). Although a similar downward trend in beat frequency was observed over time for all the cells, including those treated with water control samples, two findings were statistically different from those for the water controls: The Manhattan winter fine sample caused a statistically significant increase in spontaneous beat frequency at 15 minutes compared with the baseline; this effect did not persist at the later time point. The Manhattan winter ultrafine sample caused a statistically significant decrease in spontaneous beat frequency at 24 hours compared with the baseline.

Cellular and Mouse Exposures to PM from Five Airsheds

Table 7. Significant Changes^a in mRNA Expression After PM Exposure in HPMEC-ST1.6R Cells^b

Sample ID	Description of Sample	HO-1	ICAM-1	IL-8	TXNRD1	VEGF-A
6-Hour Exposure						
C-104	MWC	4.69				
C-109	MWC	10.05				
C-140	ASC	7.49				
C-148	ASC	4.51				
C-29	MSC	6.41				
C-45	SSC	7.71				
C-51	LWC	15.62				
C-53	LWC	19.15				
F-104	MWF	5.38				
F-109	MWF	12.05				
F-11	TSF	3.98				
F-127	LSF					-3.09
F-140	ASF					-3.86
F-151	LSF	4.76	3.36	5.16	4.68	
F-200	ASF	3.65				
F-29	MSF	4.68				
F-38	SSF	11.97	4.19	5.33	4.57	
F-44	SSF	5.76				
F-51	LWF	4.00				-3.52
F-53	LWF	5.14				-3.37
UF-147	ASU	13.37				
UF-42	SSU	5.67				
UF-53	LWU	17.04		10.95	7.50	3.10
24-Hour Exposure						
C-109	MWC	3.46				
C-130	LSC	3.11				
C-140	ASC					-3.10
C-151	LSC				-3.03	-8.20
C-29	MSC	3.54				
C-36	SSC					-4.08
C-45	SSC	4.26				
C-51	LWC	6.70				
C-53	LWC	6.24				
F-104	MWF	6.38	5.75	6.93	5.95	3.86
F-11	TSF					-3.91
F-25	MSF					-3.52
F-38	SSF	3.05				-3.72
F-53	LWF					-3.22
F-6	TSF					-3.84
UF-104	MWU	8.13	5.27	8.81	6.19	5.44
UF-147	ASU		-3.29			
UF-53	LWU	3.58				

^a Greater than threefold.

^b M = Manhattan, T = Tuxedo, A = Ann Arbor, S = Seattle, L = LA Basin, S = summer, W = winter, C = coarse, F = fine, and UF = ultrafine.

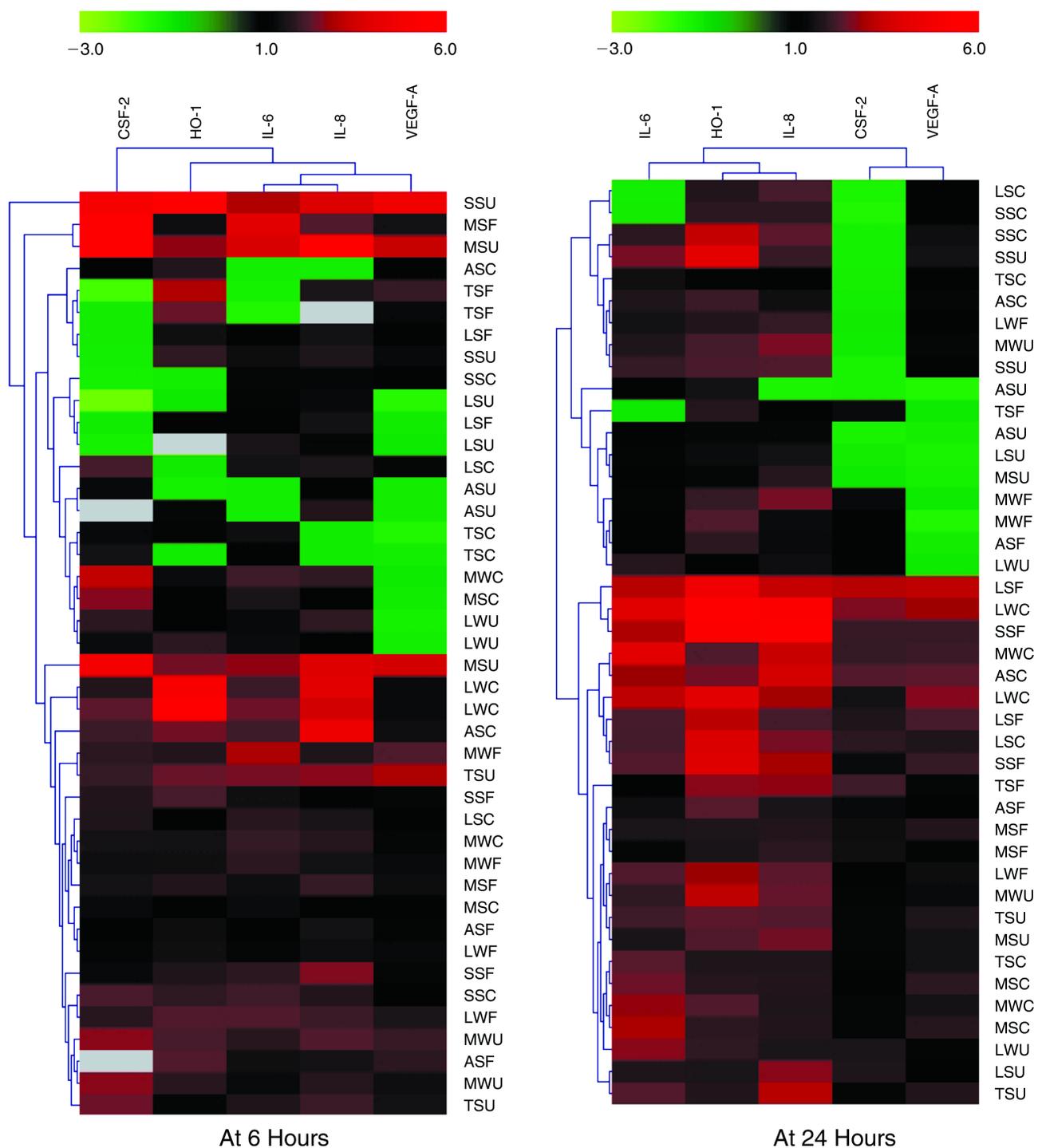


Figure 9. Heat maps of mRNA expression, by PM sampling site, sampling season, and particle size, in BEAS-2B cells at 6 hours (left) and 24 hours (right) after 50- μ g/mL exposures to PM from a subset of PM samples. Sample code: (first position) M = Manhattan, T = Tuxedo, A = Ann Arbor, S = Seattle, L = LA Basin; (second position) S = summer, W = winter; (third position) C = coarse, F = Fine, and U = ultrafine. Brackets show hierarchical clustering.

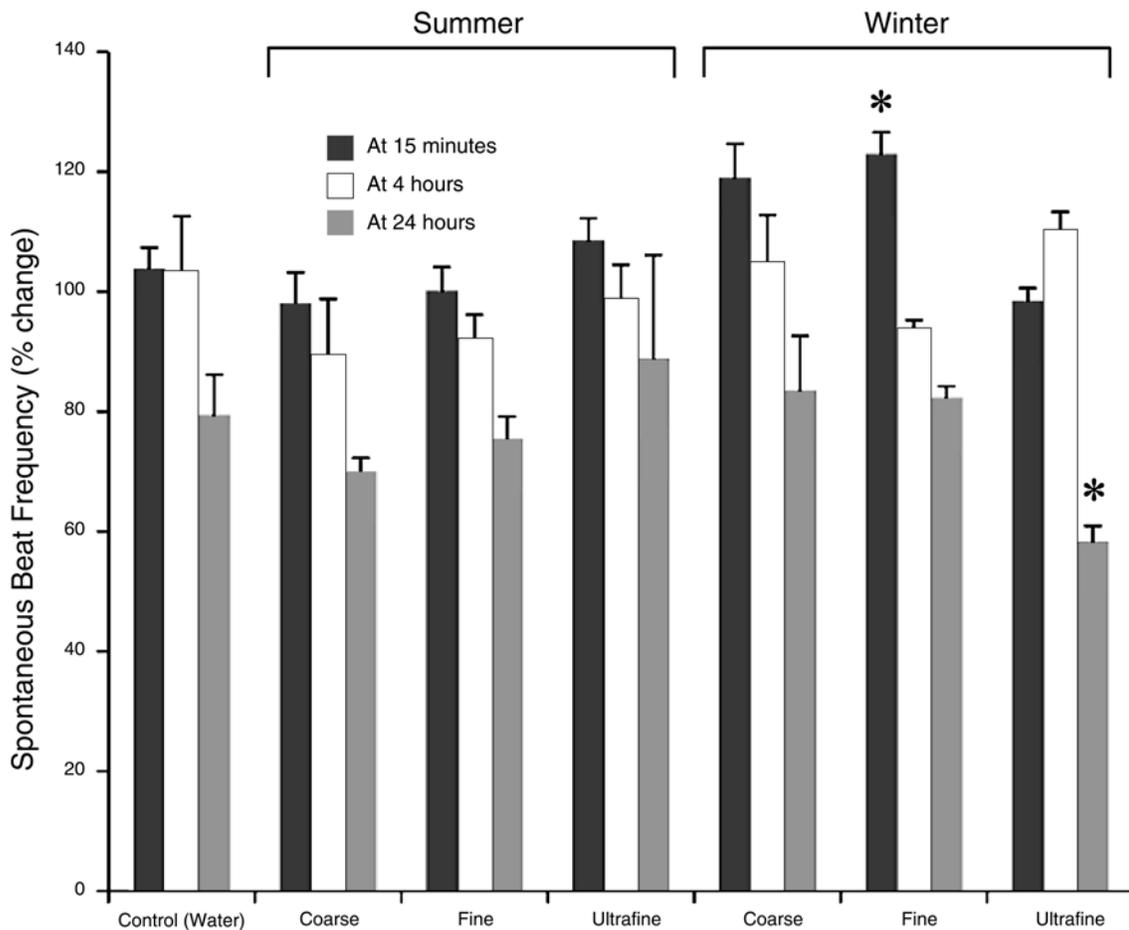


Figure 10. Changes in spontaneous beat frequency for cardiomyocytes derived from mouse stem cells after 50-µg/mL exposures to the soluble components of size-fractionated summer and winter PM from Manhattan. Values are reported as the mean percentage of the baseline beat frequency (\pm SE) of three biologic replicates. * indicates statistically significant changes ($P \leq 0.05$) compared with the control (water) when using one-way ANOVA followed by the Dunnett test.

PM Effects on Conduction Velocity and Action Potential Duration Optical mapping of confluent monolayers of neonatal rat cardiomyocytes was conducted after 24-hour treatment with the soluble components of the Manhattan winter fine sample, and a significant 13% decrease in conduction velocity was observed (Figure 11). In addition, a downward trend in action potential duration was observed in the PM-treated cultures at both 50% and 70% repolarization; however, these trends did not reach statistical significance (Figure 11).

IN VIVO 12-DAY STUDY

PM Effects on Lung Inflammation and Injury

Mice were treated by aspiration to the control vehicle (water) or extracted PM ($n = 60$ samples and $n = 5$ to 6 mice per sample) using a subset of summer samples from the 12-day study, and lavage fluid parameters were studied 24 hours later. Although the PM samples had no significant effects on total protein in lavage fluid (an index of lung injury; data not shown), statistically significant

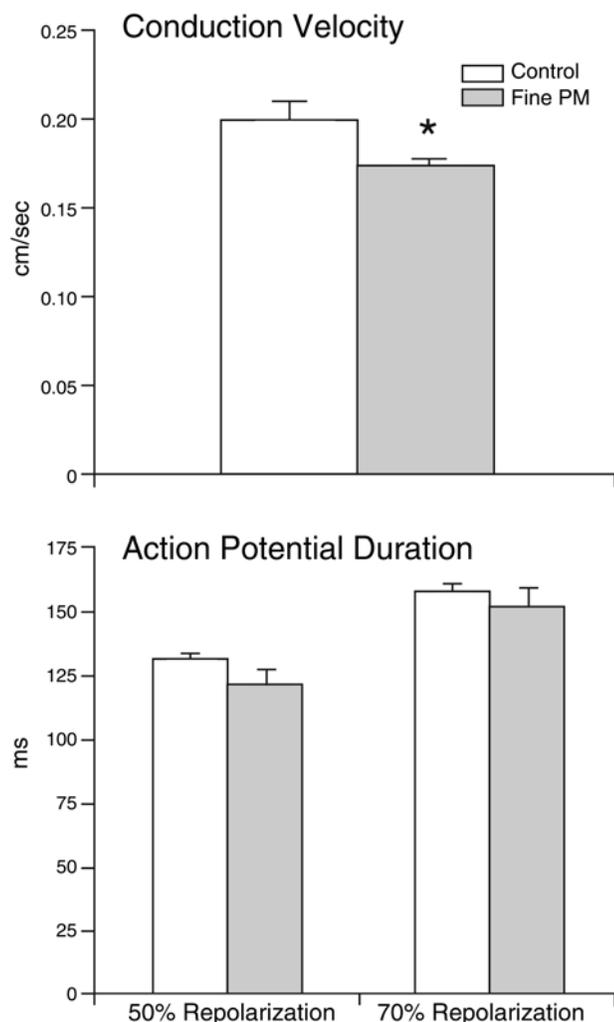


Figure 11. Changes in conduction velocity (top) and action potential duration (bottom) in primary rat cardiomyocytes after 24-hour 50- $\mu\text{g}/\text{mL}$ exposures to the soluble components of a Manhattan winter fine PM sample. Values are reported as the mean (\pm SE) of multiple biologic replicates, some of which were repeated ($n = 11\text{--}20$). * indicates statistically significant changes ($P \leq 0.05$) compared with the control (water) when using the Student *t* test.

changes in % PMNs (an index of lung inflammation) were observed; these effects were dependent on site and particle size. As shown in Figure 12, the induction of PMN influx into the lungs was similar among the PM samples collected at the five sampling sites, with the exception of the LA Basin. The change in % PMNs resulting from the aspiration of PM from the LA Basin was approximately 45% less than that produced by PM from the other sites. As observed in vitro for the ROS production endpoint, PM size had a significant effect on the lung inflammation endpoint. As seen in Figure 12, coarse

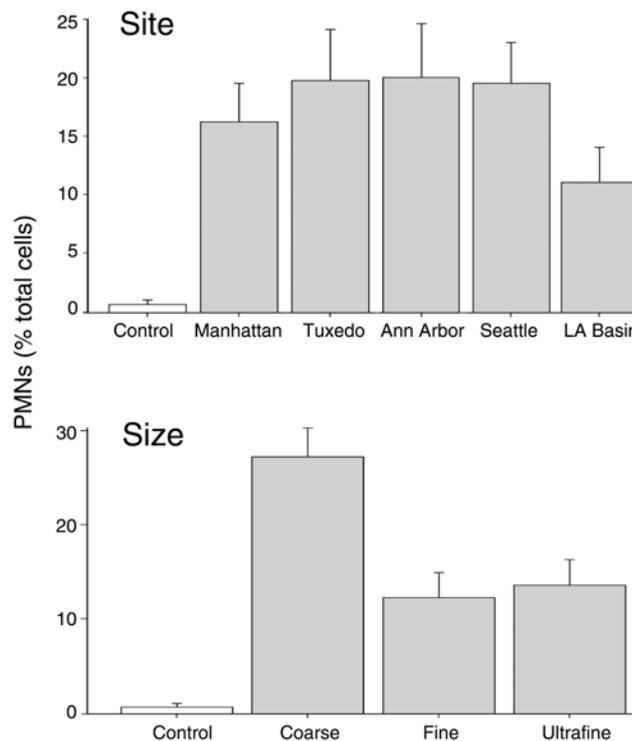


Figure 12. Effects of PM sampling site (top) and particle size (bottom) on in vivo increases in % PMNs in the lavage fluid of mice exposed to size-fractionated PM by aspiration. Values are reported as means with 95% confidence limits.

PM produced significantly greater changes in % PMNs than did fine and ultrafine PM. This size-dependent effect on PMNs, however, varied among the sampling sites. Figure 13 shows that the PM collected at Manhattan and the LA Basin, the two large urban centers, produced effects different from those of the PM collected at the other three sites. The coarse and ultrafine PM from Manhattan and the LA Basin induced degrees of lung inflammation that were roughly equivalent to each other and greater than those produced by the fine PM. In contrast, at Tuxedo, Ann Arbor, and Seattle, the coarse PM produced more lung inflammation than both the fine and ultrafine PM. These findings suggest that composition differences among the three sizes of PM collected in summer at the five sites modulated the inflammatory responses seen in vivo.

Despite the significant changes observed in lung inflammation in the mice, there was no correlation between the in vitro ROS and in vivo PMN changes for the 60 samples (Figure 14). This suggests that the composition of PM that

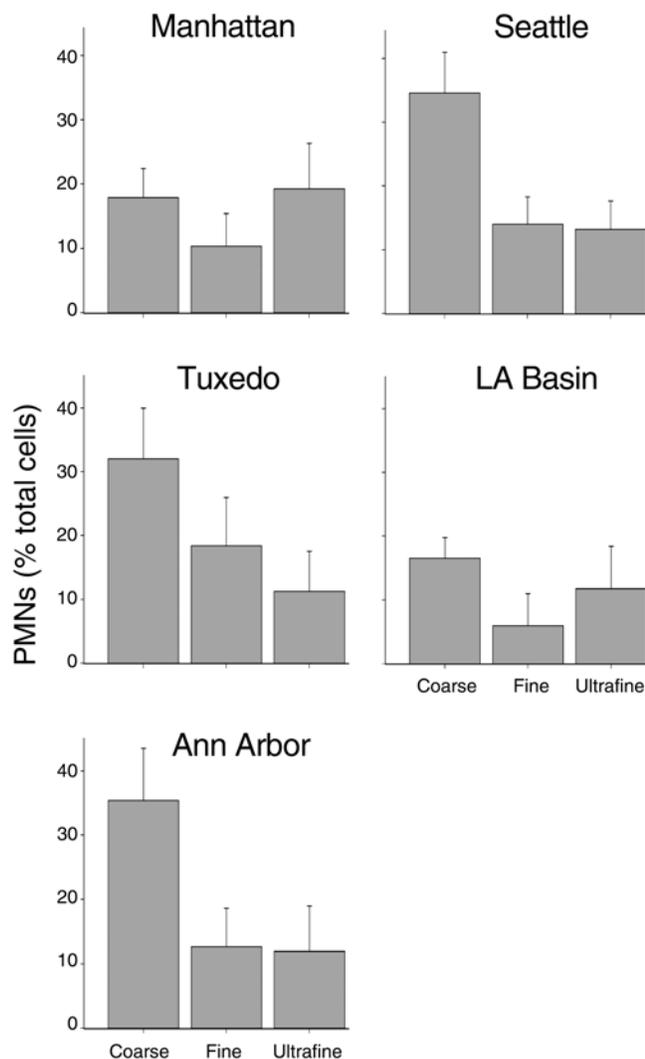


Figure 13. Effects of particle size, by sampling site, on in vivo % PMNs in the lavage fluid of mice treated with size-fractionated PM by aspiration. Values are reported as means with 95% confidence limits. Values are reported as means (\pm SE). Error bars indicate 95% confidence limits.

drove the pathways responsible for the changes in the ROS and the % PMNs was different for each endpoint. Indeed, as described earlier and below, a variety of trace elements and source categories were correlated with some of the observed in vitro and in vivo effects.

ANALYSIS OF PM MASS, ELEMENTAL COMPOSITION, AND ENDOTOXIN CONTENT

PM Mass and Size Fractions

In the 12-day study, daily weekday and full-weekend PM samples were collected at each site in both summer

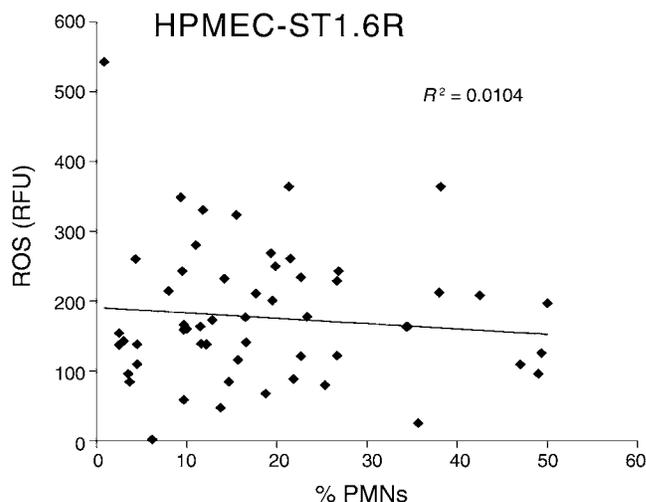


Figure 14. Correlation between in vitro ROS production in HPMEC-ST1.6R cells and in vivo %PMNs in the lavage fluid of mice treated with PM by aspiration ($n = 60$ PM samples).

and winter using a high-volume cascade impactor. As expected, differences in PM mass were observed based on site and season (Table 8). The LA Basin winter samples had the highest mean daily PM mass compared with those of the other sites; the lowest winter PM mass was found at Seattle. For all sites except the LA Basin, the mean total PM mass collected (i.e., coarse, fine, and ultrafine combined) was higher in summer than in winter. Also, as expected, variations in the ratio of the mass of the various size fractions to total PM were found across the sites and seasons. In the LA Basin, for example, the coarse PM mass fraction constituted 58% of the total mass in summer and decreased to 48% in winter, and the ultrafine fraction was 9% in summer compared with 16% in winter. A pattern of decreased coarse and increased ultrafine fractions in winter was observed at Manhattan, Tuxedo, and the LA Basin and could likely be attributed to increased combustion processes used for heating in winter or differences in atmospheric stability.

Metals Content of PM Samples

Concentrations (ng/mL) of trace elements in the extracted PM samples averaged by season and size are shown in Table 9; concentrations averaged by site and season are shown in Table 10. Site, season, and size all had significant influences on the metal content of the samples, and these

Table 8. Mean Daily Total Mass and Size-Fraction Percentage for PM Collected in the 12-Day Study^a

	Mean Total Mass (mg)		Percent of Total Mass					
			Coarse (%)		Fine (%)		Ultrafine (%)	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Manhattan	33.1	31.8	42	35	42	43	16	22
Tuxedo	26.8	19.0	37	29	52	47	11	24
Ann Arbor	28.6	24.6	34	35	44	46	22	19
Seattle	21.1	17.6	41	38	38	39	22	23
LA Basin	25.1	43.1	58	48	33	36	9	16

^a Percent mass is reported as the mass (mg) of a given size fraction divided by the total mass collected. Data are expressed as the average over 10–12 samples for each season–size combination.

differences were, as expected, trace-element dependent. For some elements the total concentrations were not different between seasons (e.g., S, K, P, Pb, and Cd showed < 6% differences between seasons), whereas other elements showed large differences between seasons (e.g., Ti and La concentrations were 41% and 70% lower, respectively, in winter compared with summer). Differences in seasonal concentrations for all other elements ranged from 11% to 45%; the majority of these elements had higher concentrations in the summer.

Clear differences in the abundance of metals were seen among the three size fractions. Compared with the coarse fraction, the fine and ultrafine fractions had more similar concentrations of many elements. The coarse fraction had concentrations of Fe, Mg, and Ti, for example, that were 4- to 30-fold higher than those of the fine or ultrafine fractions regardless of season; the differences between the fine and ultrafine fractions for these elements ranged only from 1.5- to 3-fold. Additionally, the coarse fraction showed less variation in trace-element concentrations between seasons. Although mean concentrations did vary between seasons, the elements with the highest mean concentrations in the coarse fraction in summer also had the highest mean concentrations in this fraction in winter (e.g., Ca, Fe, Mg, Cu, Sn, Mn, Ti, Sr, Sb, and La). The highest mean concentrations of elements related to fossil fuel combustion, such as Zn, V, and Ni, were observed, as expected, in the fine and ultrafine fractions in both seasons. These data suggest that, compared with the coarse fraction, there was more seasonal variability in the trace elements, and therefore in the source categories, of the fine and ultrafine fractions of ambient PM. It must be noted, however, that the similarity in the fine and ultrafine fractions might also have been caused by a less effective size cut-off for the final-stage impactor that collected the ultrafine PM, leading to a slightly larger size

cut-off for the ultrafine fraction (< 0.2 μm) that might therefore be more chemically similar to the fine fraction.

Intersite variation in the concentrations of trace elements was also found. Of the 22 elements measured, eight of the highest mean concentrations were found in samples from Manhattan (five in winter, three in summer) and the LA Basin (six in winter, two in summer); Tuxedo had only one highest concentration (in winter). Ann Arbor and Seattle had two and three highest concentrations, respectively, all in summer. Of particular note, the highest mean concentrations of Ni were found in Manhattan in both seasons, and the winter Ni concentrations were nearly double the summer concentrations, presumably because of increased use of residual oil for heating. All of the other sites had much lower concentrations of Ni — two to nine times lower in summer and six to 12 times lower in winter — thus suggesting that there was a particular source category that was unique for Manhattan (Peltier and Lippmann 2010).

Endotoxin Content of PM Samples

Endotoxin is a cell-membrane component of Gram-negative bacteria and might contribute to the inflammatory potential of ambient PM. Using a chromogenic LAL assay, endotoxin concentrations were measured in the subset of samples (60 samples and 6 field blanks) studied in the *in vitro* gene expression and *in vivo* bioassays. Overall, the majority of endotoxin concentrations were low, ranging from 0.0098 EU/mL to 16.81 EU/mL (data not shown). The highest concentration was measured in a summer coarse sample from Ann Arbor; the next highest (6.37 EU/mL) was measured in a summer coarse sample from Manhattan. As expected, given the size of the bacteria and cell-wall fragments, the coarse fraction had the highest mean EU/mL (\pm SD) (2.11 ± 0.86) compared with the fine (0.22 ± 0.08) and ultrafine (0.12 ± 0.05) fractions.

Cellular and Mouse Exposures to PM from Five Airsheds

Table 9. Elemental Composition of PM Samples Averaged by Season and Particle Size^a

	Winter				Summer			
	Coarse	Fine	Ultrafine	Total	Coarse	Fine	Ultrafine	Total
S	467 (143, 1813)	1505 (318, 3860)	1447 (351, 3406)	1129	455 (111, 1766)	1533 (407, 2806)	1525 (380, 3298)	1167
Ca	734 (328, 1790)	166 (30, 1167)	342 (86, 1033)	416	581 (124, 1272)	91 (10, 327)	421 (34, 1138)	366
K	142 (90, 369)	142 (46, 576)	173 (65, 353)	152	167 (78, 429)	130 (25, 1101)	189 (74, 805)	162
Fe	359 (48, 949)	88 (9, 801)	38 (8, 152)	166	293 (58, 704)	46 (7, 512)	23 (4, 103)	122
Mg	211 (57, 5090)	47 (6, 3450)	28 (8, 94)	98	200 (48, 365)	32 (3, 157)	27 (7, 114)	87
Zn	20 (-4, 108)	34 (1, 127)	71 (21, 197)	40	17 (0, 61)	42 (-8, 1001)	83 (4, 531)	47.0
P	22 (7, 840)	14 (3, 50)	22 (6, 94)	19	33 (6, 129)	11 (2, 97)	16 (5, 43)	20
Cu	12 (1, 49)	6 (1, 48)	4 (2, 10)	7	11 (1, 31)	4 (1, 32)	5 (1, 28)	6.0
Sn	5.0 (2.1, 9.6)	4.3 (1.2, 34.4)	1.2 (0.3, 5.2)	3.6	5.4 (2.6, 12)	6.3 (1.6, 26.9)	2.7 (0.1, 42.2)	4.8
Mn	8 (3, 15)	6 (1, 55)	3 (1, 6)	6	7 (2, 14)	3 (0, 17)	2 (0, 15)	4
Ti	12.5 (0.9, 51.0)	2.9 (0.2, 36.9)	0.8 (-0.1, 11.1)	5.6	8.7 (2.0, 24.2)	0.9 (0.3, 3.1)	0.3 (-0.1, 1.7)	3.3
V	1.5 (0.1, 8.6)	2.9 (0.1, 10.6)	3.7 (0.1, 13.5)	2.7	1.0 (0.1, 8.7)	3.7 (0.1, 20.4)	5.1 (0.0, 25.0)	3.3
Pb	3 (0.0, 53)	5 (0.0, 88)	2 (0.0, 6)	3	2 (0, 17)	4 (0, 15)	4 (1, 19)	3
Ni	2.5 (0.3, 14.3)	3.2 (0.0, 16.7)	5.8 (0.5, 26.2)	3.7	1.2 (0.02, 5.3)	3.4 (0.1, 71.9)	4.2 (0.0, 16.2)	2.9
Sr	4.4 (1.5, 10.8)	1.8 (0.3, 31.1)	1.1 (0.2, 3.5)	2.5	3.3 (0.6, 8.1)	1.1 (0.2, 18.2)	1.1 (0.2, 11.6)	1.9
Cr	1.2 (0.2, 2.3)	0.9 (0.0, 5.8)	1.3 (0.2, 10.3)	1.1	0.9 (0.1, 2.7)	1.9 (-0.1, 82.5)	1.3 (-0.1, 13.0)	1.4
Sb	2.0 (0.1, 8.0)	1.5 (0.2, 6.9)	1.5 (0.1, 6.8)	1.7	1.6 (0.3, 6.0)	0.9 (0.2, 3.4)	1.2 (0.2, 4.6)	1.2
As	0.2 (0.00, 0.6)	0.8 (0.2, 2.8)	1.1 (0.2, 4.0)	0.7	0.1 (0.0, 0.3)	0.9 (0.1, 4.1)	1.7 (0.1, 17.1)	0.9
Se	0.0 (-0.5, 1.0)	1.6 (-0.1, 40.3)	0.6 (0.04, 3.2)	0.7	0.1 (0.4, 1.0)	0.8 (-0.4, 5.3)	0.6 (-0.3, 1.9)	0.5
La	1.51 (0.3, 12.62)	0.29 (0.00, 2.53)	0.11 (0.00, .075)	0.7	0.5 (0.03, 2.26)	0.1 (0.00, 0.52)	0.1 (0.00, 0.60)	0.2
Co	0.33 (0.02, 1.57)	0.24 (-0.01, 1.54)	0.40 (-0.01, 2.32)	0.32	0.21 (0.02, 1.84)	0.26 (-0.01, 3.24)	0.20 (-0.01, 1.11)	0.22
Cd	0.06 (-0.01, 0.26)	0.27 (0.06, 1.36)	0.29 (0.07, 1.37)	0.20	0.05 (-0.1, 0.51)	0.17 (0.02, 1.18)	0.37 (0.04, 7.02)	0.20

^a Values are given as the mean ng/mL in the extracted PM samples (with minimum and maximum values underneath, in parentheses). The elements are ranked according to their average total summer abundance.

Table 10. Elemental Composition of PM Samples Averaged by Sampling Site and Season^a

	Manhattan			Tuxedo			Ann Arbor			Seattle			LA Basin			
	Summer	Winter		Summer	Winter		Summer	Winter		Summer	Winter		Summer	Winter		
S	1443 (254, 3298)	1413 (168, 2689)	1325 (3, 3265)	1785* (215, 6860)	1113 (157, 2731)	1253 (265, 2532)	691 (210, 2551)	530 (238, 1735)	1248 (393, 2544)	468 (143, 1358)						
Ca	512* (52, 1094)	468 (102, 1790)	362 (3, 1138)	307 (30, 1165)	469 (25, 1272)	517 (45, 1267)	299 (43, 945)	362 (33, 755)	186 (16, 430)	500 (38, 1225)						
K	110 (37, 201)	121 (64, 183)	153 (111, 429)	159 (86, 369)	141 (40, 378)	103 (46, 175)	204* (122, 582)	189 (92, 576)	202 (50, 1101)	182 (59, 437)						
Fe	213 (18, 704)	162 (25, 6050)	66 (25, 3130)	74 (8, 437)	101 (7, 339)	138 (10, 425)	137 (6, 6210)	151 (8, 5480)	91 (4, 5120)	343* (22, 949)						
Mg	74 (6, 297)	92 (12, 454)	42 (10, 228)	76 (6, 509)	90 (5, 302)	113 (9, 309)	96 (7, 3090)	123 (8, 444)	133* (16, 3650)	110 (9, 333)						
Zn	55 (15, 232)	61 (2, 197)	26 (0, 111)	38 (5, 159)	77* (1, 531)	18 (1, 72)	26 (1, 1390)	30 (-4, 135)	51 (-8, 1001)	43 (7, 127)						
P	23 (7, 48)	41* (19, 94)	30 (0, 129)	12 (3, 26)	19 (3, 101)	9 (3, 21)	20 (5, 55)	14 (5, 32)	9 (2, 17)	18 (6, 50)						
Cu	9 (3, 25)	7 (3, 19)	4 (0, 9)	4 (1, 26)	3 (1, 6)	3 (1, 6)	10 (2, 31)	8 (2, 20)	7 (1, 32)	16* (2, 49)						
Sn	3.4 (0.9, 7.0)	3.2 (0.9, 6.9)	5.6 (0.0, 17.2)	3.0 (0.5, 9.6)	3.7 (0.3, 9.0)	3.9 (1.9, 8.2)	7.3* (0.1, 42.2)	5.0 (0.5, 34.4)	4.2 (0.5, 26.9)	3.9 (0.3, 11.2)						
Mn	6 (1, 11)	6 (2, 12)	2 (0, 10)	3 (1, 8)	4 (1, 13)	6 (1, 14)	5 (1, 17)	7 (1, 43)	2 (0, 10)	9* (1, 55)						
Ti	5 (0, 16)	4 (0, 14)	2 (4, 10)	2 (0, 14)	2 (0, 7.6)	4 (0.2, 9.4)	4 (0, 24)	5 (0, 23)	4 (0, 17.5)	15* (0.4, 51.0)						
V	5.9 (1.0, 25.0)	6.6* (1.8, 13.5)	1.3 (0.0, 6.1)	1.7 (0.1, 10.4)	0.6 (0.0, 8.7)	0.7 (0.1, 2.1)	3.2 (0.1, 24.9)	1.7 (0.1, 10.6)	5.1 (0.5, 14.0)	2.7 (0.5, 9.5)						
Pb	5* (2, 17)	5* (1, 88)	2 (0, 19)	4 (0, 53)	2 (0, 6)	2 (0, 7)	3 (0, 17)	2 (0, 19)	4 (0, 12)	3 (0, 5)						
Ni	6.3 (1.9, 16.2)	11.6* (3.9, 26.2)	1.4 (0.8, 5.7)	1.8 (0.0, 7.9)	0.7 (-0.1, 5.4)	1.0 (0.1, 3.0)	2.2 (0.6, 12.5)	1.3 (0.1, 6.6)	3.9 (0.3, 71.9)	2.0 (1.0, 5.0)						
Sr	2.1 (0.3, 5.9)	2.5 (0.7, 5.6)	0.9 (-3.4, 2.3)	1.3 (0.2, 4.9)	1.7 (0.2, 7.2)	3.0 (0.3, 8.4)	1.7 (0.2, 4.8)	2.9 (0.3, 31.1)	2.9 (0.4, 18.2)	3.4* (0.4, 10.8)						
Cr	1.1 (0.1, 3.5)	0.8 (0.2, 2.6)	0.6 (0.0, 2.6)	0.7 (0.0, 2.9)	0.6 (-0.1, 2.4)	1.1 (0.2, 2.2)	1.9 (0.2, 13.0)	1.2 (0.1, 5.1)	2.8* (-0.1, 82.5)	1.5 (0.4, 5.8)						
Sb	1.8 (0.8, 3.3)	2.1 (0.9, 4.5)	0.7 (0.2, 1.7)	0.6 (0.1, 2.6)	0.6 (0.2, 1.4)	0.5 (0.2, 0.9)	1.9 (0.5, 6.0)	1.8 (0.4, 4.6)	1.2 (0.3, 4.6)	3.5* (0.7, 8.0)						
As	0.6 (0.1, 1.7)	0.5 (0.1, 1.0)	0.5 (0.0, 2.6)	0.5 (0.1, 1.5)	0.9 (0.1, 3.3)	0.6 (0.2, 1.6)	2.3* (0.0, 17.1)	1.2 (0.0, 4.0)	0.2 (0.0, 0.6)	0.5 (0.1, 1.9)						
La	0.69 (0.03, 2.3)	2.40* (0.1, 12.6)	0.08 (0.2, 0.4)	0.20 (0.0, 2.3)	0.08 (0.0, 0.8)	0.13 (0.0, 0.5)	0.11 (0.0, 0.5)	0.08 (0.0, 0.6)	0.20 (0.0, 0.8)	0.42 (0.01, 2.5)						
Co	0.65 (0.2, 3.2)	1.13* (0.4, 2.3)	0.09 (0.0, 1.1)	0.10 (0.0, 0.5)	0.04 (-0.1, 0.2)	0.07 (-0.1, 0.2)	0.05 (-0.1, 0.2)	0.06 (-0.1, 0.4)	0.26 (0.02, 2.0)	0.22 (0.0, 1.6)						
Cd	0.15 (0.0, 0.5)	0.13 (0.0, 0.3)	0.09 (0.0, 0.4)	0.21 (0.0, 0.6)	0.31* (-0.1, 7.0)	0.17 (0.0, 1.0)	0.30 (0.1, 1.3)	0.29 (-0.1, 1.4)	0.13 (0.0, 0.4)	0.19 (0.0, 0.5)						

^a Values are given as the mean ng/mL in the extracted PM samples (with minimum and maximum values underneath, in parentheses). The elements are ranked according to their average total summer abundance. * indicates the largest value for each element.

EFFECTS OF PM COMPOSITION AND SOURCE CATEGORIES ON BIOLOGIC MARKERS

Effects of PM Composition on in Vitro ROS Production

As suggested by the significant modifying effects seen for sampling site, season, and particle size on PM-induced ROS production, analyses were performed to examine the role in ROS production of individual PM components, including trace elements and particle-associated endotoxin. Data from all size fractions were combined, and Pearson correlation coefficients (*r*) between specific elements and ROS production induced by all locations together (for both cell lines) and by samples among sites (for the HPMEC-ST1.6R cells) were determined. Aggregating results from all test samples together, significant correlations ($P \leq 0.05$) were observed for 18 and 12 out of 25 elements

for the HPMEC-ST1.6R and BEAS-2B cells, respectively (Table 11). Cu, Sb, V, Co, Be, and Ni had correlations with $P \leq 0.001$ in both cell lines.

Stronger correlations were generally observed in the HPMEC-ST1.6R cells. This was in line with a study by Kasper and colleagues (2011) that demonstrated greater sensitivity of endothelial cells compared with epithelial cells following treatment with amorphous silica nanoparticles. The mechanisms behind cell sensitivity to PM are unclear. Correlations for sites and particle sizes are presented for the ROS production of the more sensitive HPMEC-ST1.6R cells. Many of the correlations were size-dependent. ROS production was found to be significantly correlated with, for example, Cu concentrations in the coarse and fine fractions, Sb concentrations in the coarse fraction, and V concentrations in the ultrafine fraction (Figure 15). The

Table 11. Correlations Between Trace Elements and ROS Production^a

	Total ROS Production		ROS Production by Site				
	HPMEC-ST1.6R	BEAS-2B	Manhattan	Tuxedo	Ann Arbor	Seattle	LA Basin
Cu	0.47***	0.28***	0.28*	0.16	0.34**	0.60***	0.47***
Sb	0.36***	0.21***	0.30*	0.07	0.18	0.53***	0.06
K	0.32***	0.15**	0.33**	0.02	0.38**	0.08	0.54***
Sr	0.31***	0.07	0.01	0.10	0.16	0.12	0.64***
V	0.30***	0.33***	0.26*	0.23	0.04	0.09	0.01
Fe	0.30***	0.12*	0.03	0.06	0.11	0.55***	0.27*
Co	0.30***	0.19***	0.19	0.25*	0.08	0.53***	0.10
Be	0.29***	0.18***	0.25*	0.18	0.10	0.49***	0.18
Ti	0.29***	0.12*	0.02	0.05	0.09	0.54***	0.28*
Ca	0.26***	0.15*	0.21	0.17	0.25*	0.45***	0.14
Sc	0.24***	0.11	0.06	0.06	0.07	0.50***	0.27*
Mg	0.23***	0.06	0.02	0.11	0.14	0.30*	0.21
Ni	0.22***	0.18***	0.26*	0.36**	0.08	0.05	0.06
P	0.21***	0.13*	0.15	0.17	0.20	0.41***	0.05
Mn	0.17**	-0.04	0.04	0.05	0.07	0.17	0.06
Zn	0.16**	0.13*	0.23	0.22	0.42***	0.10	0.05
Sn	0.11*	-0.01	0.31**	0.12	0.24*	0.25*	0.21
La	0.11*	0.08	0.11	0.01	0.19	0.26*	0.13
S	0.09	0.11	0.03	0.02	0.12	0.21	0.04
Pb	0.09	0.06	0.01	0.04	0.00	0.03	0.07
Cr	0.03	0.02	0.24*	0.13	0.03	0.23	0.05
As	0.00	0.04	0.09	0.25*	0.12	0.19	0.23
Cd	-0.03	0.03	0.12	0.13	0.04	0.29*	0.00
Se	-0.04	-0.07	0.19	0.10	0.08	0.14	0.11
Tl	-0.06	-0.04	0.23	0.02	0.13	0.03	0.15

^a Pearson correlation coefficients (*r*) are given for total ROS production in the two cell lines and for ROS production by site in HPMEC-ST1.6R cells. Significance is indicated by * for $P \leq 0.05$, ** for $P \leq 0.01$, and *** for $P \leq 0.001$.

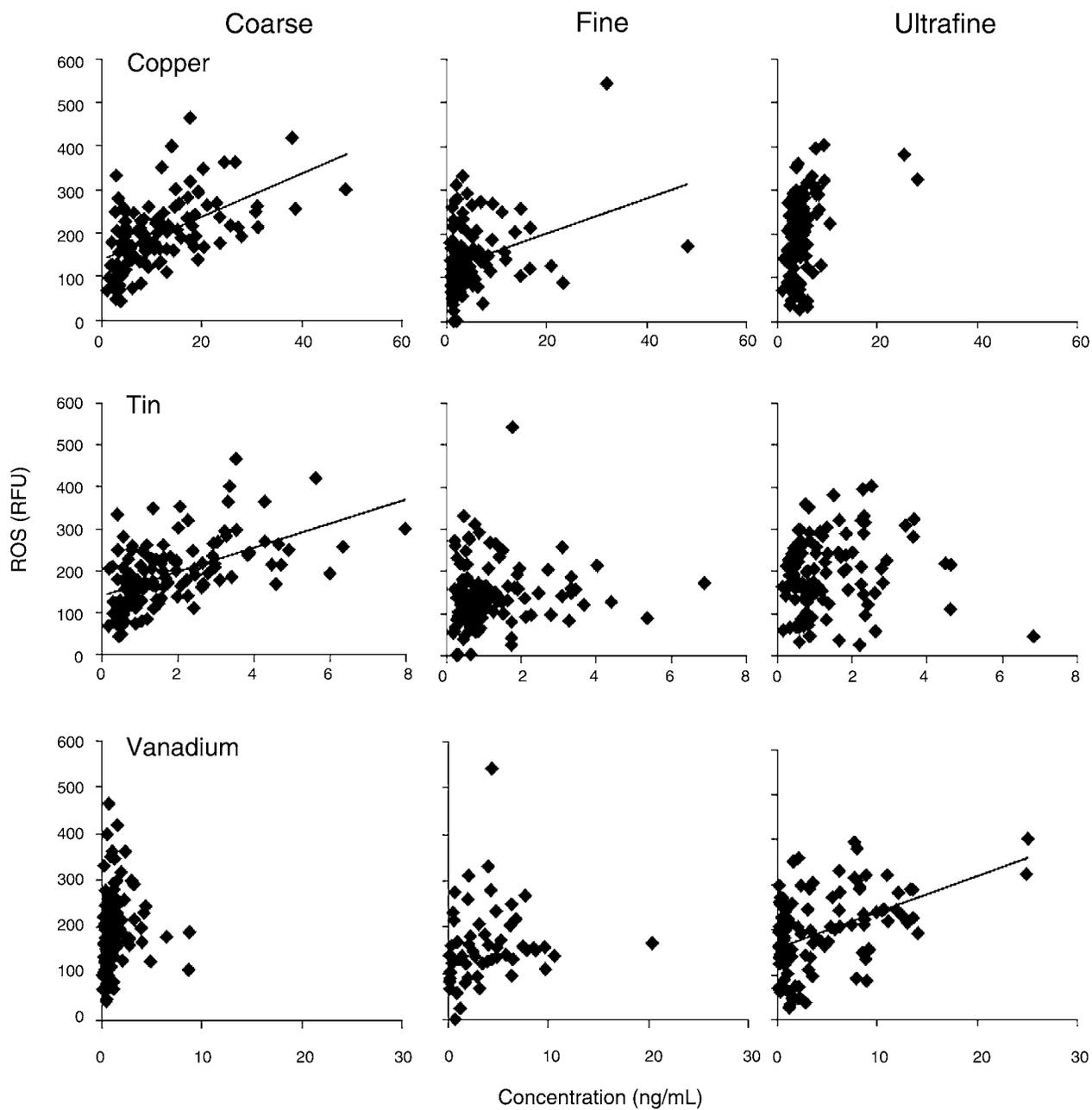


Figure 15. Correlations, by PM size fraction, between ROS production in HPMEC-ST1.6R cells and Cu (top), Sb (middle), and V (bottom) concentrations in PM. Diagonal lines indicate a significant correlation as assessed by linear regression ($P < 0.05$). Note that the x-axis scales differ from panel to panel.

statistical sensitivity of these relationships was examined by removing data points that appeared to be outliers, and the relationships were found to be unaffected (except for that of Cu in the fine fraction: when the data point with the highest ROS response was removed from analysis, the significance of the relationship was lost).

Of the 18 elements that had significant correlations when considering samples from all sites together in the HPMEC-ST1.6R cells (Table 11, first column), six elements had significant correlations with ROS production at only a single site (e.g., V at Manhattan; Zn at Ann Arbor; P, Mg, and La at Seattle; and Sr at the LA Basin). Some of these correlations at individual sites (e.g., V in ultrafine PM at Manhattan) appear to have been driven by a specific size fraction; for other elements there were no clear distinctions among the results for the various size fractions in the correlations (e.g., Sr in the LA Basin samples; Figure 16). It is interesting to note that, although Seattle PM did not induce the highest levels of ROS production, the Seattle samples did show high correlations for ROS with a number of elements (e.g., Cu, Sb, Fe, Co, Be, Ti, Ca, and Sc).

Cu had the highest correlations with ROS production in both cell lines. As shown in Figure 16, the coarse fraction from multiple sites appeared to drive this correlation. Interestingly, the highest ROS response was produced by an LA Basin fine sample collected on July 4, 2008 (this sample also had the fifth highest Cu concentration). This identifies a potential effect for a unique source category: fireworks displays. When considering the highest correlations for all sites, two elements (Cu and K) were significantly correlated with ROS response for the same three sites (Manhattan, Ann Arbor, and the LA Basin; Table 11), suggesting that similar source categories of these elements (e.g., Traffic) might be present at all three locations. Ni was also found to be significantly correlated but only for samples from Manhattan and Tuxedo, suggesting that a similar regional source category (e.g., Residual Oil Combustion) might be affecting these locations.

Association of Source Categories with ROS Production

Although analysis of individual elements provides some insight into the possible PM source categories related to ROS production, it does not fully explain the biologic mechanism involved, because metals that are not associated with Fenton-like (i.e., oxidating) reactions were also significantly associated with ROS production. This might be attributable to the fact that some of the metals were themselves correlated with each other, making it difficult to identify a single element, or even a few, responsible for the variations in ROS production. Fe, for example, was highly correlated with Ti ($r = 0.92$) and Cu ($r = 0.77$), and Cu was highly correlated with Ti ($r = 0.75$) and Sb ($r = 0.78$) (data not shown). These

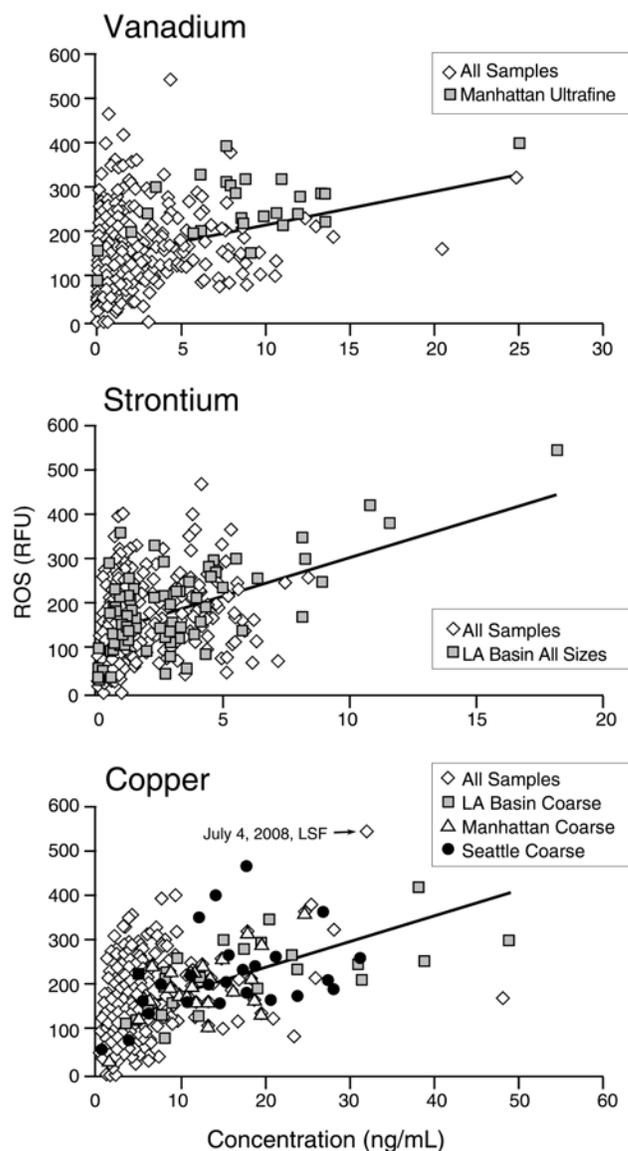


Figure 16. Correlations, by element, between ROS production in HPMEC-ST1.6R cells and V (top), Sr (middle), and Cu (bottom) in PM from all sites together. An outlying LA Basin summer fine (LSF) sample of interest, collected on July 4, 2008, is shown. Note that the x-axis scales differ from panel to panel.

elements were highly correlated with each other, which is suggestive of a Traffic/Brake Wear source category (i.e., tailpipe emissions, resuspended particles from brake and tire wear, and other road dust). To gain a better understanding of the variations in ROS response, therefore, a factor analysis was conducted.

When using all metals in the analysis, five factors were identified, although clear source categories could not be interpreted for all five. A subset of 15 relevant metals was

therefore selected for analysis. When considering all size fractions together, five factors were again identified (of which four pointed toward clear source categories; Figure 17).

- Traffic/Brake Wear (factor 1: Cu/Fe/Ti/Sb),
- Cr/Ni/Zn (factor 2),
- Residual Oil Combustion (factor 3: V/Ni/S),
- Coal Combustion (factor 4: K/As/Se/S), and
- Soil Dust (factor 5: Ca/Ti/Mn/Fe)

When an analysis was conducted separately by size fraction (data not shown), five factors were again identified for each fraction, and one new source category (Fireworks) was identified in the fine fraction. In the coarse fraction:

- Traffic/Brake Wear (factor 1),
- Ca/Mn/As/Zn (factor 2),
- Residual Oil Combustion (factor 3),
- Coal Combustion (factor 4), and
- Ti/Fe/Mn (factor 5).

In the fine fraction:

- Traffic/Brake Wear (factor 1),
- Cr/Ni/Zn (factor 2),
- Coal Combustion (factor 3),
- Fireworks (factor 4), and
- Residual Oil Combustion (factor 5).

In the ultrafine fraction:

- Residual Oil Combustion/Traffic (factor 1),
- As/Cr/K (factor 2),
- Soil Dust (factor 3),
- S/Se/Pb (factor 4), and
- Coal Combustion (factor 5) (data not shown).

Factor scores computed for these source categories were then correlated with intracellular ROS generation levels in the HPMEC-ST1.6R cells. As shown in Figure 18 (in which the July 3–6 data point was removed because of potential fireworks influence), significant correlations were identified for the Traffic/Brake Wear source category (factor 1;

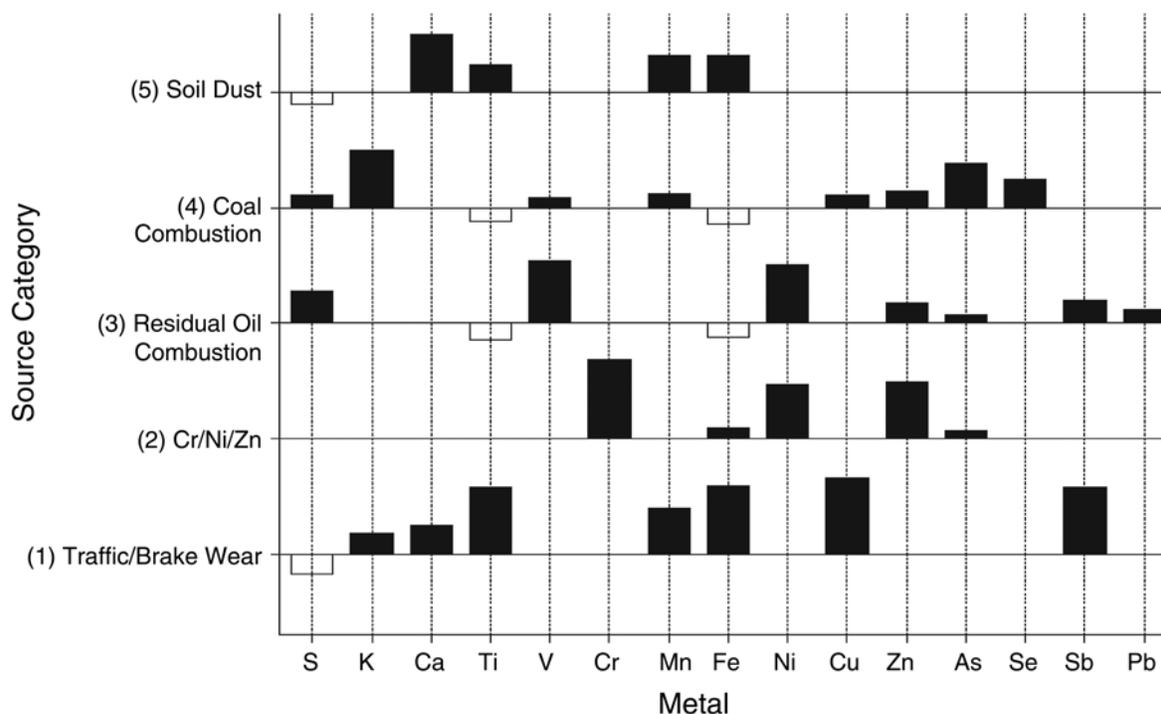


Figure 17. Factor analysis identifying source categories for a subset of metals from all PM size fractions. The black and white bars (factor loadings) are positive and negative correlations, respectively, between the metals and factors. Correlations less than 0.1 are not shown. Factor numbers indicate the rank order of the sum of squared correlations for the factor (eigenvalue). Factor 1 had the largest sum of squared correlations.

$r = 0.39$, $P < 0.0001$, both seasons combined) and the Residual Oil Combustion source category (factor 3; $r = 0.30$, $P < 0.0001$, both seasons combined). Cu and K were found to be significantly correlated with the PM samples from Manhattan, Ann Arbor, and the LA Basin, suggesting that a

Traffic-related source category was present at all three sites; these three appeared to drive the correlation between intracellular ROS and the Traffic/Brake Wear source category (factor 1). Additionally, V was found to be significantly correlated with Manhattan PM. Not surprisingly, the ultrafine

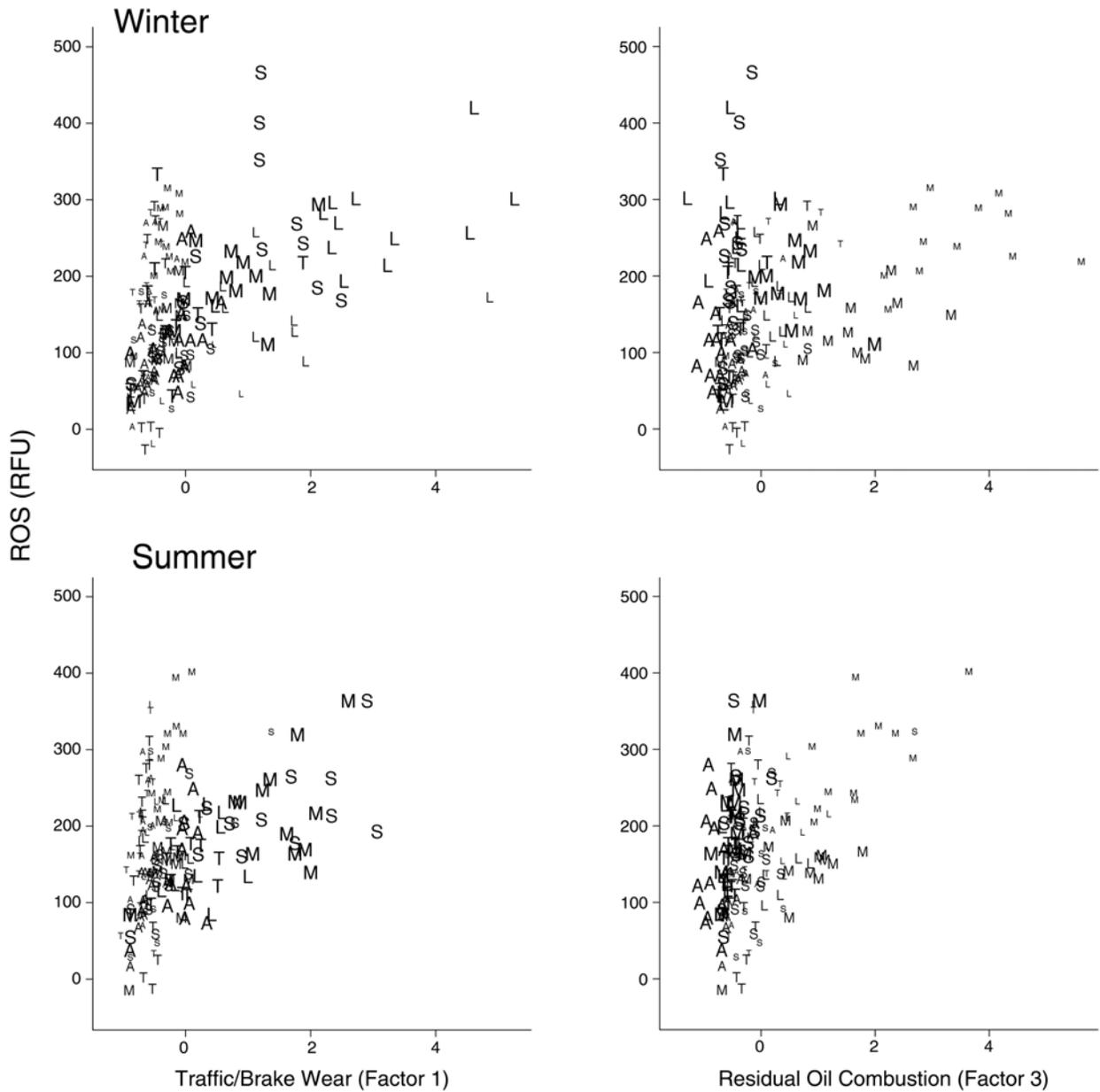


Figure 18. Significant correlations, by source category and sampling season, between ROS production in HPMEC-ST1.6R cells and factor scores for Traffic/Brake Wear (factor 1; left) and Residual Oil Combustion (factor 3; right) in winter (top) and summer (bottom) PM. Letter sizes (large, medium, and small) reflect particle sizes (coarse, fine, and ultrafine). Locations of individual samples are indicated by the following: M = Manhattan, T = Tuxedo, A = Ann Arbor, S = Seattle, and L = LA Basin.

Manhattan fraction appeared to be driving the correlation between intracellular ROS and the Residual Oil Combustion source category (factor 3), suggesting a source category of Residual Oil Combustion that might be specific to Manhattan.

ROS and Endotoxin Content

As shown in Figure 19, the Pearson correlation coefficients for ROS production and endotoxin concentrations were very low for both the BEAS-2B cells ($r = 0.017$; $P = 0.3$) and the HPMEC-ST1.6R cells ($r = 0.11$; $P = 0.4$), suggesting that the endotoxin concentrations of the PM samples did not influence ROS production in either cell type.

Effect of PM Composition on mRNA Expression of ROS and Markers of Inflammation

Correlations were found between mRNA expression and the concentrations of individual trace elements. For each cell

type, data for the size fractions were combined and Pearson correlation coefficients determined. Metals with a significant correlation with one or more changes in mRNA expressions of ROS and inflammation markers at 6 or 24 hours are shown in Table 12 for HPMEC-ST1.6R cells and Table 13 for BEAS-2B cells (the markers shown are only those whose mRNA expression was found to have significantly increased or decreased after PM treatment [see Figure 8]). HO-1 was significantly induced in the HPMEC-ST1.6R cells at both 6 and 24 hours (Figure 8), and HO-1 mRNA expression were significantly correlated with Cu, Sb, Fe, Ti, Mn, and Cr (Table 12). The strongest correlation was found for Sb at 24 hours ($r = 0.73$). Significant positive correlations with HO-1 levels were also identified for Co, Be, Sc, Ni, Pb, and endotoxin at 24 hours.

HO-1 was also induced in the BEAS-2B cells; however, this induction reached significance only at 24 hours (Figure 8) when it was found to be significantly correlated with Cu, Sb, Sr, Fe, Ti, Mn, Sn, and Cr (Table 13). These metals, with the exception of Sr and Sn, were also correlated with HO-1 in the HPMEC-ST1.6R cells. The only other significant effects on mRNA in BEAS-2B cells were on IL-8 levels at 24 hours (Figure 8), which were found to be significantly correlated with Cu, Sb, Sr, Fe, Ti, Mn, and Cr. These metals were largely the same ones as those that were significantly associated with HO-1, suggesting that similar mechanisms might have led to the induction of HO-1 and IL-8 in BEAS-2B cells.

Correlation of Trace Elements with Cardiomyocyte Beat Frequency

As shown in Table 14, examination of the metal composition of the six Manhattan PM samples tested for their effects on cardiac beat frequency revealed that the winter ultrafine sample contained the highest concentration of Zn (146 ng/mL) compared with the other samples. Zn concentrations in the other samples ranged from 39 to 79 ng/mL. Zn was the only element that was much higher in concentration in this sample compared with the other Manhattan samples, suggesting that Zn might have been involved in the observed effects on cardiac beat frequency.

Correlation of PM Constituents with in Vivo Results

As suggested by the lack of correlation between the in vitro ROS response and the in vivo inflammatory response, there was only partial overlap in the composition correlations between the ROS increases and the % PMN responses (Table 15). Phosphorus and endotoxin had the highest correlations for the % PMN responses. Surprisingly, S and V had significant inverse correlations with % PMN responses. It is not clear what components of PM

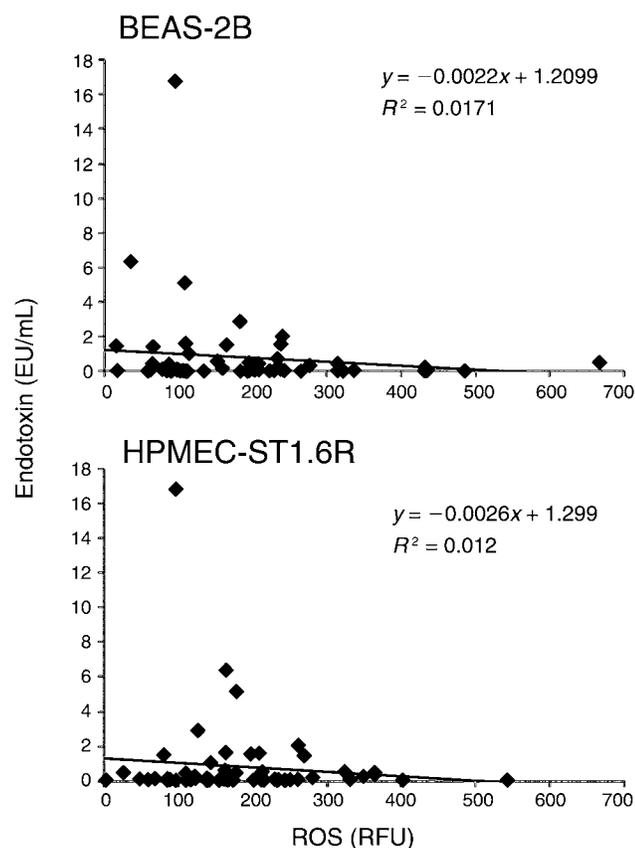


Figure 19. Correlations between ROS production and endotoxin concentrations in BEAS-2B cells (top) and HPMEC-ST1.6R cells (bottom).

Table 12. Correlations Between Trace Metals or Endotoxin and mRNA Expression in HPMEC-ST1.6R Cells at Two Time Points^a

	HO-1			ICAM-1			IL-8			TXNRD1			VEGF-A							
	6 Hours	24 Hours																		
	Cu	0.52***	0.48**	0.23	-0.01	0.01	0.17	0.24	-0.08	0.15	-0.14	0.52***	0.73***	0.00	0.27	0.33*	0.11	0.24	-0.08	0.01
Sb	0.55***	0.73***	0.00	0.27	0.33*	0.11	0.13	0.25	0.01	0.23	0.36*	0.39*	-0.13	0.48**	0.48**	0.39*	0.34*	-0.12	0.37*	-0.14
K	0.04	-0.12	0.39*	-0.14	-0.11	0.36*	0.39*	-0.13	-0.11	0.48**	0.36*	0.39*	-0.13	0.48**	0.48**	0.39*	0.34*	-0.12	0.37*	-0.14
Sr	0.14	0.08	0.41**	-0.07	-0.05	0.31	0.34*	-0.12	-0.05	0.37*	0.31	0.34*	-0.12	0.37*	0.37*	0.31	0.34*	-0.12	0.37*	-0.15
Fe	0.51***	0.53***	0.06	-0.01	0.00	0.04	0.06	-0.09	0.00	-0.14	0.04	0.06	-0.09	-0.14	-0.14	0.04	0.06	-0.09	-0.14	-0.15
Co	-0.05	0.56***	0.08	0.70***	0.76***	0.01	-0.07	0.70***	0.76***	-0.02	0.01	-0.07	0.70***	-0.02	0.71***	0.01	-0.07	0.70***	-0.02	0.71***
Ti	0.52***	0.54***	0.03	-0.01	0.00	0.02	0.06	-0.08	0.00	-0.13	0.02	0.06	-0.08	-0.13	-0.15	0.02	0.06	-0.08	-0.13	-0.15
Be	-0.06	0.55***	0.06	0.72***	0.76***	-0.01	-0.08	0.72***	0.76***	-0.02	-0.01	-0.08	0.72***	-0.02	0.74***	-0.01	-0.08	0.72***	-0.02	0.74***
Sc	0.20	0.33*	0.09	0.09	0.12	0.12	0.01	0.04	0.12	-0.01	0.12	0.01	0.04	-0.13	-0.01	0.12	0.01	0.04	-0.13	-0.01
Ni	-0.09	0.45**	0.05	0.63*	0.70***	0.03	-0.06	0.63***	0.70***	0.01	0.03	-0.06	0.63***	0.01	0.66***	0.03	-0.06	0.63***	0.01	0.66***
Mn	0.48**	0.55***	0.41**	0.13	0.14	0.23	0.21	0.04	0.14	0.00	0.23	0.21	0.04	0.05	0.00	0.23	0.21	0.04	0.05	0.00
Sn	0.18	0.14	0.37*	-0.05	-0.09	0.08	0.15	-0.15	-0.09	0.08	0.08	0.15	-0.15	0.03	-0.22	0.08	0.15	-0.15	0.03	-0.22
S	-0.38*	-0.17	-0.19	0.25	0.22	-0.22	-0.25	0.28	0.22	-0.22	-0.22	-0.25	0.28	-0.08	0.32	-0.22	-0.25	0.28	-0.08	0.32
Pb	0.03	0.39*	0.21	0.72*	0.57***	0.04	0.10	0.66***	0.57***	0.04	0.04	0.10	0.66***	0.16	0.52***	0.04	0.10	0.66***	0.16	0.52***
Cr	0.44**	0.36*	0.38*	-0.05	-0.02	0.31	0.30	-0.12	-0.02	0.31	0.31	0.30	-0.12	0.21	-0.17	0.31	0.30	-0.12	0.21	-0.17
Tl	0.31	0.03	0.44**	0.00	0.05	0.65***	0.57***	0.03	0.05	0.65***	0.65***	0.57***	0.03	0.49**	0.04	0.65***	0.57***	0.03	0.49**	0.04
Endotoxin	0.33	0.43*	0.42*	0.15	0.00	0.29	0.25	-0.16	0.00	0.29	0.29	0.25	-0.16	0.08	-0.24	0.00	0.25	-0.16	0.08	-0.24

^a Pearson correlation coefficients (*r*) are given. Significance is indicated by * for $P \leq 0.05$, ** for $P \leq 0.01$, and *** for $P \leq 0.001$.

Table 13. Correlations Between Trace Metals and mRNA Expression in BEAS-2B Cells at Two Time Points^a

	CSF-2		HO-1		IL-6		IL-8		VEGF-A	
	6 Hours	24 Hours								
Cu	0.00	0.47**	0.28	0.67***	0.16	0.62***	0.22	0.50***	-0.11	0.66***
Sb	0.10	0.21	0.39*	0.53***	0.28	0.47**	0.30	0.39*	0.03	0.36*
K	-0.12	0.40**	0.00	0.30	-0.20	0.22	-0.10	0.09	-0.10	0.40**
Sr	-0.11	0.59***	-0.03	0.45**	-0.07	0.49***	-0.03	0.31*	-0.19	0.64***
Fe	-0.02	0.20	0.18	0.38*	0.16	0.61***	0.17	0.36*	-0.21	0.48**
V	0.61***	0.12	0.08	-0.03	0.49***	-0.04	0.46**	0.12	0.38*	-0.04
Ti	-0.03	0.21	0.21	0.45**	0.16	0.55***	0.16	0.38*	-0.21	0.47**
Be	0.34*	-0.06	-0.02	-0.04	0.30	0.02	0.24	0.06	0.22	-0.07
Ca	0.10	0.05	0.10	0.01	0.15	0.50***	0.25	0.19	0.00	0.30
Sc	0.06	0.20	0.00	0.11	0.14	0.54***	0.05	0.24	-0.15	0.29
Ni	0.46**	-0.01	0.03	0.00	0.40**	0.04	0.30	0.11	0.33*	-0.06
Mn	0.06	0.19	0.19	0.47**	0.23	0.62***	0.23	0.53***	-0.09	0.40**
Sn	0.04	0.14	0.53***	0.49***	0.11	0.28	0.16	0.27	0.23	0.27
S	0.31	-0.07	-0.10	-0.30	0.23	-0.41**	0.13	-0.22	0.38*	-0.27
Cr	0.12	0.06	0.73***	0.52***	0.30	0.47**	0.28	0.31*	0.41**	0.32*
As	0.27	-0.23	0.67***	0.17	0.22	-0.01	0.24	-0.07	0.57***	-0.15
Tl	0.11	0.05	0.47**	0.18	0.21	0.23	0.18	0.10	0.36*	-0.05

^a Pearson correlation coefficients (*r*) are given. Significance is indicated by * for $P \leq 0.05$, ** for $P \leq 0.01$, and *** for $P \leq 0.001$.

Table 14. Elemental Composition (ng/mL) of Manhattan PM Samples Used in Spontaneous Beat Frequency Assays

	Summer			Winter		
	Coarse	Fine	Ultrafine	Coarse	Fine	Ultrafine
Mg	175.32	12.46	27.96	298.82	17.64	25.62
P	26.43	13.22	24.80	39.06	28.83	44.97
S	328.22	2250.82	2117.51	596.06	1349.86	1979.37
K	124.22	69.93	149.06	159.82	80.89	161.91
Ca	994.52	125.96	296.88	867.81	143.20	432.84
Sc	0.07	0.01	0.02	0.11	0.02	0.01
Ti	15.59	1.19	1.21	14.33	1.16	0.52
V	2.22	20.44	25.04	3.17	5.17	8.19
Cr	1.58	0.47	0.82	2.15	0.88	0.84
Mn	9.69	3.44	4.22	10.35	3.63	4.48
Fe	501.33	50.23	70.82	608.17	62.30	37.05
Co	0.49	0.42	0.98	0.81	0.89	1.38
Ni	3.53	9.03	16.48	6.84	9.79	14.10
Cu	14.93	5.54	9.48	19.65	3.98	5.35
Zn	42.07	60.33	73.14	39.18	78.75	145.60
Sr	3.71	0.62	1.02	5.12	0.93	1.14
Cd	0.53	0.17	0.25	0.08	0.11	0.18
Sn	3.23	2.80	2.13	5.43	2.30	0.95
Sb	1.60	1.03	2.52	3.23	1.90	2.31
La	1.99	0.52	0.60	6.28	0.49	0.23
Pb	3.40	4.72	5.14	3.25	2.85	3.01

drove the in vivo response, because the endotoxin concentrations were very low (only five out of 59 samples were greater than 2 EU/mL).

IN VIVO 100-DAY STUDY

Although no in vitro bioassays were performed on the 1600 PM samples from the 100-day study (the HEI NPACT Oversight Committee requested that we concentrate on our vivo studies), the study’s in vivo results are consistent with the findings from the 12-day in vivo studies. More than 1600 mice ($n = 3$ mice per extracted PM sample) were

treated with PM collected at Irvine or vehicle (water), and PMNs and total protein were evaluated in lavage fluid 24 hours later. As seen in Figure 20, coarse PM samples from Manhattan and Irvine produced greater lung inflammation (i.e., changes in % PMNs) than the fine or ultrafine samples from these sites. At both sites, the differences were greater between the coarse and fine samples. Interestingly, the ultrafine samples from Manhattan produced nearly four times as much lung inflammation as the ultrafine samples from Irvine and nearly twice that of the Irvine coarse samples. Unlike the protein results from the 12-day study, increases in lung injury (i.e., total protein content in lavage fluid) were observed in the mice in the 100-day study. Although these protein increases were not as dramatic as the changes in % PMNs, sampling site and particle size influenced the outcome (Figure 21).

At the time this report was published, the PM samples from the 100-day study had not yet been analyzed for chemical composition, and therefore no correlations or source category data are presented. Although composition data are not yet available, given the site- and size-dependent PMN responses reported earlier, composition data could point toward significantly different source categories for the ultrafine PM from the two sites.

Table 15. Correlations Between Elemental Composition or Endotoxin and in Vitro and in Vivo Responses^a

	In Vitro ROS Response		In Vivo Response
	HPMEC-ST1.6R Cells	BEAS-2B Cells	% PMN
Cu	0.47***	0.28***	0.10
Sb	0.36**	0.21***	0.16
K	0.32***	0.15**	-0.01
Sr	0.31***	0.07	0.07
Fe	0.3***	0.12*	0.45***
Co	0.3***	0.19***	-0.09
V	0.3***	0.33***	-0.29*
Ti	0.29***	0.12*	0.43***
Be	0.29***	0.18***	0.07
Ca	0.26***	0.15**	0.28*
Sc	0.24***	0.11*	0.38**
Mg	0.23***	0.06	0.47***
Ni	0.22***	0.18***	-0.25
P	0.21***	0.13**	0.53***
Mn	0.17**	-0.04	0.41**
Zn	0.16**	0.13**	-0.19
Sn	0.11*	-0.01	0.29*
La	0.11*	0.08	0.11
S	0.09	0.11*	-0.58**
Pb	0.09	0.06	-0.21
Cr	0.03	0.02	-0.07
As	0.00	0.04	-0.23
Se	-0.04	-0.07	-0.49**
Cd	-0.03	0.03	-0.26*
Endotoxin	0.02	-0.19	0.48**

^a Values of the Pearson correlation coefficient (r) are given. Data are ranked by highest correlation in HPMEC-ST1.6R cells. Significance is indicated by * for $P \leq 0.05$, ** for $P \leq 0.01$, and *** for $P \leq 0.001$.

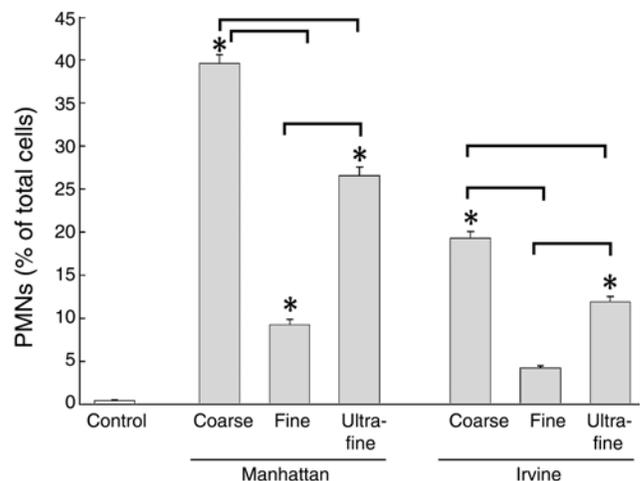


Figure 20. Comparison of % PMNs in the lavage fluid of mice treated by aspiration with size-fractionated PM from Manhattan and Irvine ($n = 180-278$ treated mice/group; $n = 28$ controls). * indicates significantly different changes ($P < 0.05$) compared with those of the control group (when using the Student t test). Brackets indicate a significant difference ($P < 0.05$) between the two groups linked by that bracket.

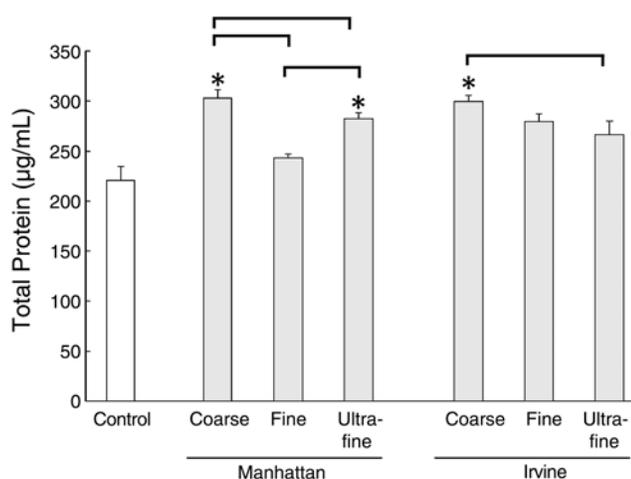


Figure 21. Comparison of increases in total protein in the lavage fluid of mice treated by aspiration with size-fractionated PM from Manhattan and Irvine ($n = 180$ – 278 treated mice/group; $n = 28$ controls). * indicates significantly different changes ($P < 0.05$) compared with those of the control group. Brackets indicate a significant difference ($P < 0.05$) between the two groups linked by that bracket.

PROBLEMS AND ISSUES

1. The sampling sites in Manhattan were different for our 12-day study (for which samples were collected at Mount Sinai) and our 100-day study (for which samples were collected at Hunter College) because the Mount Sinai building was closed for demolition between the first and second studies.
2. The sampling sites in Michigan were different for our 12-day study (University of Michigan, Ann Arbor) and the chronic CAPs study reported in Chen Study 1 (Michigan State University, East Lansing) because of Drs. Chen, Harkema, and Kleinman's decision to move the exposure trailer to the Michigan State University campus to facilitate the CAPs exposures.
3. The sampling sites in the LA area were different for our 12-day study (Anaheim), our 100-day study (Irvine), and the subchronic CAPs exposure study reported in Chen Study 1 (also at Irvine) because of Drs. Chen, Harkema, and Kleinman's decision to move the exposure trailer to the Michigan State University campus, administrative issues, and the CAPs exposures in Michigan and California needing to be done at about the same time. As a result, the ChemVol sampling and CAPs exposures were both done at the University of California–Irvine.
4. There were complications in the ChemVol sampling for the 100-day study, with the result that fewer than the desired 600 size-fractionated samples were collected at Manhattan and Irvine. At the Hunter College

site in Manhattan, improper flow rates occurred on 67 days out of 100, and therefore these samples were not included in the in vivo bioassay. At Irvine, the pump quit working after 93 days of sampling. In addition, the chemical analyses for the 100-day study have not yet been completed, and therefore trace-element data and source-category analyses were not presented in this report.

5. The experiments on total versus soluble PM in the primary airway epithelial cells were also conducted in primary microvascular endothelial cells, but despite validation of a positive response in pre-testing, the remaining vials of these cells did not respond to the PM extracts by producing ROS. In addition, the centrifugation of the samples ($12,000 \times g$ for 10 min) might not have been sufficient to remove all particles from suspension.
6. The definition of ultrafine PM is generally accepted as $0.1 \mu\text{m}$ in diameter. However, the physical characteristics of the ChemVol impactor (as well as other impactors of this size and flow rate) limited its effective size cut-off for ultrafine PM to particles up to $0.15 \mu\text{m}$, resulting in the partial introduction of fine PM into the ultrafine fraction.

DISCUSSION

Of the overall NPACT study's four initial hypotheses, three were applicable to the current study. The first was that coarse, fine, and ultrafine PM (in this case, $\text{PM}_{0.2}$) are each capable of producing acute health effects of public health concern but that the effects might differ according to particle-size range and particle composition within each size range.

Our results using PM collected at five sites in three particle-size ranges in cells in vitro and in mice treated in vivo by aspiration clearly showed that each size range was capable of producing acute effects. Importantly, the effects were often greater for coarse and ultrafine PM than for fine PM; they also varied significantly by site and season.

The second hypothesis was that the source-apportionment techniques that we developed and refined in recent years can provide a useful basis for identifying major PM air pollution source categories and specific chemical components having the greatest impacts on ROS production. Our findings in the current study clearly support this hypothesis by demonstrating associations between specific source categories and various in vitro or in vivo outcomes. Surprisingly, a wider range than expected of trace elements and endotoxin was significantly associated with the measured outcomes. The study thus met its objective of illustrating that the acute

effects of PM collected in various regional airsheds, seasons of the year, and particle-size ranges can usefully be studied in cells *in vitro* and in animal models treated *in vivo* with PM delivered by aspiration.

The overall aim of this study was to investigate the role of PM composition on toxicity *in vitro* and *in vivo*. The study has provided unique information on the role played by particle size in the adverse effects of PM. Our sampling strategy paralleled that of Chen Study 1's 6-month CAPs inhalation studies and the regional data analyses of Ito Study 3 and Thurston Study 4, the two epidemiologic studies. These three other studies, however, could not address the role of particle size as well as this study did, because of (1) the inability of inhaled coarse PM to penetrate past the nasal passages of mice and (2) the lack of size-specific concentration data and corresponding composition information in the air monitoring datasets used in the epidemiologic studies.

Although coarse PM typically makes up a large portion of total PM mass, it has been suggested that fine PM might be more toxic because of its greater toxic-metals content or that ultrafine particles within the fine fraction are more potent because of their greater surface-to-mass ratio compared with larger particles (Hetland et al. 2001). In addition, coarse PM has less penetration into the lung's gas-exchange region, and its components thus have fewer chances of being taken up systemically. Although some studies have shown that smaller particles can induce a greater biologic response, others have shown that coarse PM produces greater effects (Becker et al. 2005; Hetland et al. 2005; Gilmour et al. 2007), suggesting that the impact of surface area and size might not be as important as that of PM composition. Because the general population is exposed to a myriad of local and regional sources, each of which can emit different mixtures of PM, it has become clearer that mass concentration might not be the best regulatory metric for PM-induced health effects. There are also different patterns of particle deposition for the three particle-size fractions, with coarse and ultrafine PM having more deposition in the conductive airways than does fine PM.

To test the hypothesis that the particle size and elemental composition of ambient PM drive its biologic toxicity and subsequent adverse cardiopulmonary effects, we identified associations between the trace-element concentrations and source categories for a large number of PM samples in specific size ranges and a number of short-term biologic endpoints. The *in vitro* endpoints were measured in biologically relevant cell types (i.e., bronchial epithelial cells, endothelial cells, and cardiomyocytes) and included ROS production, mRNA abundance of markers of ROS and inflammation, and cardiomyocyte function. The *in vivo* endpoints included lung inflammation and lung injury in a

murine model treated by aspiration with PM samples in specific size ranges.

The coarse and fine mass concentrations were greater than the ultrafine concentrations at all five sampling sites; comparable results have been found by numerous other studies. In terms of trace-element composition, the coarse fraction showed less variation for individual elements between seasons than did the other size fractions, suggesting that there was more variability in the seasonal sources of the fine and ultrafine PM fractions than of the coarse fraction. This can be attributed to the fact that source contributions to coarse PM, such as wind-blown soil and resuspended road dust, might be less dependent on season and that combustion-related elements associated with fine and ultrafine PM are more dependent. In addition, we observed that, compared with the coarse fraction, the fine and ultrafine fractions had more nearly similar concentrations of many elements, suggesting that these two fractions might have been generated from similar source categories. It should be noted again, however, that the size cut-off for ultrafine PM in the Chem-Vol sampler used in the study did not strictly conform to that of the commonly used definition of ultrafine PM (i.e., $< 0.10 \mu\text{m}$). Because ambient PM typically has a bimodal distribution of PM mass, with one mode around $0.5 \mu\text{m}$, there might in fact have been some accumulation-mode PM in our ultrafine fraction. This size cut-off difference would mean that the ultrafine's relative mass concentration of total ambient PM, and therefore its role in toxicity, was potentially overestimated in the study.

This study has also provided evidence that — as observed by other investigators (Becker et al. 2005; Gilmour et al. 2007) and the other three NPACT studies — certain PM components are more associated with adverse effects than are other components and that PM composition varies greatly by sampling site, season, and particle size. In this study, clear source category contributions were identified through factor analysis. These source categories included Traffic/Brake Wear, Residual Oil Combustion, Coal Combustion, and Soil Dust. The Traffic/Brake Wear and Residual Oil Combustion source categories were identified as being statistically significantly correlated with *in vitro* intracellular ROS production. Evaluation of inflammatory markers suggested that these source categories might also be causal in the induction of mRNA levels of genes related to oxidative stress and inflammation. Correlation analyses of individual components of PM demonstrated that a wide variety of trace elements were significantly correlated with *in vitro* ROS production. The number of PM components with significant contributions to the endpoints in this study was larger than observed in the other three NPACT studies, and because the number of significantly correlated individual components was so large, it would be difficult

to utilize these data for risk assessment. Thus, the source-apportionment analyses might have greater utility.

One limitation of the study's design was the procedure used for aqueous extraction of PM samples from the collection substrates. The extraction method, developed at Harvard, the EPA, and RIVM, was relatively efficient in removing PM mass from the substrates used to collect coarse and fine PM (at typically greater than 80% efficiency for both size fractions) but was less efficient for the substrate used to collect ultrafine PM (at approximately 60% efficiency). As a result, the cells and mice were not treated with the complete composition of ambient PM in each size fraction. This limitation somewhat hinders the extrapolation of our study results to real-world sources. Nonpolar organic materials, for example, are not efficiently extracted by water and were thus not presented to the cells or mice in the bioassays. Although the potential role of organic compounds, such as organic and elemental carbon, might not have been discoverable in our bioassays, one-third of each substrate was removed before aqueous extraction and archived for future studies that might examine the comparative contributions of nonpolar components to the adverse health effects of ambient PM.

Importantly, the study's *in vitro* and *in vivo* response data did not correlate with each other, either in terms of the *in vitro* oxidative stress or *in vivo* lung inflammation responses that were produced by each sample or in terms of the individual components or source categories that drove each response. This suggests that the use of *in vitro* toxicity data to predict *in vivo* PM toxicity should be done with caution. Alternatively, the lack of correlation might be explained by the possibility that oxidative-stress pathways leading to ROS formation and acute lung inflammation are not strongly linked in mice. Another alternative hypothesis is that the PM components driving each response are significantly different. Trace metals, for example, drove a significant portion of the *in vitro* ROS response, whereas endotoxin was highly correlated with the *in vivo* response. Research has supported the notion that endotoxin in coarse PM contributes to the adverse pulmonary effects of ambient PM, and the current study showed that coarse PM is more inflammatory than the other size fractions for most of our sampling sites. However, we do not believe that endotoxin was responsible for the observed *in vivo* effects. The highest endotoxin concentration in our PM samples was 16 EU/mL; most samples were below 2 EU/mL. Additional groups of mice were treated with endotoxin by aspiration, and no significant lung inflammation was observed at endotoxin concentrations far above those present in the size-fractionated samples we collected. The percentage changes in % PMNs in

lavage fluid for 0, 20, 100, and 200 EU/mL endotoxin (assuming 1 ng endotoxin = 10 EU) were 0.5%, 1.3%, 0.5%, and 2.8%, respectively. Thus, a co-correlated component of PM might have been responsible for the observed lung inflammation induced by the coarse PM. The strongest correlations with endotoxin were for P, Ca, Mn, and Fe (with correlation coefficients of 0.49, 0.31, 0.34, and 0.30, respectively; see Table 16). The latter three elements are markers of a Soil Dust source category that would logically be associated with bacterial endotoxin. Another limitation of *in vitro* studies is the interaction of PM and its components with serum and other components of cell culture media. To reduce the potential for variation in interactions with serum, the *in vitro* experiments were conducted without (or with lowered) serum concentrations.

Another concern in the understanding of the adverse cardiopulmonary effects of inhaled ambient PM is the relative contribution of soluble and insoluble PM components to the causation of injury or stimulation of cellular responses. In the current study, we found that the soluble components of PM accounted for approximately half (53% to 60%) of the ROS-inducing ability of the total PM samples used to treat primary airway epithelial cells. Because our centrifugation ($12,000 \times g$ for 10 min) of the suspended PM might not have removed all insoluble PM components, particularly the ultrafine fraction of PM, it is possible that the contribution of soluble components to *in vitro* ROS production might have been even less than was measured. Regardless, the experimental results suggest that both the soluble and insoluble fractions of PM can induce oxidative stress *in vitro* in airway epithelial cells. It is unlikely, however, that a significant portion of the solid fraction of inhaled and deposited ambient particles reaches nonpulmonary targets, such as the liver or cardiovascular system. The experiments that investigated the effects of ambient PM on cardiomyocytes were therefore conducted with the soluble fraction of the size-segregated PM collected in Manhattan. Beat frequency results were mixed, with no significant increases or decreases in four of the six samples. The Manhattan fine PM collected in winter caused a significant increase in beat frequency after 15 minutes of treatment, and the Manhattan winter ultrafine PM produced a significant decrease in beat frequency at 24 hours. This latter finding for the soluble PM fraction was consistent with the statistically significant decrease in conduction velocity observed in the treatment of primary rat cardiomyocytes with the soluble fraction of the Manhattan winter fine PM. Although the percentage decrease in conduction velocity was not large, even a small slowing of electrical signals through heart tissue is biologically important (Dr. Gregory Morley, NYU School of Medicine, personal communication).

Table 16. Correlation Matrix of Elements and Endotoxin^a

	Be	Mg	P	S	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Sr	Cd	Sn	Sb	La	Pb	Endo
Be	1.00																							
Mg	0.00	1.00																						
P	0.53	0.20	1.00																					
S	0.18	-0.50	-0.15	1.00																				
K	-0.12	0.15	0.19	-0.07	1.00																			
Ca	0.24	0.56	0.37	-0.38	0.12	1.00																		
Sc	0.30	0.54	0.43	-0.39	-0.07	0.60	1.00																	
Ti	0.08	0.56	0.25	-0.46	0.05	0.51	0.76	1.00																
V	0.49	-0.22	0.17	0.34	0.08	-0.07	-0.04	-0.17	1.00															
Cr	-0.03	0.06	-0.02	0.05	0.07	0.07	0.01	0.04	0.09	1.00														
Mn	0.19	0.47	0.31	-0.30	0.19	0.51	0.50	0.59	0.05	0.15	1.00													
Fe	0.13	0.63	0.30	-0.48	0.00	0.62	0.81	0.92	-0.16	0.17	0.64	1.00												
Co	0.80	0.01	0.42	0.16	-0.13	0.16	0.32	0.10	0.48	0.20	0.16	0.17	1.00											
Ni	0.63	-0.12	0.28	0.26	-0.05	0.04	0.08	-0.11	0.58	0.68	0.10	0.02	0.71	1.00										
Cu	0.09	0.39	0.23	-0.33	0.32	0.37	0.51	0.75	0.05	0.09	0.56	0.77	0.13	0.03	1.00									
Zn	0.16	-0.16	0.07	0.19	0.15	0.07	-0.13	-0.13	0.19	0.70	0.03	-0.04	0.27	0.63	-0.02	1.00								
As	-0.08	-0.25	-0.06	0.17	0.35	-0.04	-0.25	-0.22	0.21	0.14	-0.01	-0.24	-0.11	0.06	-0.05	0.19	1.00							
Sr	0.08	0.63	0.19	-0.31	0.49	0.50	0.47	0.49	0.01	0.06	0.62	0.53	0.07	-0.02	0.53	-0.08	-0.12	1.00						
Cd	-0.07	-0.20	-0.11	0.17	0.16	-0.12	-0.18	-0.15	0.03	0.03	-0.06	-0.17	-0.09	0.00	-0.03	0.11	0.32	-0.09	1.00					
Sn	-0.12	0.25	0.19	-0.05	0.28	0.13	0.12	0.17	0.06	0.12	0.41	0.20	-0.06	-0.05	0.25	-0.10	0.39	0.35	0.00	1.00				
Sb	0.27	0.20	0.26	-0.24	0.16	0.24	0.39	0.60	0.21	0.04	0.45	0.59	0.28	0.17	0.78	0.00	0.04	0.28	0.03	0.18	1.00			
La	0.29	0.26	0.36	-0.18	-0.07	0.33	0.76	0.30	0.11	0.01	0.28	0.43	0.35	0.20	0.25	-0.05	-0.13	0.26	-0.09	0.04	0.20	1.00		
Pb	0.11	-0.11	-0.01	0.18	0.01	-0.04	-0.02	-0.05	0.18	0.06	-0.01	-0.03	0.13	0.13	0.02	0.07	0.15	-0.03	0.06	0.10	0.05	0.05	1.00	
Endo	0.02	0.27	0.49	-0.28	0.08	0.31	0.21	0.26	-0.14	0.05	0.34	0.30	-0.02	-0.12	0.10	-0.10	-0.10	0.14	-0.13	0.24	0.07	0.13	-0.07	1.00

^a Pearson correlation coefficients (*r*) applied pairwise. Strong correlations (coefficients greater than *r* = 0.55) are in **bold**.

Additional *in vitro* investigations examined the mRNA expression of key ion-channel proteins for heart cells, but unfortunately the results of four separate high-throughput experiments were highly variable and thus inconclusive (data not shown). More research will be needed to understand the mechanisms by which the components of ambient PM alter heart rate and heart-rate variability, as demonstrated in both human and animal studies.

Another consideration in evaluating the relative toxicity of ambient PM is the role of particle size. Considerable research and conjecture have suggested that the ultrafine fraction of ambient or occupationally related PM is more toxic than the larger PM fractions. The current study's experiments were designed to test this possibility, and they consistently showed that coarse particles *in vivo* were more potent on an equal-mass basis. In the 12-day study, coarse particles produced significantly greater lung inflammation than the other size fractions when the data from all sampling sites were combined. This was confirmed in the *in vitro* experiments in which the relative weight concentration, as determined by the relative mass collected on the ChemVol substrates, was used to dose cells.

Interestingly, the greater *in vivo* potency of coarse PM was dependent on its source category. Although the coarse PM fraction was more potent than the fine fraction for all five sites, the ultrafine PM fractions from Manhattan and the LA Basin produced lung inflammation equivalent to that of the coarse PM fraction. Traffic/Brake Wear, a source category that includes both resuspended particles and fresh combustion products, is likely a more important contributor to ambient PM concentrations in Manhattan and the LA Basin than at the other three sites, and therefore the role of resuspended particles should receive more study, especially with respect to the determination of the differences between the toxicities of coarse PM from urban and rural sources. The data from our 100-day study point to a greater inflammatory response in mice treated with coarse PM collected at Manhattan and Irvine compared with the other size fractions. These data are surprising because the Manhattan and LA Basin PM samples used in the 12-day study showed equivalent toxicity for the coarse and ultrafine PM fractions for these two sites. For various reasons beyond our control, however, the sampling sites at both Manhattan and the LA area were different in the 12-day and 100-day studies, which might impair direct comparisons. Overall, we have observed complex interactions among particle sizes and

source categories that drove PM-induced inflammatory responses in the mouse lung.

The design of the study has allowed a toxicologic comparison of coarse, fine, and ultrafine fractions of ambient PM. Although similar experiments in cells and in rodents *in vivo* have been reported with PM collected over longer time periods (Hetland et al. 2001; Gilmour et al. 2007), our study collected uniquely size-fractionated ambient PM on a daily basis during two seasons. To achieve the goals of the study, bioassays and chemical analysis protocols were first optimized to test the small amounts of size-fractionated PM collected on a daily basis. The results of the study suggest, as expected, that PM components and source categories, in addition to PM mass, drive the adverse effects of inhaled ambient PM. In some of the *in vitro* experiments, a daily sampling effect appeared to drive variability in response at some sites but not others (Figure 5). Overall, however, the study's data demonstrate that the toxicity of PM is driven by complex interactions among sampling site, season of the year, and particle size. These findings suggest that, as hypothesized in the overall study objectives, PM components — as dictated by particle source categories — are responsible for the adverse effects of ambient PM. Importantly, as demonstrated here and in the other studies, the responsible components and sources categories can vary with the cardiovascular or pulmonary endpoint being assessed.

We conclude that the continued use of PM mass concentrations as the metric for the PM NAAQS, although generally useful, might be insufficient for future, more informative assessments of the human health effects of ambient PM. Although regional and seasonal influences on PM composition would be complicated to quantify, we think it would be important to incorporate them into future risk-assessment evaluations of ambient PM. Our data also suggest that a NAAQS for coarse PM should be considered and that it would also be important to acquire speciation data for coarse PM. Although the study was not designed to compare the toxicity of urban and rural coarse PM, our findings demonstrate that coarse PM from different source categories — as would be found at various urban versus rural sites — produce different degrees of toxicity. Some of our *in vitro* and *in vivo* results also suggest that it would be useful to consider implementing a separate nationwide monitoring program for ultrafine PM, at least for initial research purposes.

NPACT Study 3. Time-Series Analysis of Mortality, Hospitalizations, and Ambient PM_{2.5} and Its Components

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ABSTRACT

BACKGROUND

A number of recent multicity studies have reported associations between ambient PM* and all-cause mortality and cardiovascular and respiratory hospitalizations. Some of these studies also suggested that specific components were more strongly associated with the health outcomes than others. However, specific components of PM responsible for the observed associations have not been established, and uncertainty remains about the methods to identify them. Furthermore, as regulatory decisions move toward the assessment of multiple air pollutants, the role of gaseous pollutants in the observed associations of PM and health outcomes needs to be examined.

METHODS

We assembled a multicity database of air pollution, weather, mortality (years 2001–2006), and elderly hospitalizations (years 2000–2008) in 150 U.S. cities, based on the availability of PM_{2.5} chemical speciation data. We also analyzed a subset of 64 cities for which data for PM_{2.5} and

its components, as well as for the gaseous criteria pollutants nitrogen dioxide (NO₂), sulfur dioxide (SO₂), and carbon monoxide (CO), were available. We first characterized the quality of the data for the components of PM_{2.5} (e.g., the percentage of measurements of component concentrations below the detection limit) and performed temporal correlation of monitors for those cities with multiple monitors. We conducted factor analysis of the key components of PM_{2.5} and the gaseous criteria pollutants to reduce the dimensionality of multiple pollutants and also to facilitate source-oriented interpretation of multipollutant results. We estimated short-term risks associated with these pollution indices for all-cause mortality and for cardiovascular (CVD) and respiratory hospitalizations at 0-through 3-day lags, for individual cities. We used Poisson regression models, adjusting for temporal trends, immediate and delayed temperature, and day-of-week pattern, for entire years and for warm (April–September) and cold (October–March) seasons. We then combined the risk estimates (i.e., percentage excess risk, the percentage of increased risk associated with the stated increase in exposure) from individual cities using a second-stage random-effects model, and the city-to-city variations were also modeled as a function of city-specific characteristics, including land use and other pollution- and exposure-related variables such as seaport berth volume and traffic.

RESULTS

The components of PM_{2.5} showed varying degrees of quality in terms of their percentages of measurements below the detection limit. Monitor-to-monitor temporal correlations were high for the components of PM_{2.5} associated with secondary aerosols (e.g., sulfate [SO₄²⁻]) but poor for those associated with local combustion sources (e.g., Ni). Nationwide factor analysis of short-term fluctuations of the components of PM_{2.5} and gaseous pollutants identified six major source categories: Traffic, Soil, Coal Combustion, Salt, Metals, and Residual Oil Combustion.

This Investigators' Report is one part of Health Effects Institute Research Report 177, which includes Investigators' Reports of three other studies, a Commentary by the NPACT Review Panel, an HEI Statement about the research project, and a Synthesis of the NPACT Initiative relating this report to Research Report 178. Correspondence concerning the Investigators' Report may be addressed to Dr. Kazuhiko Ito, Bureau of Environmental Surveillance and Policy, New York City Department of Health and Mental Hygiene, 125 Worth Street, Room 326, New York, NY 10013. kito1@health.nyc.gov.

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* A list of abbreviations and other terms appears at the end of the Investigators' Report.

Source apportionment conducted in 64 individual cities identified Traffic and Soil source categories in the majority of these cities, but other source categories in smaller fractions of the cities. In time-series analyses, the components of PM_{2.5} and gaseous pollutants were often at least as strongly associated with the health outcomes as were PM_{2.5} mass concentrations. More pollutants were associated with all-cause mortality in the warm season (NO₂, SO₂, EC, OC, Pb, Si, and V) than in the cold season (SO₂, Cu, K, OC, and Si). In contrast, the pollutants' associations with CVD hospitalizations occurred mostly at 0-day lag in the cold season. The pollutants associated with CVD hospitalizations at 0-day lag in the cold season were PM_{2.5}, NO₂, SO₂, CO, Cu, EC, Fe, OC, SO₄²⁻, Se, Si, and Zn. V showed associations at 1- and 3-day lags, and it also showed a nearly significant association at 0-day lag. Several pollutants with 0-day-lag associations (NO₂, SO₂, CO, EC, and OC) also showed associations at 3-day lags. NO₃⁻ (0-day lag) and Na (2-day lag) showed associations in the warm season. In contrast to CVD hospitalizations, respiratory hospitalizations were associated with the pollutants both in the warm (PM_{2.5}, CO, As, K, OC, and SO₄²⁻) and cold (PM_{2.5}, CO, Cu, EC, K, and Si) seasons. Based on the factor scores for the six source categories examined, the Traffic source category showed most consistent associations with all-cause mortality, CVD hospitalizations, and respiratory hospitalizations, though their significance was less than that for some of the individual components of PM_{2.5} and gaseous pollutants associated with traffic. In the second-stage analysis, to examine the heterogeneity of risk estimates associated with PM_{2.5} exposure across the cities, SO₄²⁻, V, the berth volume of seaports within 60 miles, and the sum of road lengths were important predictors in explaining the variation in PM_{2.5} all-cause mortality risk estimates. For CVD hospitalizations, Cu, NO₂, V, Ni, Fe, and the extent of land development were important positive predictors. For respiratory hospitalizations, Cu, Ni, V, SO₂, and the extent of land development ranked high as positive predictors of PM_{2.5} risk estimates.

DISCUSSION AND CONCLUSIONS

We provided a source-oriented assessment of multipollutant effects on mortality and morbidity outcomes through characterizations of daily concentrations of the components of PM_{2.5}, factor analysis, multicity analysis of both daily mortality and daily hospitalizations, and a second-stage evaluation of the variation in the estimated risks across cities of these outcomes associated with PM_{2.5} exposure. This study presents an approach to reduce the dimensionality of the multiple pollutants and suggests

that a major fraction of variations in multiple pollutants could be attributable to traffic-related sources, and that the temporal variations of these sources affect their associations with daily mortality and CVD and respiratory hospitalizations. We also found that secondary aerosols were associated with these outcomes.

INTRODUCTION AND SPECIFIC AIMS

Many observational time-series studies have reported short-term associations between PM and morbidity and mortality (U.S. EPA 2004, 2009). Recent research has focused on PM_{2.5} because the particles in this size range can penetrate deep into the pulmonary system and, thus, are likely to cause adverse effects in a variety of organ systems. However, PM_{2.5} is a chemically heterogeneous pollutant that may originate or be derived from various types of emission sources. As a result, PM_{2.5} toxicity may vary depending on its source and chemical composition. If the toxicity of PM_{2.5} could be determined based on specific components or source types, the regulation of PM_{2.5} to protect health might be implemented more effectively and efficiently. Thus, recent studies of the health effects of air pollution have begun to examine the effects of individual components of PM_{2.5}, rather than those of its total mass concentrations. An alternative approach to examining individual components is to study the source apportionment of PM_{2.5}, using chemical speciation data, and to thereby examine the associations between source apportioned PM_{2.5} and health outcomes. The process of apportioning sources applies statistical methods to data on chemically speciated PM_{2.5} mass in order to apportion particle mass to various types of emission sources. Source-apportionment analysis results in an estimate of daily PM_{2.5} mass contributions from identified source categories. Source-apportionment methods based on multivariate factor analysis have been widely used to analyze chemical speciation data (e.g., Watson et al. 2008), but their applications to epidemiologic studies have been limited to date.

A small, but growing, number of studies have analyzed the health effects associated with source-apportioned fine particles (e.g., Özkaynak and Thurston 1987; Özkaynak et al. 1996; Tsai et al. 2000; Laden et al. 2000; Mar et al. 2000; Thurston et al. 2005; Ito et al. 2006; Mar et al. 2006; Sarnat et al. 2008). Recent toxicologic studies from our laboratory also have used source-apportionment techniques to evaluate source-specific effects (e.g., Maciejczyk and Chen 2005; Lippmann et al. 2005c). These studies have provided some suggestive evidence that PM_{2.5} from certain combustion sources (e.g., secondary aerosols, fossil fuel combustion, and

traffic), but not from other sources (e.g., soil), were associated with daily mortality. However, the results, to date, are far from conclusive because the data analyzed in these studies were collected as part of special studies in a few cities or by a network of air quality monitors for characterizing visibility, and therefore may not be widely representative.

Clearly, more analyses of speciation data using multiple cities with larger populations and a range of PM_{2.5} source types are needed. Fortunately, a large number of monitors started collecting chemical speciation data from PM_{2.5} filters starting circa 2000 in the United States. The EPA's PM_{2.5} CSN is one of the monitoring requirements set forth by the Federal Register (62 FR 38763) as part of the PM_{2.5} National Ambient Air Quality Standards (NAAQS) review completed in 1997 (U.S. EPA 1999). In 2010, over 230 monitors in the United States were collecting these chemical speciation data.

Several recent multicity studies have examined the relationships between individual PM_{2.5} components from the new CSN data and morbidity and mortality (Lippmann et al. 2006; Dominici et al. 2007a; Ostro et al. 2007, 2008; Franklin et al. 2008; Zanobetti et al. 2009; Bell et al. 2009; Peng et al. 2009). However, all of these studies, except the ones by Ostro and colleagues (2007, 2008) and Peng and colleagues (2009), used the annual or seasonal means of the CSN data for the components of PM_{2.5} in a second-stage regression analysis to explain the city-to-city heterogeneity of short-term PM_{2.5} or PM₁₀ risk estimates obtained in a first-stage time-series regression conducted for individual cities. Presumably, the studies were designed this way because the every-third-day collection frequency for the CSN data did not provide data that were as statistically powerful as the daily PM_{2.5} mass concentration data. The Peng and colleagues study (2009) examined data on PM_{2.5} components collected every third day, but focused its analysis only on the seven major components having appreciable mass. None of these multicity studies considered source-apportioned PM_{2.5} mass concentrations in their health effects analyses. Furthermore, the majority of the time-series studies published in the past 10 years have focused on PM_{2.5} and ozone, the two pollutants that most frequently exceed the established NAAQS for them. Thus, our analyses, which are of source-apportioned PM_{2.5} mass concentrations as well as of gaseous copollutants, aim to comprehensively fill in the gaps in knowledge about the adverse health effects of PM_{2.5} components — including those present in minor mass concentrations — and about the types of PM_{2.5} sources.

In setting research priorities for studying the health effects of ambient air PM_{2.5}, the National Research Council

(NRC) acknowledged the value of receptor-oriented source-apportionment models, such as those used here, but they also pointed out that "...a number of approaches have been presented in the literature, but they are typically applied to only a single location or region. There has not been an extensive effort to test the effectiveness of these alternative methods..." (NRC Committee on Research Priorities for Airborne Particulate Matter 2001). The NRC's update on the progress in PM_{2.5} health effects research further emphasized the importance of characterizing emission sources (NRC 2004). Recent U.S. EPA advancements in chemical speciation technology and increases in the number of monitoring sites have led to the creation of an as-yet-underused data set that describes potential exposures of populations to a wide array of the components of PM_{2.5}. These data can be used to identify specific chemical tracers (and thus specific source categories of aerosols that are associated with PM_{2.5}) and their corresponding health-impact end points. Thus, the results of this work will help identify specific methods for determining the components and source categories of PM_{2.5}-associated aerosols that have specific effects on human health.

Our specific approach involved the use of source-apportionment and land-use regression techniques to identify the components and sources of PM_{2.5} most likely to have significant associations with mortality and morbidity. We have exploited the available nationwide database that has collected data on PM_{2.5} and its chemically speciated components, estimated source categories and computed associated factor scores for each city, and then applied these results to a multivariate regression model. The results from this investigation provide relevant insights into the efficacy of source-oriented evaluations of PM_{2.5} health effects.

HYPOTHESES

Our posited hypothesis was that one or more components of PM_{2.5} or source-related components, or both, are more strongly associated than PM_{2.5} mass concentrations with the health effects associated with PM_{2.5} exposure previously reported for CVD and respiratory morbidity and mortality.

This study addresses three of our four initial overall hypotheses restated in terms of acute human responses to short-term exposures. These hypotheses were as follows:

1. PM_{2.5} is capable of producing acute health effects of public health concern, but the effects may differ according to its composition.
2. The source-apportionment techniques that we have developed and refined in recent years are useful for

identifying the categories of sources of PM_{2.5} air pollution and specific components that have the greatest impacts on a variety of acute health effects.

3. The acute health effects due to ambient PM_{2.5} exposures can best be seen in elderly populations (who are more likely to be sensitive to the effects of the pollution) within human populations.

SPECIFIC AIMS

Our specific aims were to (1) characterize PM_{2.5} components to help interpret the results of time-series analyses of health effects data and these air pollution indices; (2) conduct factor analyses of the components of PM_{2.5} and local gaseous pollutants to characterize local-pollution source types and to reduce the dimensionality of the large number of air pollution indices examined; (3) conduct time-series analyses of mortality and elderly hospital admissions to estimate short-term risk; (4) model the city-to-city variation of risk estimates (using the analyses of aim 3) as a function of city-specific characteristics including pollution levels, land use, and other exposure-related information; and (5) evaluate the consistency of results (based on the analyses and models from aims 3 and 4) about which PM_{2.5} components and source types are associated with the health outcomes.

METHODS

OVERVIEW OF APPROACH

We used four methods to analyze the data in this project:

1. Factor analysis to characterize nationwide air pollution data, including PM_{2.5} chemical speciation and gaseous-pollutant data.
2. Multicity time-series analysis of air pollution (i.e., of its mass, components, and source categories) and mortality for the years 2001 through 2006.
3. Multicity time-series analysis of air pollution (i.e., of its mass and components) and Medicare hospitalization data for the years 2000 through 2008.
4. Second-stage regression to investigate the city-to-city heterogeneity in the health effect risk estimates associated with PM_{2.5} components.

While these analyses examined both mortality and Medicare hospitalizations, we did not design the study to link Medicare hospitalization records to mortality records (i.e., linkage identifiers were not used).

SELECTION OF THE CITIES FOR ANALYSIS

Because the main objective of this project was to examine the associations between the components of PM_{2.5} and health outcomes, we chose 150 U.S. metropolitan statistical areas (MSAs) with at least 100,000 residents and where the data from at least one CSN monitor were available. The list of these 150 MSAs is provided in Appendix Table B.1. However, because we were also interested in determining the health effects of air pollution in a multipollutant atmosphere, we also analyzed a subset of 64 cities for which data on PM_{2.5} components and gaseous criteria pollutants (NO₂, ozone [O₃], SO₂, and carbon monoxide [CO]) were all available. These 64 cities are also identified in Appendix Table B.1. The locations of the 150 cities and the 64 cities are shown in Figure 1. Characteristics of the cities (such as population density and area) are shown Appendix Table B.1. Thus, this project provides results from data analyses of both of these two data sets. Note that the 64 cities tend to be larger cities, a feature that we needed to consider when we compared the results from these two sets of data.

In addition to the analyses for the individual PM_{2.5} components and gaseous pollutants for the 64 cities where all of the pollutants were available, we conducted a factor analysis and used the derived factor scores for the health effects analyses as alternative source-oriented air pollution indices. Note that daily data on the gaseous criteria pollutants were available (the daily values were computed from

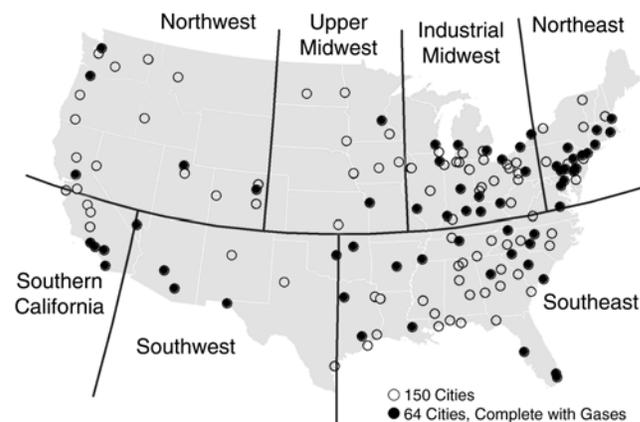


Figure 1. U.S. cities analyzed for mortality and hospitalization. Included are the locations of the 150 cities (for which speciation data exist) and the subset of 64 cities (for which data on speciation and all the gaseous pollutants exist) selected for data analysis.

hourly data), and PM_{2.5} mass concentrations data (that were not part of the CSN data) were available daily or every third day. However, the CSN data for PM_{2.5} components were available either every third day or every sixth day. The pattern of sampling frequency in these cities is shown in Appendix Figure B.1. As a result of the CSN sampling frequency, the sample sizes (in terms of the number of days of data available for each lag day of the analyses involving the PM_{2.5} components) were substantially smaller than those for the analysis sets that involved only PM_{2.5} mass concentrations and gaseous pollutant data. Thus, it was not feasible to conduct time-series analyses for individual cities with a combination of specific subcategories of CVD or respiratory outcomes and smaller daily counts and PM_{2.5} component data, because such a combination would make the generalized linear model (GLM) fail to converge (resulting in biased estimates or no estimate). Therefore, we selected and analyzed combinations of health outcomes, pollution indices, and data on the 150 cities or the 64-cities subset so that we had a sufficient number of observations for meaningful comparisons of results. Table 1 shows these analysis subsets, indicating which combinations were analyzed and which figures illustrate the results. Note that, although we use “150-cities data set” and “64-cities data set” throughout this report, several sets of analyses using the GLM model failed to converge for a few cities for specific pollutants at specific lag times. In such cases, we excluded these risk estimates when calculating combined estimates and in the meta-regression. For each set of analyses, Appendix Tables B.9 and B.10 list the combinations of city, pollutant, and lag day that failed to converge (and therefore were excluded from the combined estimates) for each set of analyses. We opted to use this approach (rather than redefining the number of cities based on the results) because the cities listed had either small daily counts of health outcomes or small numbers of observations for specific components and therefore were unlikely to substantially influence the overall combined estimates.

DATA USED FOR TIME-SERIES HEALTH EFFECTS ANALYSIS

Air Pollution Data

We obtained the CSN data for PM_{2.5} and its components as well as hourly NO₂, SO₂, and CO data from the U.S. EPA Air Quality System (AQS) archive database (U.S. EPA 2012). From the hourly data on gaseous pollutants, we computed daily maximum 8-hour average values for CO, and 24-hour average values for NO₂ and SO₂. Because the AQS archive site at the time did not contain all of the data for PM_{2.5} mass concentrations, we obtained all available

nationwide AQS PM_{2.5} mass data from Mr. Nick Mangus at the U.S. EPA; the data provided were based on the Federal Reference Method (FRM; 24-hr average values) for this analysis. When the data from multiple monitors were available in a given city, we computed the average of the daily values from multiple sites after standardizing each site’s data using the mean and standard deviation of the site’s data, as was done in Zanobetti and Schwartz (2009).

Weather Data

We obtained weather data for the 150 MSAs from the National Climatic Data Center, Climate Data Online (National Oceanic and Atmospheric Administration 2013). We retrieved the global summary of the day files for one weather station per city. In general, we chose the data from a large airport with the most complete observations for each MSA.

Mortality Data

We obtained the nationwide multiple-cause-of-death files for the years 2001 through 2006 from the National Center for Health Statistics (NCHS) through the EPA’s arrangement with NCHS to support research. The data for Hawaii and Idaho were not included in the database, and therefore, the analysis of mortality data was conducted for only up to 148 cities. Using *International Classification of Diseases, 10th Revision* (ICD-10) codes (NCHS 2008), we aggregated daily death counts for the major underlying causes of deaths frequently analyzed in time-series mortality studies (e.g., Peng et al. 2008, 2009; Zanobetti and Schwartz 2009): (1) nonaccidental all-cause deaths (A00–R99); (2) CVD deaths (I01–I79); and (3) respiratory deaths (J00–J99). The daily counts of these deaths are shown in Appendix Table B.2.

Medicare Hospitalization Data

We obtained the nationwide Medicare hospitalization data for research use from the Center for Medicare and Medicaid Services after our study protocol was approved. For each city, we aggregated daily counts of emergency (i.e., admission type = emergency) hospitalizations for the elderly (those 65 and older) according to CVD and respiratory categories. The choice of these categories was based on the working hypothesis that air pollution has acute effects on these conditions and is consistent with the past epidemiologic studies of air pollution, including our past analysis of Medicare data (Lippmann et al. 2000; Ito 2003) as well as recent multicity analyses (Dominici et al. 2006; Peng et al. 2008; Bell et al. 2009). The ICD-9 (NCHS 2010) CVD and respiratory categories were as follows: CVD includes ischemic heart disease (IHD; 410–414), acute myocardial infarction (410),

Table 1. Overview of Analyses and Data Sets for Health Outcomes and Indices of Air Pollution Exposure^a

City Sets / Health Outcomes	Air Pollution Indices				
	PM _{2.5}	NO ₂ , SO ₂ , CO ^b	PM _{2.5} Speciation ^c	Factor Scores Individual Cities ^d	Factor Scores Nationwide ^d
150 Cities Data Set					
Mortality					
All-cause	Figure 4^e	NA	Figure B.4	NA	NA
CVD	Figure 4	NA	NA	NA	NA
Respiratory	Figure 4	NA	NA	NA	NA
Hospitalizations					
CVD	Figure 5^e	NA	Figure B.4	NA	NA
Specific CVD	Figure 5	NA	NA	NA	NA
Respiratory	Figure 6^e	NA	Figure B.4	NA	NA
Specific respiratory	Figure 6	NA	NA	NA	NA
64 Cities Data Set^f					
Mortality					
All-cause	Figure G.1 Figure 7	Figure G.1 Figure 7	Figure 7	Figure 8	Figure B.6
CVD	Figure G.2	Figure G.2	NA	Figure G.3	NA ^g
Respiratory	Figure G.4	Figure G.4	NA	Figure G.5	NA ^g
Hospitalizations					
CVD	Figure G.6 Figure 9	Figure G.6 Figure 9	Figure 9	Figure 10	Figure B.6
Respiratory	Figure G.7 Figure 11	Figure G.7 Figure 11	Figure 11	Figure 12	Figure B.6

^a **Bold** figure numbers indicate that analyses included data for all possible days for PM_{2.5} and gaseous pollutants. Unbolded figure numbers indicate that data were analyzed for only those days (every third or sixth day) when speciation data were available.

^b NA indicates that analyses were not conducted because data for gases were not consistently available for the 150 cities.

^c NA indicates that these data were not analyzed because of a small sample size resulting from the relatively small counts of health outcomes and the sampling frequency (every third or sixth day) of the speciation data.

^d NA indicates that factor analysis was not performed because gaseous pollutant data were not consistently available in the 150-city data set.

^e The risk estimates for the individual cities summarized in these figures were used in the second-stage analyses shown in Figure 14.

^f See Appendix G (available on the HEI Web site).

^g NA indicates that these data were not analyzed because of the relatively small daily counts for mortality for CVD and respiratory causes.

dysrhythmias (427), heart failure (428), and cerebrovascular diseases (431–437); respiratory includes pneumonia (480–486) and chronic obstructive pulmonary disease (COPD; 490–496). The daily counts of hospitalizations for these causes are shown in Appendix Tables B.3 and B.4.

The New York University School of Medicine Institutional Review Board reviewed our study description and determined that this study did not constitute research involving human subjects as defined in federal regulations at 45 CFR 46.102.

DATA USED FOR CHARACTERIZATIONS OF PM_{2.5} AND FOR THE SECOND-STAGE REGRESSION ANALYSIS

Land-Use Data

We obtained the 2001 National Land Cover Data (NLCD) (Homer et al. 2007; United States Geological Survey [USGS] 2007), which is derived from Landsat Thematic Mapper satellite data. The NLCD is a 20-class land-cover classification scheme applied consistently over the United States. We used these classification categories to compute the percentage of each MSA's land cover in seven land-use categories: (1) developed; (2) high-density development; (3) farm; (4) wetland; (5) barren; (6) green; and (7) water.

Traffic Data

We used traffic data two ways: (1) as part of the characterization of PM_{2.5} and (2) as an explanatory variable in the second-stage regression analysis with risk estimates obtained from multiple cities. We used the Highway Performance Monitoring System (HPMS) of the Federal Highway Administration (National Transportation Atlas Database 2006). The sum of HPMS road lengths was computed for each city (MSA) and used in the second-stage analysis as a predictor to explain city-to-city variation in PM_{2.5} risk estimates.

Port-Related Data

To examine the potential influence of air pollution from ships burning "bunker oil" (heavy residual oil) in major seaports, we compiled several variables using the centroid of an MSA as a reference point: (1) distance to nearest coast, (2) distance to nearest large-berth seaport, and (3) sum of total berths in ports within 60 miles of the MSA centroid. We obtained the data for (1) from USGS coastline data on the vulnerability of coasts to sea-level rise (U.S. Department of the Interior 2005). We obtained the data for (2) and (3) from the Institute for Water Resources Navigation Data Center (Navigation Data Center 2011).

The land-use, traffic, and port-related data in the 150 cities are shown in Appendix Tables G.1 and G.2. (Appendix G is available on the HEI Web site.)

DATA ANALYSES

Characterization of PM_{2.5} and Its Components

To help interpret the estimated health effects of PM_{2.5} components, we examined six characteristics of the measured components data: (1) percentage of values below the detection limit, (2) fraction of measured readings equaling 0, (3) monitor-to-monitor temporal correlation of measured

values in the 21 cities where multiple monitors were located, (4) correlation with the temperature at 0-day lag, (5) correlation with the average of 1- through 3-day lag temperatures, and (6) correlation with the day-of-week variable (through regression). The results from analyses (1) through (6) above were considered in order to narrow down the key components for the health effects analysis. We initially examined the data for the following 27 PM_{2.5} components in terms of the first three characteristics listed above: As, Al, Ba, Br, Cd, Ca, Cr, Cu, Fe, Pb, Mn, Ni, Mg, Hg, P, Se, V, Si, Zn, S, K, Na, NH₄⁺, OC, NO₃⁻, EC, and SO₄²⁻. For these components, we also assessed potential toxicologic considerations, known associations with source types, and information from past epidemiologic studies. Note that because Na⁺ measurements by ion chromatography (IC) were more reliable than Na measurements by XRF, we replaced Na by XRF with Na⁺ by IC. For brevity, however, in the following text and in the referenced tables and figures, "Na" indicates "Na⁺."

Factor Analysis

We used factor analysis to reduce the dimensionality of multiple air pollutants and facilitate the characterization of the PM_{2.5} component and gaseous pollutant concentration data into source categories. The derived factor scores were further used in the health effects analyses as alternative indices of air pollution source categories. Note that, as in the factor analysis conducted in Study 4 of this report, this factor analysis focused on identifying MSA-level air pollution source categories and excluded the PM_{2.5} components associated with secondary aerosols that could travel long distances. Thus, we excluded S, SO₄²⁻, NO₃⁻, and NH₄⁺. However, we did separately analyze the secondary aerosols SO₄²⁻ and NO₃⁻ in the health effects analysis. Likewise, we included the gaseous pollutants NO₂, SO₂, and CO, but not O₃, in the factor analysis. Because we needed to consistently include these gaseous pollutants, we limited the factor analysis to the 64-cities data set in which all of the cities had these gaseous pollutants and PM_{2.5} components. We conducted factor analysis in two ways: (1) by a nationwide analysis of the combined data for all of the 64 cities and (2) by separate analyses for the 64 individual cities. The nationwide analysis was conducted to characterize and identify all major source categories. Note that, as in the time-series health effects analysis (further explained in the section Time Series Analysis Model, below), the pollution variables that we used were the deviations from the monthly means. For the nationwide analysis, using the deviation variables reduced the influence of city-to-city spatial contrasts in the pollution levels, as well as the influence of seasonal cycles,

though it did not eliminate them, as the variances of pollution variables were related to the means. In contrast to this factor analysis, the factor analysis conducted in Study 4 did not eliminate the spatial contrasts of the pollutants, since the health effects analysis of the long-term effects of pollutants in fact relied upon the spatial contrasts of the pollution levels, rather than the temporal contrasts considered in this acute-effects analysis.

We conducted the factor analysis with the SAS (version 9.2) (SAS, Cary, NC) PROC FACTOR procedure with orthogonal rotation using the Harris-Kaiser rotation with “hkpower = 1.0” (this is equivalent to the varimax rotation except that it includes an option to change the rotation for varying extents of obliqueness). Thus, the resulting factors were uncorrelated with each other. We ran five sets of the nationwide factor analysis, by varying the number of factors from four to eight, and assessed the interpretability of factors. We chose the solution with the number of factors that resulted in the least number of cases of mixing and splitting of the presumed major factors. The naming and identifying of the factors as major source categories were mainly based on an exercise in which several research groups independently conducted source apportionment using the same data sets from two cities and reported their results in a workshop in 2003 (Thurston et al. 2005; Hopke et al. 2006). It was also based on the results from the factor analysis conducted in Study 4. In addition, we added the gaseous pollutants to the analysis to further strengthen the source category identification. The six major U.S.-wide source categories identified (and their respective key PM_{2.5} components and gaseous pollutants) were Traffic (EC, OC, and NO₂); Soil (Al, Si, and Ti); Coal Combustion (As, Se, and SO₂); Residual Oil Combustion (Ni and V); Salt (Na and Cl); and Metals (Fe, Mn, and Zn).

In the factor analysis conducted for individual cities, we analyzed data for the 64 cities for which data on all gaseous pollutants were available, which meant that the likelihood of finding a Traffic source category was high. This was because the gaseous pollutant NO₂, though the least frequently measured NAAQS pollutant in the United States, is a good indicator of traffic, and the measurement of NO₂ in a given city implies that the impact of traffic was likely to be high. However, the other source categories identified in the nationwide analysis, such as Residual Oil Combustion and Metals, had a smaller chance of being identified in the factor analysis of individual cities. We applied consistent criteria for naming the source categories by using an algorithm we wrote that classified each factor into one of the six major source categories based on clusterings of factor scores above a cut-off level (0.4, based on our nationwide analysis) for PM_{2.5} components and

gaseous pollutants. We then scored the extent of matching (measured as the ratio of the number of matched components to the total number of components for the source category profile) to prioritize the candidate source categories. For example, if one factor listed components EC, OC, NO₂, Al, and Si as having factor scores of 0.4 or higher, then the score for Traffic is 1.0 (because three of these components match the ones identified with traffic in the nationwide analysis), which is higher than the score for Soil (0.67, for two out of the three components matching), and the factor would be named Traffic. When the same component was identified in multiple factors, the algorithm picked the factor with the largest eigenvalue (i.e., the sum of the square of the factor score for that factor) for the component. The number of factors to be extracted was fixed across cities so that the size of the eigenvalues for a given factor could be kept relatively consistent across cities. Based on the algorithm for this factor analysis of individual cities, all six source categories were “identified” for some cities, whereas for others, two source categories might have been identified, which would likely be the case in reality, as not all the source categories would be identified in a given city, depending on the existence and the strength of the emission sources. This is in contrast to the nationwide factor analysis, in which the low scores would be assigned for nonexistent source categories. The advantage of this “automatic source category identification” approach is that it uses a consistent criterion to “name” source categories. However, while this analysis performed in individual cities allows a more local characterization of nationally important source categories, it does not identify unique local sources of pollutant emissions.

Time-Series Analysis Model

We analyzed air pollution indices (PM_{2.5}, its components, factor scores, and gaseous pollutants) for their associations with mortality and emergency hospital admissions in individual cities. To model count data, we used the Poisson GLM (McCullagh and Nelder 1989). Our base model specification is

$$\begin{aligned} \log[E(Y)] = & \text{intercept} + \beta \text{ pollutant concentration} \\ & + ns(\text{study days [df = 8 df/yr]}) \\ & + as.\text{factor}(\text{day-of-week}) \\ & + ns(\text{0-day-lag temperature [df = 3]}) \\ & + ns(\text{average of 1- through 3-day-lag} \\ & \quad \text{temperatures [df = 3]}), \end{aligned}$$

where Y is a daily health outcome (all-cause mortality or hospital admission counts), $E(Y)$ is the expected value of Y , ns indicates the smoothing term for the natural cubic

spline, and *as.factor* indicates that *day-of-week* was treated as a categorical variable. The model is based on our past studies of mortality and morbidity (e.g., Ito and Thurston 1996; Thurston and Ito 2001; De Leon et al. 2003; Ito 2003; Silverman et al. 2005; Ito et al. 2005b; Ito et al. 2007; Silverman and Ito 2010; Metzger et al. 2010). Through these studies, we have developed model-building strategies whose steps include (1) modeling of confounding temporal trends (annual cycles and influenza epidemics) and day-of-week patterns; (2) developing weather-effects models; and (3) developing models of air pollution effects for multiple-day lags as well as distributed lags. These approaches are generally consistent with strategies used in other recent studies (e.g., Peng et al. 2009), but our model is more parsimonious than some of the other models used in recent multicity studies. For example, compared to the NMMAPS model (Health Effects Institute 2003), our model does not include dew-point temperature because we found dew-point temperature to be highly correlated with temperature ($r > 0.9$) in most cities, and because we intended to evaluate (and interpret) the shape of the fitted temperature–outcome relationship, which becomes more difficult with correlated temperature terms. Also, our preliminary analysis using an alternative weather model specification suggested that including additional dew-point terms did not significantly change the risk estimates associated with the pollution. We also conducted sensitivity analysis using the NMMAPS model, which included dew-point terms. The main reason we used 8 degrees of freedom per year (df/yr) for the temporal adjustment is that we determined that this extent of smoothing could fit relatively sharp influenza peaks in the mortality and morbidity time series. This extent of smoothing for the temporal trend is also consistent with recent multicity time-series studies (e.g., Air Pollution and Health: A European and North American Approach [APHENA]; Katsouyanni et al. 2009). Additional sensitivity analyses included (1) alternative degrees of freedom to adjust for temporal trends and seasonal cycles, (2) alternative degrees of freedom to model the extent of nonlinear relationships between the weather variables and the outcomes, and (3) alternative weather variables (e.g., apparent temperature rather than temperature).

We also estimated pollution risks for the warm (April–September) and the cold (October–March) seasons using the season–pollution interaction terms, as was done in other recent multicity studies (e.g., Peng et al. 2005; Bell et al. 2009), except that we used two seasons rather than four.

Using the above model, we examined the air pollution indices at 0- through 3-day lags separately. It is important to note that we expressed each pollutant concentration in

the model as a deviation from the monthly mean. Note, too, that we consistently used each pollutant’s deviation from the monthly mean across the analyses in this study because the factor analysis required reducing the influence of spatial and seasonal variations on the overall correlation structure to estimate short-term variations in source categories. Using the deviation variable in the health effects model is advantageous because it reduces the influence of the seasonal cycles of the pollutants on the overall associations and helps to focus on the short-term associations. While this was addressed in part by including a smooth function of study days as a covariate in the model, such terms compete with seasonal cycles in pollution variables in the time-series analysis. Removing seasonal variations in pollutant concentrations can be important in investigating the short-term associations among multiple pollutants because many of the pollutants have strong seasonal cycles. PM_{2.5} often does not have very strong seasonal cycles compared to its components because it is often a mixture of winter-peaking components (e.g., NO₃⁻) and summer-peaking components (SO₄²⁻). However, because using deviation variables is not the most common way to include air pollution variables in recent time-series studies, we also considered using raw (untransformed) pollutant-concentration variables. We conducted the analysis with the raw pollution variables for PM_{2.5}, its components, and gaseous pollutants for all-cause mortality and CVD and respiratory hospitalizations in the 64-cities data set.

In response to the Review Panel’s request, we also evaluated two-pollutant models in which each PM_{2.5} component was included in the model along with the PM_{2.5} mass concentration because (quoting the committee’s comment) “the results would provide a reasonable way to determine the relative impact on health effects measures of the temporal variability in a given component controlled for that of PM_{2.5} as a whole.” It made sense to do this only for the key components whose associations with the health outcomes were significant or nearly significant, and only using the lags with the most significant results. The obvious limitation of such a two-pollutant analysis is the expected instability (and likely bias) of individual-pollutant risk estimates when the concentration of a component is strongly correlated with PM_{2.5} mass concentrations (e.g., SO₄²⁻ and PM_{2.5} in the warm-season model). This limitation needs to be carefully considered when interpreting these results. It renders many of the individual-component coefficients in the regressions with PM_{2.5} uninterpretable, which is why Study 4 of this report restricted multiple-pollutant comparisons to the more stable composite total risk impact (TRI) analysis when more than one pollutant was considered in those models simultaneously. An additional limitation of the two-pollutant model using the chemical speciation data

set is that, because of the every-third-day or every-sixth-day collection schedule, we could not evaluate the two pollutants at different lag days. Thus, PM_{2.5} mass was included in the model using the same lag as the one used for the component. We also recognized the value of two-pollutant models for the gaseous pollutants (each gaseous pollutant plus PM_{2.5} mass). Therefore, we conducted two-pollutant analysis using the 64-cities data set, for which gaseous pollutant data were available, but we only used the days when the chemical speciation data were available. We present the results as scatterplots of risk estimates associated with exposure to the key components and gaseous pollutants with and without PM_{2.5} mass included in the model (single-pollutant model vs. two-pollutant model).

Second-Stage Regression Model

In the first step of the second-stage analysis, we calculated risk estimates for city-specific air pollutants across cities using a random-effects model (DerSimonian and

Laird 1986) as discussed in the previous section. Table 2 shows the medians of the interquartile ranges (IQRs) across cities for PM_{2.5}, PM_{2.5} components, and gaseous pollutants. For the next step, to explain the city-to-city heterogeneity in these first-stage risk estimates, we conducted a second-stage metaregression analysis with explanatory variables (i.e., potential effect modifiers) such as land use and average air pollution levels. The regression coefficients for the air pollution variables from the time-series analysis for individual cities were the dependent variables, and the city-specific characteristics were the explanatory variables in the second-stage model:

$$\beta_j = W_j\delta + \gamma_j,$$

where β_j is a risk coefficient for a pollutant for city (MSA) j , W is a city-specific information (e.g., median monitor-to-monitor correlation, average temperature, etc.) vector, and γ is city-to-city random effects. Various methods of implementing this model, including restricted maximum likelihood estimation (Viechtbauer 2005) and the empirical Bayes estimator (Berkey et al. 1995), are available (Viechtbauer 2006). In our research, we have developed data-analytical routines to conduct these metaregressions and have been analyzing mortality data. We applied the same data-analytical routines to the analysis of the Medicare hospitalization data.

Because the city-specific mean values of the pollution variables were highly skewed to the right, we log-transformed all the pollution variables except SO₄²⁻, whose values were more normally distributed. This step kept a few cities from influencing the results (though the random-effects model should guard against this to some extent). Note that the log-transformation was done for the variables in the second-stage analysis only (not the pollution variables in the first-stage time-series analysis). To evaluate the impact of city-specific variables, we computed the percentage change in risk estimates per IQR of the variable and we computed the combined risk estimate without the dependent variable (i.e., a random-effects combined estimate) as the denominator.

As was done in recent studies of PM_{2.5} components (Lippmann et al. 2006; Dominici et al. 2007a; Franklin et al. 2008; Bell et al. 2009; Peng et al. 2009), the main second-stage analysis focused on explaining the city-to-city heterogeneity in the risk estimate associated with PM_{2.5} mass as a function of its components. We also considered gaseous pollutants and land-use variables as the explanatory variables. However, in our second-stage analysis, we also considered the risk estimates associated with PM_{2.5} components and gaseous pollutants as dependent variables, to provide a more comprehensive multipollutant assessment. For example, if a certain group of PM_{2.5}

Table 2. Medians of the IQRs of Air Pollutants Across Cities, Used to Compute and Display the Percentages of Excess Risk in Results for the 64- and 150-Cities Data Sets

Pollutant ^a	64 Cities Median IQR	150 Cities ^b Median IQR
PM _{2.5}	7.892	7.891
NO ₂ (ppb)	7.721	N/A
SO ₂ (ppb)	2.349	N/A
CO (ppm)	0.353	N/A
As	0.002	0.001
Cu	0.005	0.004
EC	0.414	0.369
Fe	0.061	0.053
K	0.070	0.053
Na	0.136	0.131
Ni	0.002	0.002
NO ₃ ⁻	0.902	0.787
OC	2.083	2.039
Pb	0.004	0.004
SO ₄ ²⁻	2.273	2.220
Se	0.001	0.001
Si	0.074	0.072
V	0.002	0.002
Zn	0.009	0.008

^a Values are µg/m³ unless noted.

^b Data on gaseous pollutants were not available for all the cities represented in the 150-city data set; therefore, risk estimates for those pollutants were not computed.

components explained the city-to-city variation in risk estimates associated with $PM_{2.5}$ mass, but also explained the same variation for NO_2 , then the interpretation would no longer be just about the potentially toxic components of $PM_{2.5}$, but instead would be about the pollution mix. However, a limitation of this analysis using the component data is that, unlike for the risk estimates for $PM_{2.5}$ mass (which often was sampled daily), the every-third-day or every-sixth-day sampling schedule for the speciation data caused the standard error of the risk estimates to be much larger, which resulted in an analysis with less statistical power than the analysis using $PM_{2.5}$ mass as the dependent variable. This limitation needs to be taken into consideration when interpreting the results. As with the two-pollutant analysis described earlier, we focused on the 64-cities data set, in which data on gaseous pollutants were also available, but we used the risk estimates associated with gaseous pollutants only for those days when the chemical speciation data were available. We did this so that all the pollutants and components had equal sample sizes, which allowed for a fair comparison. In these second-stage analyses, we used the all-year risk estimates.

Overall Evaluation

Aside from the statistical models and tests that we used to draw conclusions about specific sets of data analysis, as part of our overall evaluation we considered which pollutant ($PM_{2.5}$, $PM_{2.5}$ component, or gaseous pollutant) or source category was influential. In the first step of this analysis of city-specific components, or of source types, we considered several criteria, including the following:

- Consistency of risk estimates across cities and variables that explain their heterogeneity.
- Specificity of associations between pollutants and health outcomes.
- Influence of estimated exposure error (i.e., within-city spatial variation).
- Correlation of the pollutants with covariates, to infer potential confounding.
- Robustness of risk estimates in sensitivity analysis.

RESULTS

CHARACTERIZATION OF $PM_{2.5}$ COMPONENTS

The measurement data for the 27 $PM_{2.5}$ components exhibited a wide range of quality in terms of (1) percentage of values below detection limit, (2) fraction of zeros, and (3) monitor-to-monitor temporal correlation. Some of the components (e.g., Cd, Hg, and P) showed consistently high

percentages of values below the detection limit over the study period (Appendix Table B.5). Data for these elements also showed high percentages of zeros. The histograms of measurement data for $PM_{2.5}$ and its components (Appendix Figure B.2) suggest that the components with the highest percentages of zero values tended to have a frequency of zeros that is not consistent with the rest of the data distribution (see, for example, the histogram for As), indicating that the data distribution was truncated. Ba levels exhibited peculiar strong downward trends in many of the cities that were also reflected in the upward trends in the percentage of measurements below the detection limit and the percentage of zero measurements during the study time frame (Appendix Tables B.5 and B.6). Based on these results and considering the importance of identifying the source signatures for major pollution sources and for interpreting the toxicologic and epidemiologic background, we excluded Ba, Cr, Cd, Mg, and P from further analyses. The results of additional analyses, (1) correlation with the same-day temperature, (2) correlation with the average of 1- through 3-day temperatures, and (3) correlation with the day-of-week variable (through regression), are shown in Appendix Figures G.8 through G.13 and discussed as part of the interpretation of the health effects analysis. The mean and standard deviations of the key components in these cities are shown in Appendix Tables G.3, G.4, and G.5. A list of the AQS site identification numbers for the speciation monitors considered in each city is presented in Appendix Table B.7.

FACTOR ANALYSIS

The factor loadings for the six source categories obtained from the nationwide factor analysis of the $PM_{2.5}$ components and gaseous pollutants are shown in Figure 2 (the numerical results are shown in Appendix Table B.8). The first factor in the table shows high loadings for NO_2 , CO, EC, and OC, suggesting Traffic-related pollutants. Br (tire wear) and Cu (brake wear) also show correlations with this factor. The factor assigned to the Soil source category, with high loadings for Al, Ca, Si, and Ti, suggests soil-related components, as would be expected for resuspended roadway dust. The factor assigned to the Metals source category has high loadings for Fe, Mn, and Zn. The factor with high loadings for SO_2 , As, Hg, and Se is assigned to the Coal Combustion source category. The factor with Cl and Na is assigned to the source category Salt. The factor with high loadings for Ni and V (and showing low loadings with NO_2 and SO_2) is assigned to the Residual Oil Combustion source category. The factor analysis for which we specified five factors showed a mixing of these factors, and the analyses specifying seven and eight factors showed a splitting of these factors (see Appendix Figure G.14).

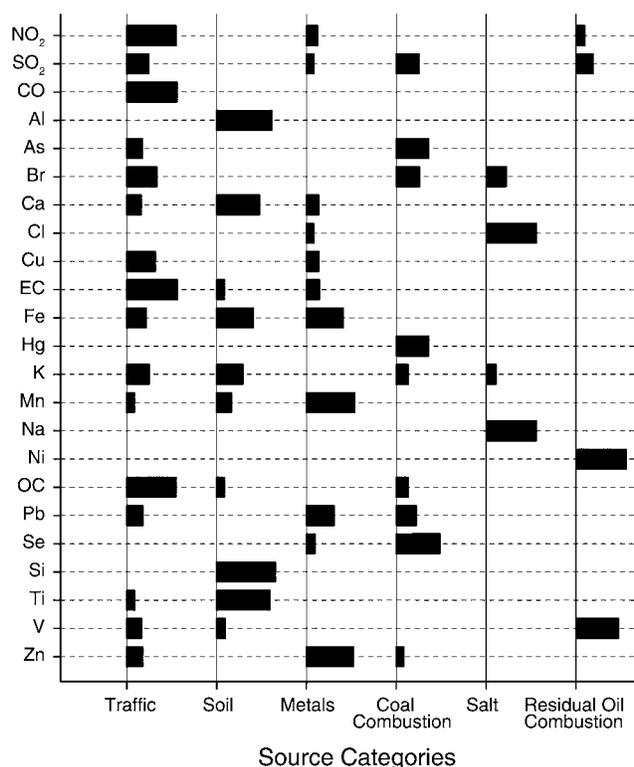


Figure 2. Factor loadings for the nationwide 64-cities factor analysis. Numerical results are shown in Appendix Table B.8.

We repeated the factor analysis for each of the 64 cities, and the factors were automatically assigned the name of one of the six major source categories identified in the national analysis using the rule described in the Methods section. Figure 3 shows the results with associated eigenvalues and illustrates which source categories were identified for each city. Traffic and Soil source categories were identified for 63 of the 64 cities. Other source categories were identified less frequently: Coal Combustion for 46 cities; Salt for 42 cities; Residual Oil Combustion for 29 cities; and Metals for 16 cities. Note that these are modeled categorizations based on the statistical model, and, therefore, an identification of a source category for a given city may not be correct. Also, these qualitative source categories still leave some ambiguities in their interpretations. For example, Salt may be marine aerosols or resuspended road dust particles containing salt sprinkled to melt ice and snow. Note that the number of cities used to compute the combined risk estimates is shown later in Figures 8, 10, and 12.

Thus, we had two sets of factor scores to be analyzed in the health effects analysis. We focused on the factor scores obtained for individual cities, but we also analyzed factor scores from the nationwide analysis for the time-series analysis of each of the 64 cities.

CITY-SPECIFIC TIME-SERIES ANALYSIS

Refer again to Table 1 to identify which set of analyses (the combination of the health outcome, the air pollution index, and the 150- or 64-cities data set) is being presented here. The table indicates the numbers of the figures (referred to in the following sections) that display information about an analysis set. Note that some of the figures are shown in Appendix B and some in Appendix G because the 150- and 64-cities data sets generated similar results. Note also that the risk estimates in all the figures showing the percentage of excess risk were computed per the median of the IQR of the pollutant computed for individual cities. In addition, all the results for the 64 cities, which are shown in Figures 7 through 13 and described in this section, are based only on the days when data on PM_{2.5} components were available. The following sections briefly describe the key features of each set of analyses. The pattern of associations of PM_{2.5} components, gaseous pollutants, and factor scores with all-cause mortality, CVD hospitalizations, and respiratory hospitalizations is summarized later in Tables 3 and 4. The combinations of city, pollutant, and lag day excluded in the computations of the combined estimates for the individual analysis sets are shown in Appendix Tables B.9 and B.10.

PM_{2.5} and All-Cause, CVD, and Respiratory Mortality in 148 Cities

Figure 4 shows that the associations were generally stronger in the warm season than in the cold, or year round, for these mortality outcomes. All-cause (nonaccidental) mortality showed the strongest associations at 0-day lag in the warm season and at 1-day lag (not significant) in the cold season. When the all-cause mortality risk estimates were combined for six U.S. regions, these 0-day lag associations in the warm season were seen in all the regional estimates except those for the Northwest and Southwest (Appendix Figure B.3, first panel). CVD mortality (a major fraction of all-cause mortality) showed a pattern of warm- and cold-season lag associations similar to those for the entire year, whereas respiratory mortality showed positive, though not statistically significant, associations on multiple days in the warm season and at 1-day lag in the cold season. Because the daily mortality counts were higher in the cold season in all of these mortality categories (presumably because of the influenza influence, cold temperature effects, and generally higher deaths in the winter), the overall all-year risk estimates tended to be influenced more strongly by the corresponding pattern of associations in the cold season. Thus, all-year all-cause mortality showed positive associations at 0- and 1-day lags, all-year CVD mortality did not show positive associations, and

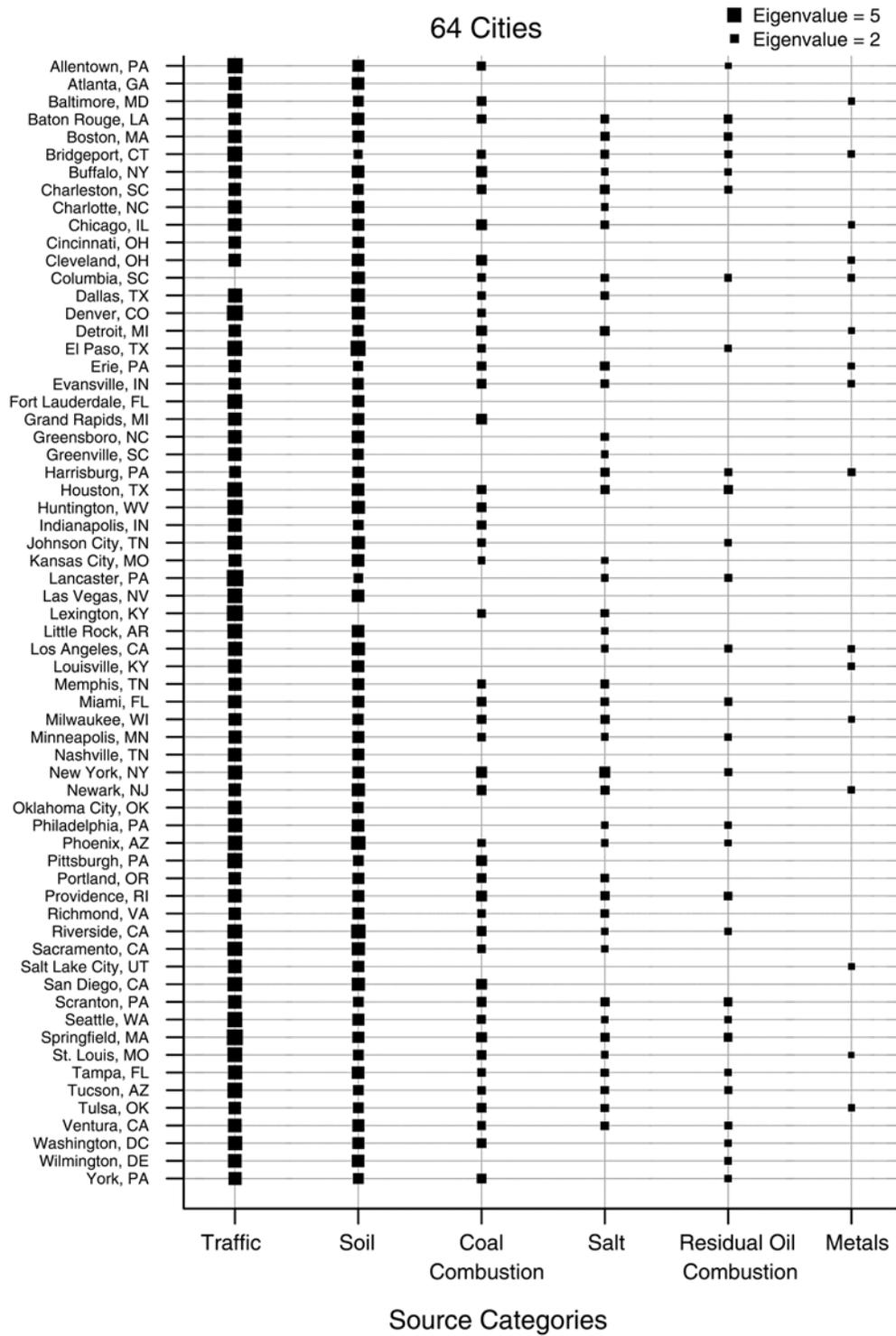


Figure 3. Major source categories identified in factor analyses conducted separately for 64 cities. The sizes of the black squares are proportional to their eigenvalues (the sum of the squares of factor loadings for a factor). Two eigenvalue sizes (5 and 2) appear in the legend for reference.

all-year respiratory mortality showed a nearly significant positive association at 1-day lag.

PM_{2.5} Components and All-Cause Mortality in 148 Cities

Results for this analysis are shown in Appendix Figure B.4 (first panel). A more complete assessment, which includes gaseous pollutants, is presented later in Figure 7 for the 64-cities data set.

The main feature of this analysis is that the sample sizes (in terms of days of available data for the pollution indices) were the same because, even for gaseous pollutants, the samples were subsets consisting only of those days when the PM_{2.5} chemical speciation data were available. The unequal lengths of the 95% confidence intervals across the pollutants are due to the variability in the skewness of the distribution of measured pollutant concentrations. For example, the confidence bands for Ni are much narrower than, say, those for PM_{2.5}, because the distribution of Ni concen-

trations was highly skewed. Note also that the confidence bands for this analysis set are much wider than those shown in Figure 4 above (and most of associations are not significant at $\alpha = 0.05$ level) because the PM_{2.5} components were collected every third or every sixth day. However, the lag structure of PM_{2.5} (strongest at 0-day lag) and seasonal pattern (stronger in the warm season) of the associations in this analysis set are the same as those shown in Figure 4 (described above). In the warm season, only NO₃⁻ and SO₄²⁻, both of which explained a major fraction of PM_{2.5}, showed positive associations at 0-day lag. EC and OC show associations at 1- through 3-day lags.

PM_{2.5} and All-CVD and Cause-Specific Elderly CVD Hospitalizations in 150 Cities

Figure 5 shows that the overall CVD hospitalization category had an apparent distinct seasonal pattern of associations with PM_{2.5}: There was no association in the warm

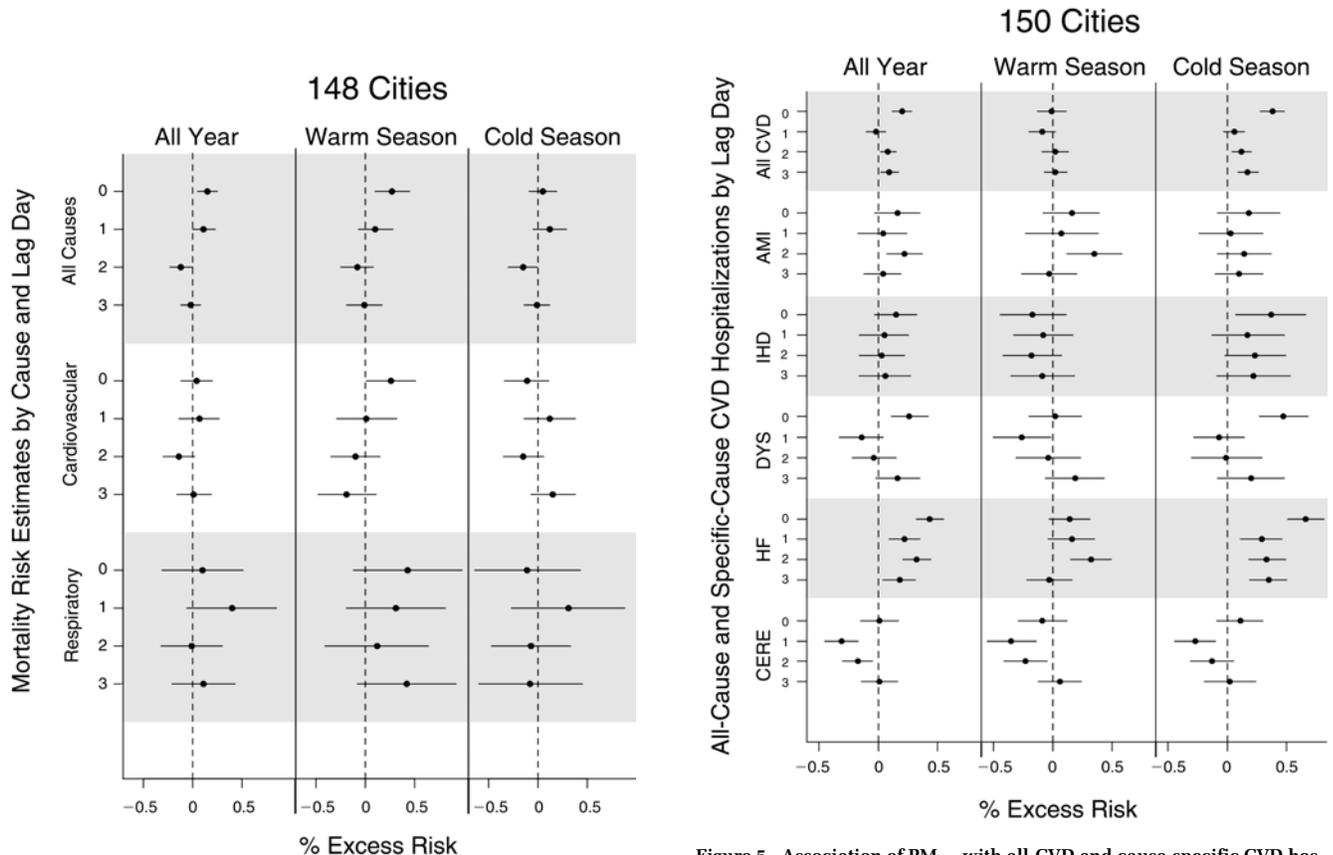


Figure 4. Association of PM_{2.5} with all-cause, CVD, and respiratory mortality in 148 cities. The estimated percentage of excess risk at 0- through 3-day lags for all-cause, cardiovascular, and respiratory mortality is per 7.4 $\mu\text{g}/\text{m}^3$ increase in PM_{2.5} (the median IQR for the cities combined).

Figure 5. Association of PM_{2.5} with all-CVD and cause-specific CVD hospitalizations of the elderly in 150 cities. The estimated percentage of excess risk at 0- through 3-day lags, for all CVD and cause-specific CVD elderly hospitalizations is per 7.4 $\mu\text{g}/\text{m}^3$ PM_{2.5} (the median IQR for the cities combined). AMI: acute myocardial infarction; IHD: ischemic heart disease; DYS: dysrhythmias; HF: heart failure; CERE: cerebrovascular disease.

season, and there was a strong association at 0-day lag and weaker positive associations at 1- through 3-day lags in the cold season. However, the figure also shows that this pattern of associations for the overall CVD category comes from mixing the distinctly different lag and seasonal associations evident in the CVD subcategories. Notably, cerebrovascular disease hospitalizations had peculiar significantly negative associations at 1- and 2-day lags. In contrast, heart failure hospitalizations showed positive associations at multiple lag days in both seasons. The hospitalizations for acute myocardial infarction showed the strongest association at 2-day lag. IHD hospitalizations showed positive associations at all lags in the cold season, although the association is statistically significant only at 0-day lag. It is important to note, in the following sections in which only the results for the “all CVD” hospitalizations are presented, that this category is a composite health-outcome indicator, and that the results presented in Figure 5 suggest the limitation of the indicator, particularly regarding lagged associations. When we combined CVD hospitalization risk estimates for six U.S. regions, this 0-day-lag association in the cold season was seen in all the regional estimates except those for the South-west (Appendix Figure B.3, center panel).

PM_{2.5} Components and CVD Hospitalizations in 150 Cities

Appendix Figure B.4, second panel, shows that the results of this analysis set yielded essentially the same pattern of associations as those in the corresponding analysis of the 64-cities data set, which also included gaseous pollutants. Therefore, we describe the results later in Figure 9.

PM_{2.5} and All-Respiratory, Pneumonia, and COPD Causes for Elderly Hospitalizations in 150 Cities

The combined respiratory category in Figure 6 shows positive associations at 0- through 3-day lags in the warm season (which is mainly a reflection of the COPD results) and an association at 0-day lag in the cold season (which is derived from both the pneumonia and COPD results). When the respiratory hospitalization risk estimates were combined for six U.S. regions, positive associations for multiday lags in the warm season were seen in all the regional estimates except those for the Northwest (for which negative associations were seen at multiday lags) and the Southeast; 0-day-lag associations in the cold season were suggested in the Northeast and Southeast (Appendix Figure B.3, right panel).

PM_{2.5} Components and Respiratory Hospitalizations in 150 Cities

The results of this analysis set, shown in Appendix Figure B.4, third panel, yielded essentially the same pattern of associations as those in the corresponding analysis of the 64-cities data set, which also included gaseous pollutants. Therefore, we describe the results below in Figure 11.

PM_{2.5}, Gaseous Pollutants, and PM_{2.5} Components and All-Cause Mortality in 64 Cities

PM_{2.5} mass concentration was significantly associated with all-cause mortality at 0-day lag during the warm season (Figure 7). Although NO₃⁻ and SO₄²⁻, the two major contributors to PM_{2.5} mass, also showed positive associations at 0-day lag in the warm season, other relatively major components (EC, OC, and Si), minor components (Fe, Pb, and V), and gases (NO₂ and SO₂) were associated with other lags in the warm season. In the cold season, SO₂, Cu, K, and Si were associated with all-cause mortality, but PM_{2.5} showed no association. The results from a corresponding analysis using raw data, rather than the deviations from the monthly means, are shown in

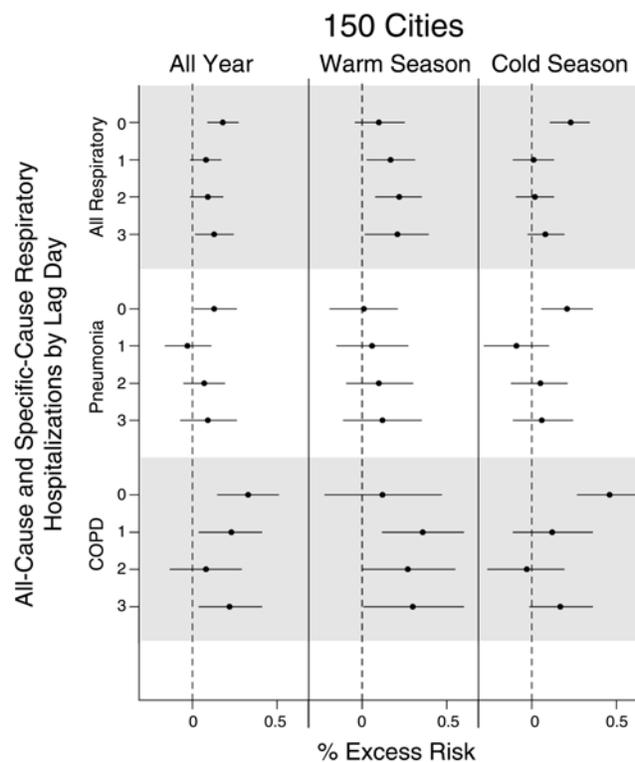


Figure 6. Association of PM_{2.5} with all-respiratory, pneumonia, and COPD hospitalizations of the elderly in 150 cities. The estimated percentage of excess risk at 0- through 3-day lags for all-respiratory, pneumonia, and COPD elderly hospitalizations is per 7.4 μg/m³ PM_{2.5} (the median IQR for the cities combined).

Appendix Figure B.5, first panel. The pattern of lag structure of associations and the magnitude of the risk estimates are very similar, though the risk estimates for the warm season for the raw variables are slightly smaller; and the risk

estimates for the cold season for the raw variables are slightly larger.

Factor Scores Derived for Individual Cities and All-Cause Mortality in 64 Cities

The results, in Figure 8, show that the number of cities for which common source categories were identified (and that therefore contributed to the combined risk estimates) varied, as shown in Figure 3: 63 cities for Traffic and Soil, 46 cities for Coal Combustion, 42 cities for Salt, 29 cities for Residual Oil Combustion, and 16 cities for Metals. The Traffic category was significantly associated with all-cause mortality at 1-day lag for the all-year analysis. The Traffic category also showed a similar pattern of lagged associations (strongest at 1-day lag) for both seasons. The Soil category also showed an association at 1-day lag for the all-year and warm season analyses. The Coal Combustion category showed a 3-day-lag association, in part consistent with the lagged (2- and 3-day) association of SO₂ seen in

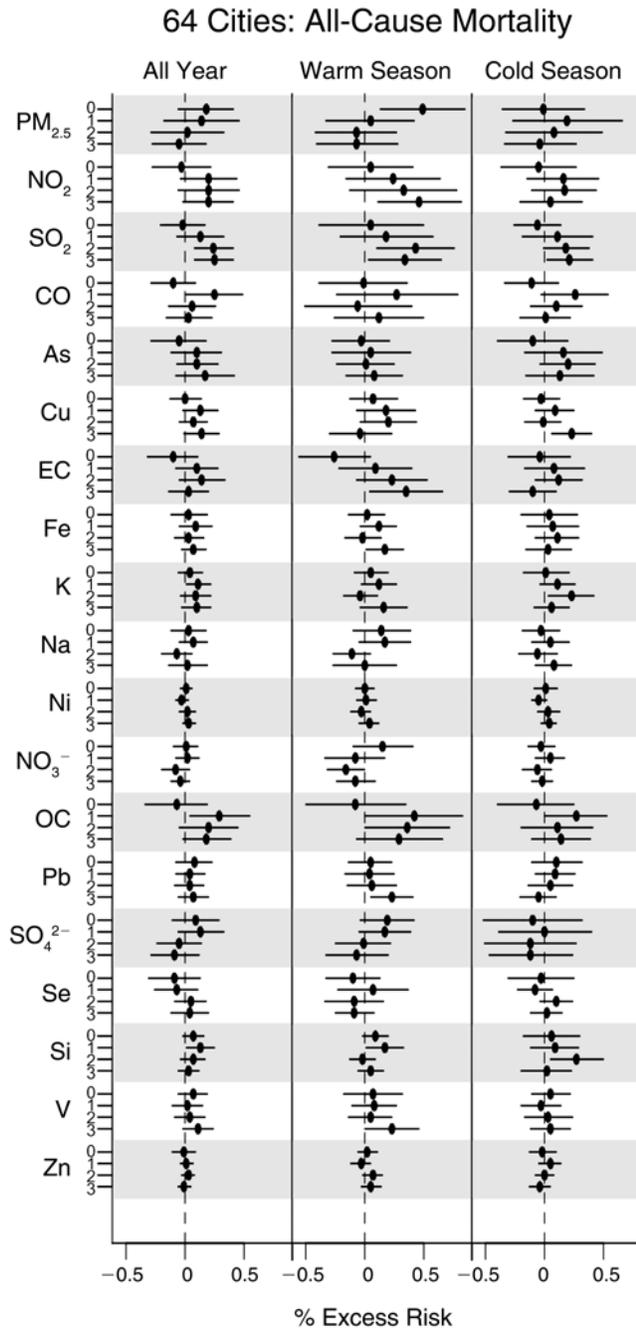


Figure 7. Association of PM_{2.5}, gaseous pollutants, and PM_{2.5} chemical components with all-cause mortality in 64 cities. The estimated percentage of excess risk at 0- through 3-day lags for all-cause mortality is per the median IQR of the pollutants for the cities combined.

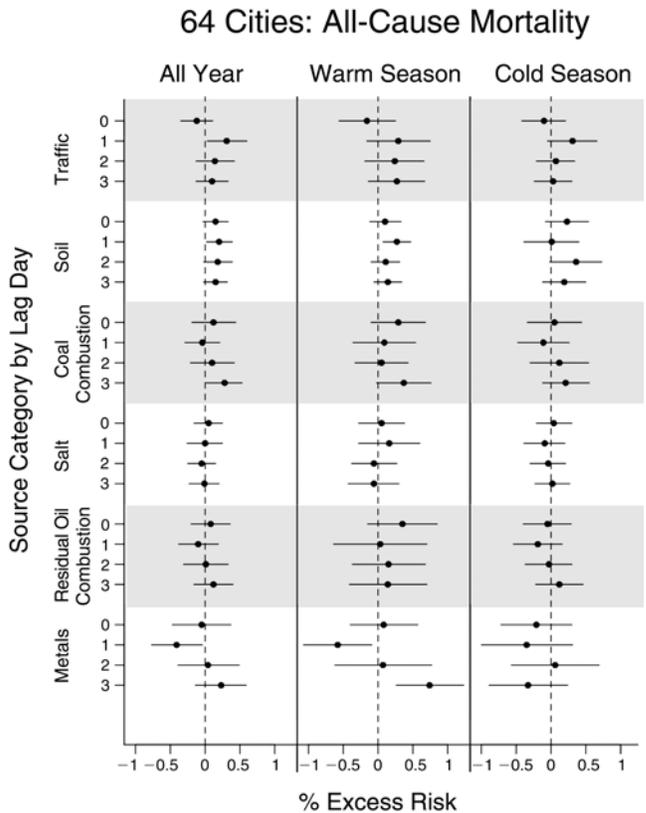


Figure 8. Association of factor scores derived for individual cities with all-cause mortality in 64 cities. The estimated percentage of excess risk at 0- through 3-day lags for all-cause mortality, based on factor scores computed from factor analysis for each city, is per the median IQR of the factor scores for the cities combined. The results are based only on the days when data on PM_{2.5} chemical components were available.

Figure 7. The Metals category was significantly negatively associated with all-cause mortality at 1-day lag for the all-year and warm season analyses, but it also showed a significantly positive association at 3-day lag in the warm season. The negative association might have been related to the fact that the Metals category was the only pollution index that was negatively associated with same-day temperature in the majority of the 64 cities during the warm season (see Appendix Figure G.8). However, although the risk estimates from the analysis using the factor scores from the nationwide (64-cities) factor analysis (Appendix Figure B.6, left panel) are very similar to those in Figure 8, especially for the Traffic and Soil source categories, the appendix figure shows no significantly negative association for the Metals category.

PM_{2.5}, Gaseous Pollutants, and PM_{2.5} Components and CVD Hospitalizations in 64 Cities

Unlike the results for all-cause mortality, the lag structure of associations with the gaseous pollutants and PM_{2.5} components was generally consistent with that for PM_{2.5}: The strongest associations were at 0-day lag (Figure 9). For the all-year analysis, NO₂, SO₂, CO, and EC were more strongly associated with CVD hospitalizations than was PM_{2.5}. V was significantly associated with CVD hospitalizations at 0-, 1-, and 3-day lags in the all-year analysis. The seasonal risk estimates indicate that most of the 0-day-lag associations seen in the all-year analysis come from the associations in the cold season. The results from a corresponding analysis using raw data, rather than the deviations from the monthly means, are shown in Appendix Figure B.5, second panel. The pattern of the lag structure of associations between these pollutants and CVD hospitalizations and the magnitude of the risk estimates are essentially the same as those in Figure 9.

Factor Scores Derived for Individual Cities and CVD Hospitalizations in 64 Cities

The Traffic category showed the most significant associations with CVD hospitalizations in the all-year and cold season analyses (Figure 10), which was not surprising because all the components associated with the Traffic category, NO₂, CO, Cu, EC, and OC, were also individually significantly associated with CVD hospitalizations (Figure 9). However, the associations of the Traffic category with CVD hospitalizations in the all-year or cold season analyses were no more significant than those for some of the individual PM_{2.5} components or gases. The risk estimates from the analysis using the factor scores from the nationwide (64-cities) factor analysis (Appendix Figure B.6, center panel) are generally consistent with those in Figure 10.

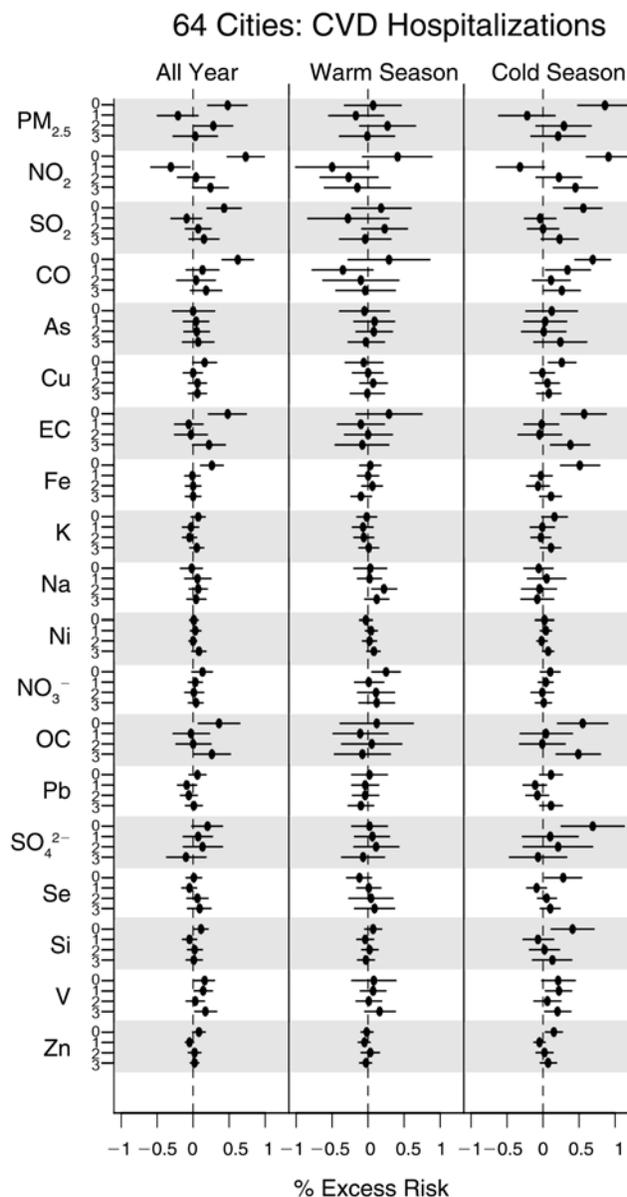


Figure 9. Association of PM_{2.5} mass concentrations, gaseous pollutants, and PM_{2.5} chemical components with CVD hospitalization in 64 cities. The estimated percentage of excess risk at 0- through 3-day lags for CVD hospitalizations is per the median IQR of the pollutants for the cities combined. The results are based only on the days when data on PM_{2.5} chemical components were available.

PM_{2.5}, Gaseous Pollutants, and PM_{2.5} Components and Respiratory Hospitalizations in 64 Cities

As with the results for CVD hospitalizations (Figure 9), the lag structure of associations for the gaseous pollutants and PM_{2.5} components in the all-year analysis was generally consistent with that for PM_{2.5} (i.e., strongest at 0-day lag; Figure 11). Unlike the results for CVD hospitalizations, some pollutants were also significantly associated with

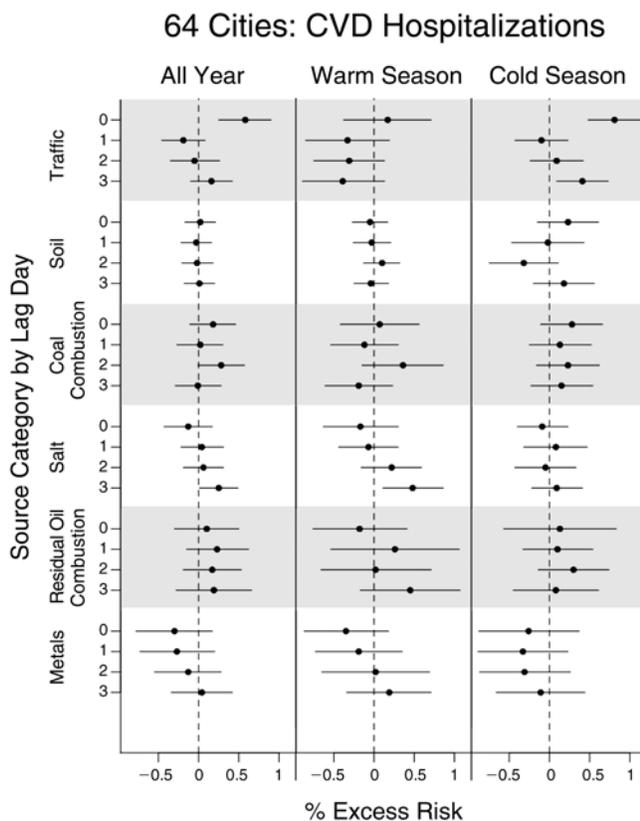


Figure 10. Association of factor scores derived for individual cities with CVD hospitalizations in 64 cities. The estimated percentage of excess risk at 0- through 3-day lags for CVD hospitalizations, based on factor scores derived from factor analysis for each city, is per the median IQR of the factor scores for the cities combined. The results are based only on the days when data on PM_{2.5} chemical components were available.

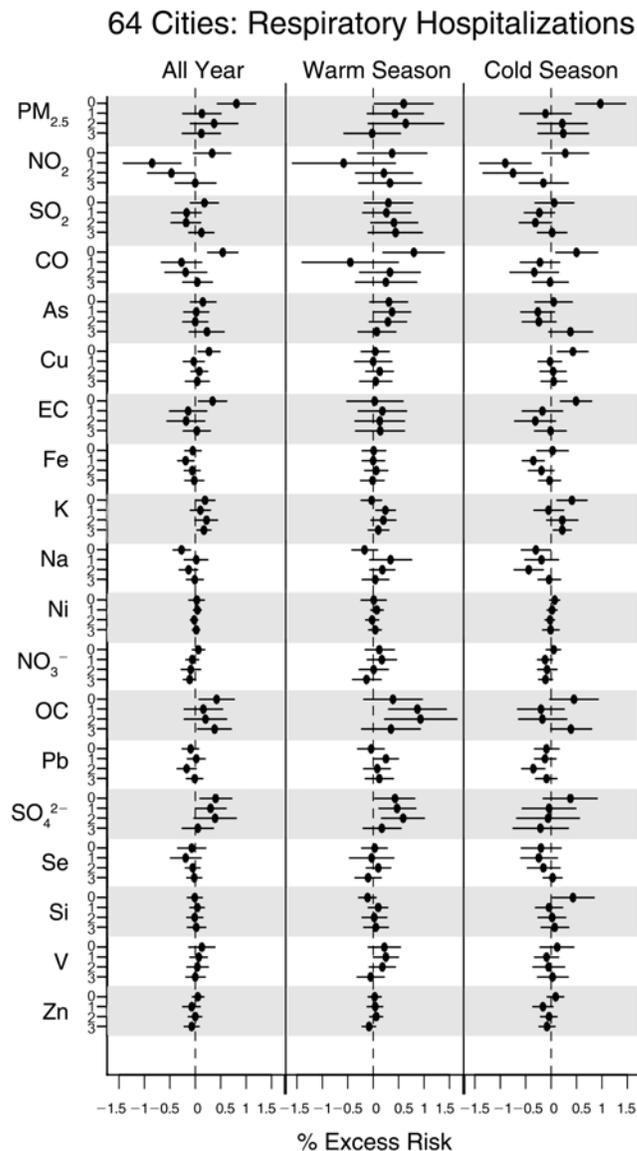


Figure 11. Association of PM_{2.5} mass concentrations, gaseous pollutants, and PM_{2.5} chemical components with respiratory hospitalizations in 64 cities. The estimated percentage of excess risk at 0- through 3-day lags for respiratory hospitalizations is per the median IQR of the pollutants for the cities combined. The results are based only on the days when data on PM_{2.5} chemical components were available.

respiratory hospitalizations in the warm season. Most notably, SO₄²⁻ showed statistically significant associations at 0- through 2-day lags in the warm season. OC also showed statistically significant associations at 1- and 2-day lags in the warm season. These multiday associations for SO₄²⁻ and OC (which are major mass contributors to PM_{2.5}) in the warm season seem to be reflected in the multiday positive (though not statistically significant) associations for PM_{2.5} in the warm season. The results from a corresponding analysis using raw data, rather than the deviations from the monthly means, are shown in Appendix Figure B.5, third panel. The pattern of the lag structure of associations and the magnitude of the risk estimates are very similar to those in Figure 11, especially for the associations in the cold season. However, the associations for SO₄²⁻ and OC in the warm season were less significant in the analysis using the raw pollution data.

Factor Scores Derived for Individual Cities and Respiratory Hospitalizations in 64 Cities

Of all the source categories, the Traffic category showed the strongest associations with respiratory hospitalizations (Figure 12). This result is consistent with the associations found for traffic-related individual pollutants (NO₂, CO, EC, and OC, in Figure 11). The risk estimates from the analysis using the factor scores from the nationwide (64-cities) factor analysis (Appendix Figure B.6, right panel) are generally consistent with those in Figure 12.

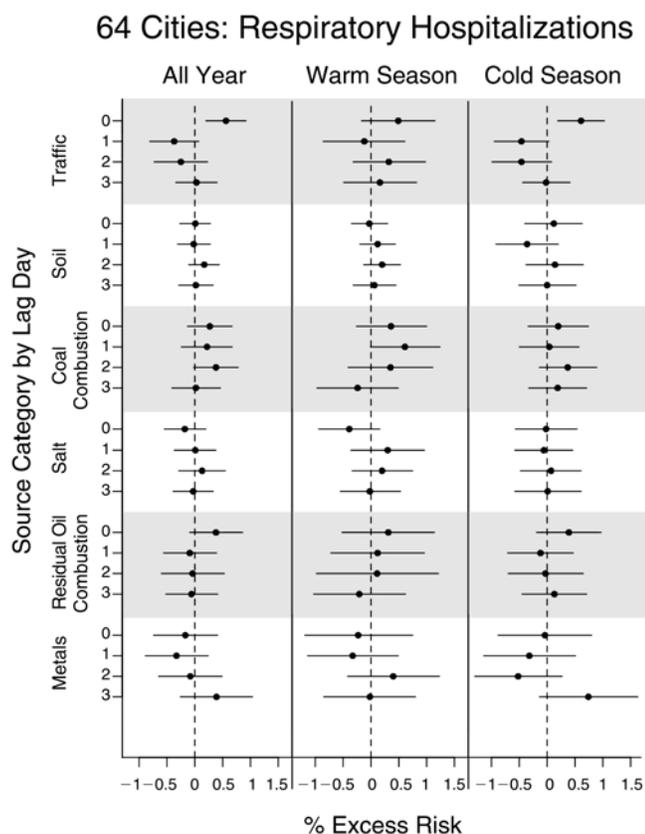


Figure 12. Association of factor scores derived for individual cities with respiratory hospitalizations in 64 cities. The estimated percentage of excess risk at lag 0- through 3-day lags for respiratory hospitalizations, based on factor scores derived from factor analysis for each city, is per the median IQR of the factor scores for the cities combined. The results are based only on the days when data on PM_{2.5} chemical components were available.

All-Cause Mortality Risk Estimates Associated with Key PM_{2.5} Components and Gaseous Pollutants With and Without PM_{2.5} in the Model in 64 Cities

Note that the results, shown in Figure 13, left figures, are based on models that either include or leave out PM_{2.5} mass. Including PM_{2.5} mass in the regression model did not meaningfully affect the all-cause mortality risk estimates for the key components and gaseous pollutants, except those for EC and SO₄²⁻, which were greatly reduced, or even became negative. This was very likely due to the high correlation of PM_{2.5} mass and these components (both significant fractions of PM_{2.5} mass), which therefore resulted in biased individual risk estimates in a two-pollutant model.

CVD Hospitalization Risk Estimates Associated with Key PM_{2.5} Components and Gaseous Pollutants With and Without PM_{2.5} in the Model in 64 Cities

Figure 13, center figures, shows the results based on models that either include or leave out PM_{2.5} mass. Including PM_{2.5} mass in the regression model did not meaningfully affect the CVD hospitalization risk estimates for the key components and gaseous pollutants, except those for OC and SO₄²⁻ in the all-year and warm season analyses. Again, the sensitivity of these components' effect estimates is likely a result of their higher intercorrelation with PM_{2.5} mass.

Respiratory Hospitalization Risk Estimates Associated with Key PM_{2.5} Components and Gaseous Pollutants With and Without PM_{2.5} in the Model in 64 Cities

Figure 13, right figures, shows the results based on models that either include or leave out PM_{2.5} mass. Including PM_{2.5} mass in the regression model did not meaningfully affect the respiratory hospitalization risk estimates for the key components and gaseous pollutants, except those for OC, NO₂, and SO₂, again likely a result of coefficient biases induced by simultaneously constructing models with two correlated pollutant variables.

SUMMARY OF THE TIME-SERIES ANALYSIS OF THE 64-CITIES DATA SET

To summarize the associations between all-cause mortality, CVD hospitalizations, and respiratory hospitalizations and PM_{2.5} mass, its components, gaseous pollutants, and factor scores consistently, the associations shown in Figures 7 through 13 are summarized in Table 3 (all-year results) and Table 4 (seasonal results). We observed the following.

All-Cause Mortality

- In the all-year analysis (Table 3), most consistent associations (in terms of significance and the number of associations with components and source categories) were found at 1-day lag, though no significant associations were found for PM_{2.5} mass.
- In the seasonal results (Table 4), the gaseous pollutants and PM_{2.5} components showed lagged associations, whereas PM_{2.5} showed the strongest association on the same day. In this case, neither the PM_{2.5} components nor the gaseous pollutants helped explain the association of PM_{2.5} mass concentration with the outcome.
- More pollutants were associated with all-cause mortality in the warm season (Table 4: NO₂, SO₂, EC, Fe, OC, Pb, Si, and V) than in the cold season (SO₂, Cu, K, OC, and Si).

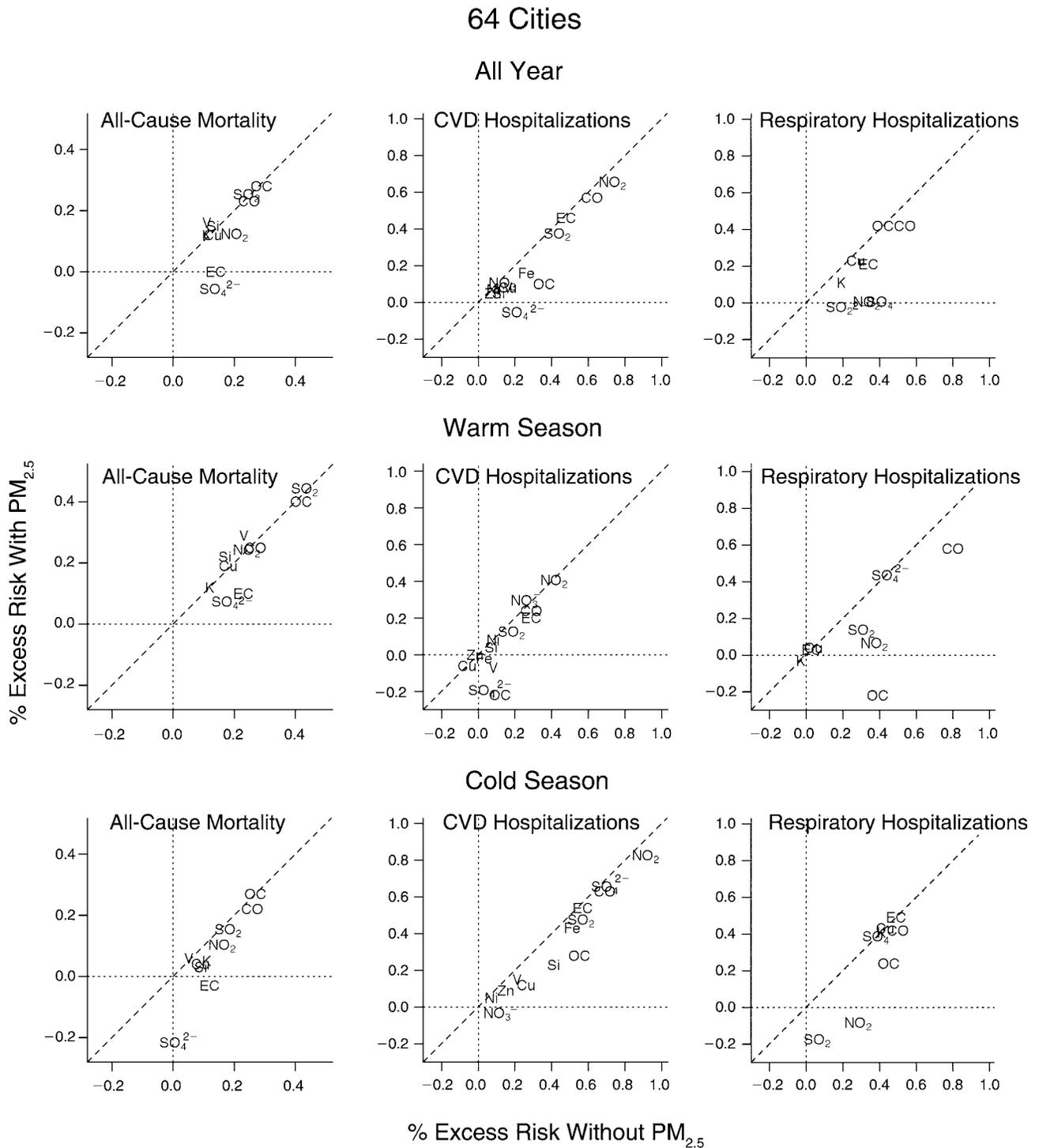


Figure 13. Estimated risks with and without PM_{2.5} mass in the model for all-cause mortality, CVD hospitalizations, and respiratory hospitalizations for the key PM_{2.5} components and gaseous pollutants. The estimated percentage of excess risk associated with the key PM_{2.5} chemical components and gaseous pollutants is per the median IQR of each of the components for the 64 cities combined, at each component's most significant lag times, with and without PM_{2.5} mass in the model. **For all-cause mortality**, the estimates without PM_{2.5} mass correspond to those in Figure 7. The lag days used (in parentheses) were NO₂ (1), SO₂ (2), CO (1), Cu (1), EC (2), K (1), OC (1), SO₄²⁻ (1), Si (1), and V (3). **For CVD hospitalizations**, the estimates without PM_{2.5} mass correspond to those in Figure 9. The lag day used was 0 day for all pollutants except Ni, for which the 3-day lag was used. **For respiratory hospitalizations**, the estimates without PM_{2.5} mass correspond to those in Figure 11. The lag day used was 0 day for all pollutants.

Table 3. Summary of the Associations Between Lagged Exposures (0–3 Lag Days) to Air Pollution Variables and Health Outcomes for the 64-Cities, All-Year Analysis^{a,b}

Pollutant or Source Category	All-Cause Mortality				CVD Hospitalizations				Respiratory Hospitalizations			
	0	1	2	3	0	1	2	3	0	1	2	3
PM _{2.5}					+		+		+			
NO ₂				+	+	-		+	+	-	-	
SO ₂			+	+	+							
CO		+			+				+			
As												
Cu		+		+	+				+			
EC					+			+	+			
Fe					+					-		
K		+							+		+	+
Na									-			
Ni												
NO ₃ ⁻					+							-
OC		+		+	+			+	+			+
Pb												-
SO ₄ ²⁻					+				+	+	+	
Se												
Si		+			+							
V				+	+	+		+				
Zn						-						
Traffic		+			+				+	-		
Soil		+	+	+								
Coal Combustion				+			+				+	
Salt								+				
Residual Oil Combustion												
Metals		-										

^a Positive or negative associations that were significant at $\alpha = 0.05$ (two-tailed) are indicated by + or - in **bold** type; those at $\alpha = 0.10$ are not bold. Blank areas denote associations with lesser statistical significance. Note that these analyses included only the days when PM_{2.5} component data were available.

^b This table is a summary of the data in Figures 7 through 12.

- The risk estimates associated with gaseous pollutants were not sensitive to inclusion of PM_{2.5} mass in the model (Figure 13). The risk estimates of the key components were robust to inclusion of PM_{2.5} mass in the model, except for EC, which is a component whose concentrations are often highly correlated with PM_{2.5} mass concentrations, and which could result in biased coefficient estimates when it is included in the same model as PM_{2.5}.
- In the all-year analysis (Table 3), the Traffic and Soil source categories were associated with all-cause mortality at 1-day lag, and the Coal Combustion source category was associated at the 3-day lag. The seasonal analysis (Table 4) found significant positive associations with all-cause mortality for Soil (1-day lag) and Metals (3-day lag) source categories in the warm season.

Table 4. Summary of the Associations Between Lagged Exposures (0–3 Lag Days) to Air Pollution Variables and Health Outcomes for the 64-Cities Warm- and Cold-Season Analyses^{a,b}

Pollutant or Source Category	All-Cause Mortality				CVD Hospitalizations				Respiratory Hospitalizations						
	Warm Season		Cold Season		Warm Season		Cold Season		Warm Season		Cold Season				
	0	1	2	3	0	1	2	3	0	1	2	3			
PM _{2.5}	+								+	+			+		
NO ₂				+				+	-		+				- - -
SO ₂			+	+			+	+			+				-
CO							+	+	+	+				+	
As											+				+
Cu			+					+						+	
EC	-		+	+				+		+				+	
Fe				+				+							-
K							+				+			+	+
Na								+			+			-	-
Ni															
NO ₃ ⁻				-				+							
OC		+	+			+				+		+	+	+	+
Pb				+											-
SO ₄ ²⁻										+		+	+	+	
Se										+					
Si		+				+				+				+	
V				+						+	+		+	+	
Zn										+					
Traffic						+				+		+			+ - -
Soil		+				+									
Coal Combustion												+			
Salt								+							
Residual Oil Combustion															
Metals		-		+											

^a Positive or negative associations that were significant at $\alpha = 0.05$ (two-tailed) are indicated by + or - in **bold** type; those at $\alpha = 0.10$ are not bold. Blank areas denote associations with lesser statistical significance. Note that these analyses included only the days when PM_{2.5} component data were available.

^b This table is a summary of the data in Figures 7 through 12.

CVD Hospitalizations

- Significant associations were found for many pollutants, mostly at 0-day lag and mostly in the cold season (Table 4).
- The pollutants associated with CVD hospitalizations at 0-day lag in the cold season were PM_{2.5}, NO₂, SO₂, CO, Cu, EC, Fe, OC, SO₄²⁻, Se, Si, and Zn. V showed

associations at 1- and 3-day lags, and it also showed a nearly significant association at 0-day lag. Several pollutants with 0-day-lag associations (NO₂, SO₂, CO, EC, and OC) also showed associations at 3-day lag. NO₃⁻ (0-day lag) and Na (2-day lag) showed associations in the warm season (Table 4).

- The risk estimates for gaseous pollutants were not sensitive to inclusion of $PM_{2.5}$ mass in the model (Figure 13). The risk estimates for the key components were not sensitive to inclusion of $PM_{2.5}$ mass in the model, except those for OC and SO_4^{2-} .
- Consistent with the results for several of the traffic-related pollutants (NO_2 , SO_2 , CO, EC, and OC), the Traffic source category showed significant associations at 0- and 3-day lags in the cold season (Table 4). The Salt source category was associated with CVD hospitalizations at 3-day lag in the warm season.

Respiratory Hospitalizations

- In contrast to CVD hospitalizations, respiratory hospitalizations were associated with the pollutants both in the warm ($PM_{2.5}$, CO, As, K, OC, and SO_4^{2-}) and cold ($PM_{2.5}$, CO, Cu, EC, K, and Si) seasons (Table 4).
- The risk estimates for OC, NO_2 , and SO_2 were sensitive to the inclusion of $PM_{2.5}$ mass in the regression model (Figure 13).
- The Traffic source category was associated with respiratory hospitalizations at 0-day lag in the cold season (Table 4).

Overall Observations Across Health Outcomes

- The associations of all-cause mortality (effect size and significance) with the pollutants were generally weaker than those for CVD and respiratory hospitalizations (Tables 3 and 4).
- Ni showed no associations with health outcomes at any lag day (Tables 3 and 4).
- In the all-year analysis, CO and OC were significantly associated with all three health outcomes (Table 3).
- In the all-year analysis, the Traffic source category was significantly associated with all of the three health outcomes (Table 3).
- In the seasonal analyses, EC, OC, and Si showed significant associations with the three health outcomes in one or both seasons (Table 4).
- The associations of the factor scores with health outcomes were less significant than those for some of the specific components or gaseous pollutants that were related to the sources of pollution (Figures 7 through 12).

Note: We also conducted an analysis of daily (rather than the EPA CSN's every third or every sixth day) $PM_{2.5}$ chemical speciation data and its associations with daily mortality

and hospitalizations in Detroit, Michigan, and Seattle, Washington, for the years 2002 through 2004 (shown in Appendix F on the HEI Web site). The daily data allowed us to examine potential multiday effects using distributed lag models. The research was made possible by HEI supplemental funding for speciation analyses of daily $PM_{2.5}$ FRM filters. The results of our mortality analysis for the two cities have been published (Zhou et al. 2011).

SECOND-STAGE ANALYSIS

In the main second-stage analysis, described in the Methods section, we regressed $PM_{2.5}$ mass risk estimates from 150 cities (148 cities for all-cause mortality) on the city-specific averages of components and gaseous pollutants (as there were fewer cities with available data for gases; the number of cities for each analysis is noted in the captions in the following figures). Note that the $PM_{2.5}$ mass concentration data were often collected more frequently (nearly daily in some cities) than were the CSN data (every third or sixth day); they thus provided more precise risk estimates (Figures 4 through 6) compared to those from the analyses using CSN data (Figures 7 through 13). Because the combined $PM_{2.5}$ risk estimates for all-cause mortality and CVD and respiratory hospitalizations all happened to show the strongest associations at 0-day lag in the all-year analyses, we used the risk estimates at 0-day lag as dependent variables. For the additional second-stage analyses in which the risk estimates for $PM_{2.5}$ components and gaseous pollutants (from Figures 7, 9, and 11) were dependent variables, the most significant lags varied across the pollutants and the outcomes. Therefore, we used the risk estimates for the most significant lags (indicated for each pollutant in the figures). Note that the sample sizes for these additional second-stage analyses were smaller than those for the main second-stage analyses both in terms of the number of cities and the number of days used. This is a limitation that needs to be considered when interpreting these additional second-stage analyses. We also regressed the risk estimates of the pollutants on the land-use variables, in the additional second-stage analysis, but we do not present these results because the confidence bands were even wider than those using city-specific average pollution levels.

All-Year, 0-Day-Lag $PM_{2.5}$ Risk Estimates

All-Cause Mortality Risk Estimates Associated with Averages of $PM_{2.5}$ Components and Gaseous Pollutants

Figure 14, left panel, shows that the average city-specific values for SO_4^{2-} , weekday excess $PM_{2.5}$ (the difference

between Wednesday–Thursday averages and Saturday–Sunday averages) and the average values for Pb and V significantly or nearly significantly positively influenced the PM_{2.5} risk estimates.

CVD Hospitalization Risk Estimates Associated with Averages of PM_{2.5} Components and Gaseous Pollutants

Figure 14, center panel, shows that the average city-specific values of Cu, Ni, V, SO₂, NO₂, Fe, Na, NO₃⁻, the weekday excess PM_{2.5}, and the average values for Zn and Pb significantly or nearly significantly positively influenced the CVD hospitalization risk estimates associated with PM_{2.5}. Because it has been pointed out (Dominici et al. 2007a) that the results for Ni and V in this type of analysis could be highly influenced by New York City data (levels of Ni and V have been particularly high in New York City, one of the largest cities in the United States), we also ran the analysis without New York City (Appendix Figure B.7, left panel), but the results were essentially unchanged.

Respiratory Hospitalization Risk Estimates Associated with Averages of PM_{2.5} Components and Gaseous Pollutants

Figure 14, right panel, shows that the PM_{2.5} components associated with the cities with higher respiratory hospitalization risk estimates were very similar to those for CVD hospitalizations: Cu, NO₂, weekday excess PM_{2.5}, V, Ni, SO₂, and Na. Again, we reran the analysis without New York City (Appendix Figure B.7, right panel), but Cu, NO₂, V, and Ni remained high in the ranking.

All-Cause-Mortality Risk Estimates Associated with Land-Use, Port-, and Traffic-Related Data

Figure 15, first panel, shows that seaport berth volume of ports within 60 miles of the cities and the sum of road lengths were the two most influential variables in explaining the variation of PM_{2.5} all-cause mortality risk estimates across the cities. The significant negative influence of the distance to a large seaport was consistent with the positive influence of the seaport berth volume.

CVD Hospitalization Risk Estimates Associated with Land-Use, Port-, and Traffic-Related Data

Figure 15, center panel, shows that the extent of land development (the percentage developed and percentage high-density development) was a significant positive predictor of PM_{2.5} CVD hospitalization risk estimates across the cities. Seaport berth volume of ports within 60 miles of the cities and the sum of road lengths were also positive predictors of this outcome, though their effect estimates were not as significant

as they were for all-cause mortality. The distance to a large seaport and the percentage of farm land were negatively associated with this health outcome.

Respiratory Hospitalization Risk Estimates Associated with Land-Use, Port-, and Traffic-Related Data

Figure 15, right panel, shows that the land-use variables associated with the percentage of developed land and the percentage of high-density development were positive (and nearly significant) predictors of the PM_{2.5} risk estimates for this outcome. However, unlike the results for all-cause mortality and CVD hospitalizations, neither the seaport berth volume nor the sum of road lengths was associated with the city-to-city variations in the risk estimates for this outcome. The percentage of farm land was the most significant negative predictor.

All-Year All-Cause Mortality Risk Estimates Associated with Key PM_{2.5} Components and Gaseous Pollutants

The results shown in Appendix Figure B.8, for the 64-city data set, are for key PM_{2.5} components and gaseous pollutants as a function of average PM_{2.5} mass, its components, and gaseous pollutants. The pollution variables from the 64-city regression are shown with the chosen lag days in parentheses; the predictor pollution variables (i.e., the city-specific averages for each pollutant) used in the second-stage regression are shown across the top x-axis. For example, the top-left corner of the figure shows the PM_{2.5} regression coefficients for mortality risk at 0-day lag versus the same coefficients calculated in a second-stage regression using the corresponding city-specific averages of PM_{2.5}. There was no indication that cities with high average levels of PM_{2.5} had larger PM_{2.5} risk estimates.

Possibly in part a result of the smaller sample size (related to the every-third- or every-sixth-day sampling schedule of the speciation data), and possibly in part because of the smaller number of cities covered (as compared to the data on which Figure 14, left panel, is based), very few estimates in Figure B.8 are statistically significant. The pattern of associations between PM_{2.5} mass risk estimates and city-specific averages of pollutants in Figure B.8 can be compared with those in Figure 14: unlike in Figure 14, neither SO₄²⁻ nor Pb in Figure B.8 was positively associated with PM_{2.5} risk estimates, though V was positively (though not significantly) associated. NO₃⁻ and Zn were significantly negative predictors of the SO₂ risk estimates; As was a significantly positive predictor of the V risk estimate.

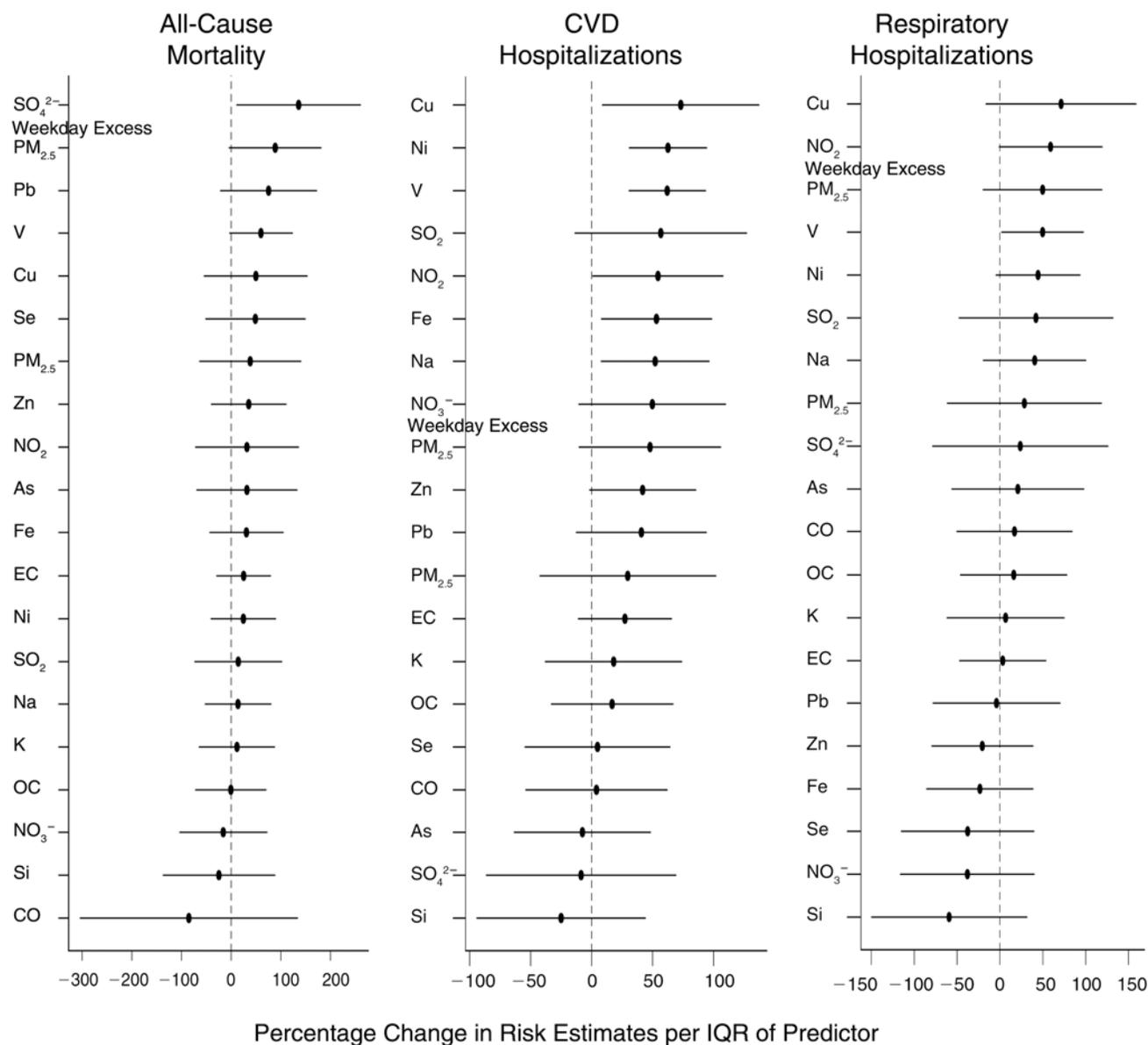


Figure 14. Percentage change at 0-day lag in all-cause mortality and CVD and respiratory hospitalization risk estimates associated with PM_{2.5} and gaseous pollutants. The results are per the IQRs of the mean levels of air pollution variables across cities in the second-stage analysis. The data analyzed are for PM_{2.5} chemical components (148 cities), NO₂ (95 cities), SO₂ (106 cities), and CO (106 cities). PM_{2.5} chemical components except for SO₄²⁻ are log-transformed. (Note that data for the gaseous pollutants are not available for all of the 150 cities, whereas data for PM_{2.5} are available for most or all of the cities.) The risk estimates for all-cause mortality and CVD and respiratory hospitalizations for the individual cities in these second-stage analyses are shown in Figures 4, 5, and 6, respectively.

All-Year CVD Hospitalization Risk Estimates Associated with Key PM_{2.5} Components and Gaseous Pollutants

The results shown in Appendix Figure B.9, for the 64-cities data set, are for key PM_{2.5} components and gaseous pollutants as a function of average PM_{2.5} mass, its components, and gaseous pollutants. The significant predictors of PM_{2.5} CVD hospitalization risk estimates across

the 64 cities were mostly consistent with those found in the larger set of cities (Figure 14, center panel): NO₂, EC, Fe, Ni, and V. Note that the city average for NO₂ was a significantly positive predictor of NO₂ risk estimates (that is, NO₂ risk estimates tended to be higher in cities with higher NO₂ levels), a pattern that is not seen in other pollutants. In contrast, Cu concentration was a significantly

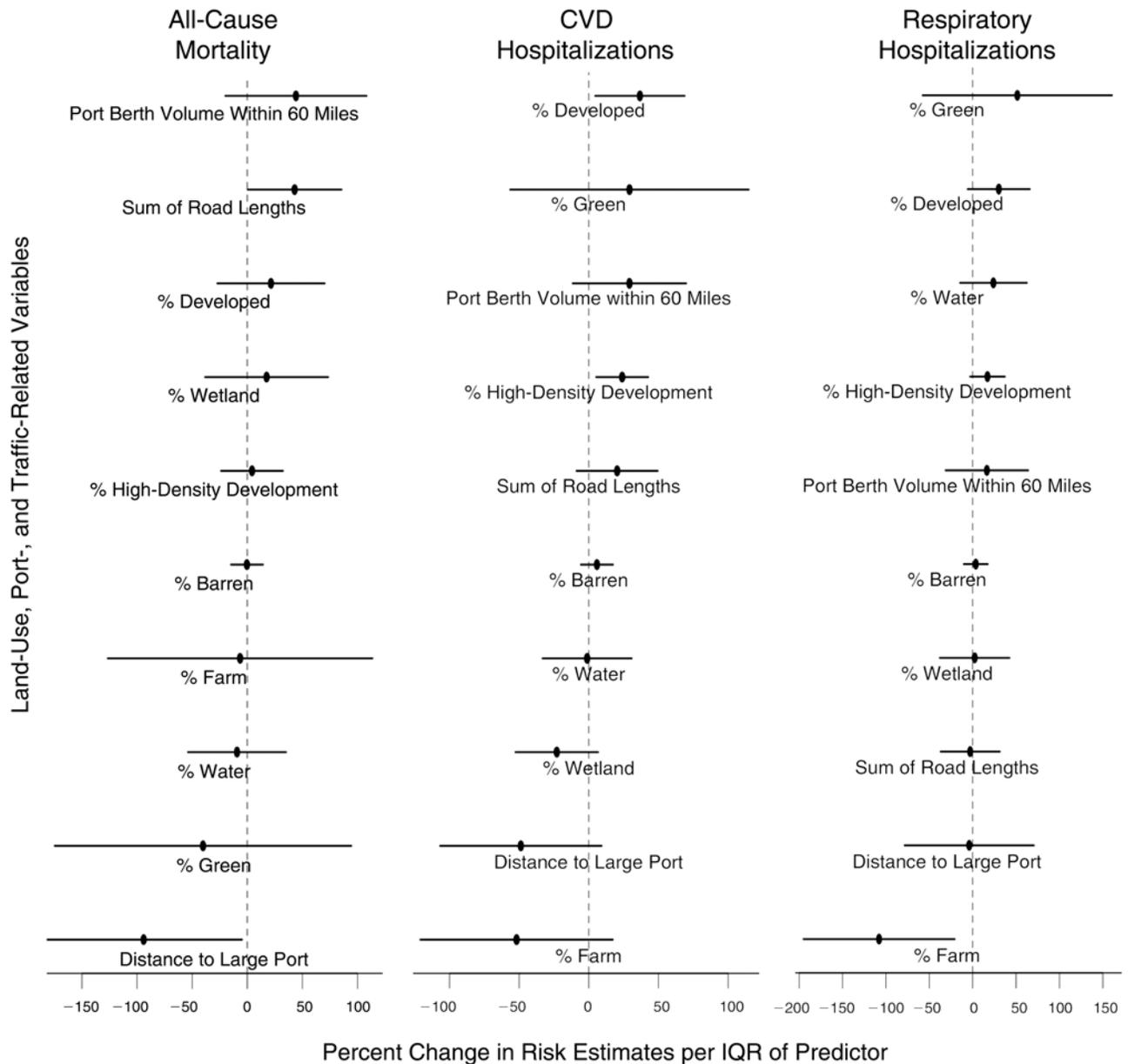


Figure 15. Percentage change at 0-day lag in all-cause mortality and CVD and respiratory hospitalization risk estimates associated with PM_{2.5}, based on land-use, port-, and traffic-related data. The results are per the IQR of the mean levels of predictor variables across cities in the second-stage analysis.

negative predictor of Cu risk estimates, EC was a significantly negative predictor of EC risk estimates, and Fe was a significantly negative predictor of Fe risk estimates. Ni was a significantly positive predictor of PM_{2.5} mass and also of CO, Fe, NO₃⁻, SO₄²⁻, and Zn. V was a significantly positive predictor of PM_{2.5} mass and also of SO₂, NO₃⁻, and SO₄²⁻.

All-Year Respiratory Hospitalization Risk Estimates Associated with Key PM_{2.5} Components and Gaseous Pollutants

The results shown in Appendix Figure B.10, for the 64-cities data set, are for key PM_{2.5} components and gaseous pollutants as a function of average PM_{2.5} mass, its components, and gaseous pollutants. No significant predictor of PM_{2.5}

respiratory hospitalization risk estimates across the 64 cities was evident, which is consistent with the results for the larger set of cities (Figure 14, right panel). However, both Ni and V were significantly positive predictors of NO₂ risk estimates, and Ni was a significantly positive predictor of EC. V was a significantly positive predictor of SO₂ risk estimates. Si was a significantly negative predictor of CO and NO₂ risk estimates. SO₄²⁻ and Se were significantly positive predictors of CO.

DISCUSSION AND CONCLUSIONS

CONSISTENCY BETWEEN THE RESULTS OF THE FIRST-STAGE AND SECOND-STAGE ANALYSES

The main question we started with was: Which component or components of PM_{2.5} are responsible for the observed effects of PM_{2.5} mass concentration? Therefore, our second-stage analysis originally focused on explaining the heterogeneity in the effects of PM_{2.5} mass across cities as a function of city-specific average levels of the components of PM_{2.5}. However, as our first-stage results showed, the associations for individual components or gaseous pollutants were often stronger than those for PM_{2.5} mass. Furthermore, the risk estimates associated with the key PM_{2.5} components and gaseous pollutants were generally not sensitive to the inclusion of PM_{2.5} mass in the regression models. As a result, we also subsequently analyzed the risk estimates associated with the key components and gaseous pollutants as dependent variables in second-stage analyses, though the sample size for this additional second-stage analysis was smaller than that for the main second-stage analysis.

All-Cause Mortality

Risk estimates for all-cause mortality associated with PM_{2.5} mass, summarized in Tables 3 and 4, do not show SO₄²⁻ as a pollutant significantly associated with all-cause mortality. However, it was, in fact, nearly significantly positively associated with all-cause mortality at 0- and 1-day lags in the 148 cities analysis (Appendix Figure B.4, first panel, and the 64-cities analysis (Figure 7), in the all-year and warm season analyses. Thus, the assumption that SO₄²⁻ is the pollutant that best explains the heterogeneity of the effects of PM_{2.5} mass is not inconsistent with the first-stage analysis results. In the second-stage analysis, using land-use, seaport- and traffic-related variables, the seaport berth volume of ports within 60 miles (i.e., potentially the largest source of residual oil burning in most seaport cities) ranked high among the predictors. This is in

part consistent with the result that V also ranked high among the PM_{2.5} components in the second-stage analysis. The sum of road lengths was also a nearly significant predictor of PM_{2.5} risk estimates. This is consistent with the result that Traffic source category was positively associated with all-cause mortality.

The average PM_{2.5} mass concentrations across the cities did not significantly predict the city-to-city variation in PM_{2.5} risk estimates, but neither was there any indication that the mortality risk estimates associated with any of the gaseous pollutants or components were higher in the cities where the levels of those pollutants were high.

CVD Hospitalizations

The PM_{2.5} components that ranked among the highest as effect modifiers of PM_{2.5} mass risk estimates in the second-stage analysis for CVD hospitalizations were Cu, Ni, and V. V was in fact associated at 0-, 1-, and 3 day lags (Tables 3 and 4), and therefore the first- and second-stage results appear to be consistent. In keeping with this result, in the second-stage analysis of PM_{2.5} mass risk estimates, the seaport berth volume of ports within 60 miles ranked high for CVD hospitalizations.

Cu was associated with CVD hospitalizations in the first-stage result in the cold season (Table 4). In the nationwide factor analysis (Figure 2), Cu was most closely associated with the Traffic category, and because NO₂ also ranked high in the second-stage results for CVD hospitalizations, and also given that many of the other traffic-related pollutants (NO₂, CO, EC, and OC) were associated with CVD hospitalizations, Cu (which can be emitted by brake wear) may be serving as a marker for traffic-associated effects in these analyses. The sum of road lengths also appeared to influence PM_{2.5} CVD hospitalization risk estimates (but not risk estimates for respiratory hospitalizations). However, as noted earlier, in the second-stage analysis, Cu concentration was a significantly negative predictor of the Cu risk estimates, which was not consistent with the direct effects of Cu. Thus, in these analyses, interpreting the association of Cu with health effects as a direct effect of source emissions was problematic.

Of the traffic-related pollutants that were significantly associated with CVD hospitalizations (NO₂, CO, Cu, EC, and OC), NO₂ was the only pollutant in the second-stage model whose risk estimates for CVD hospitalizations were significantly positively predicted by its city-specific average levels across cities (i.e., short-term NO₂ risk estimates were higher in the cities with higher average NO₂ levels).

The second-stage result that two land-use variables, percentage of developed land and percentage of high-density development, were positive predictors of PM_{2.5} risk

estimates suggests an influence of local combustion sources, including traffic.

Respiratory Hospitalizations

The pattern of associations and associated components for respiratory hospitalizations has some similarity to that for CVD hospitalizations. As with CVD hospitalizations, most of the associations were found on the same day, mostly in the cold season, except that the secondary aerosols OC and SO₄²⁻ showed multiday associations in the warm season (Tables 3 and 4). As with CVD hospitalizations, in the second-stage analysis, Cu, Ni, V, and NO₂ were among the positive predictors of risk estimates for respiratory hospitalizations associated with PM_{2.5}. Unlike for CVD hospitalizations, the sum of road lengths did not explain the city-to-city variation in PM_{2.5} respiratory hospitalization risk estimates. As for CVD hospitalizations, the percentage of farm land was a negative predictor of PM_{2.5} risk estimates.

None of the city-to-city variations of the significantly associated components or gaseous pollutants in the first-stage model (CO, Cu, EC, OC, and SO₄²⁻) could be explained by the corresponding city-specific average levels across cities in the second-stage model.

USING THE DEVIATIONS FROM THE MONTHLY MEANS

In its health effects model, this study used pollution variables in the form of deviations from the monthly means to prevent the influence of seasonal trends from interfering with the correlation structure for the factor analysis. In the health effects model, confounding by seasonal pollutant trends was in part addressed by including a smooth function of study days as a covariate, but such terms can compete with seasonal cycles in the pollution time series unless they are also detrended. Since this approach is not common practice in more recent time-series studies (although case–crossover analysis, using a time-stratified referent sampling scheme, replaces the smooth function by matching a case day with referent days by day-of-week within the same month of the year), we repeated the analysis with the raw pollution variables for PM_{2.5}, its components, and gaseous pollutants for all-cause mortality and CVD and respiratory hospitalizations in the 64-cities data set. The results for the raw and the deviation variables were generally comparable (compare Figures 7, 9, and 11 with Appendix Figure B.5). We speculate that this was the case because the extent of seasonal adjustment for the health outcomes, 8 df/yr, was sufficient to eliminate correlation between the remaining seasonal

variations in the health outcomes and seasonality in the pollution variables.

POTENTIAL INFLUENCE OF EXPOSURE ERROR

Although Ni was correlated with V, and together they constituted a factor that we designated as a Residual Oil Combustion source category (Figure 2), Ni showed no associations with all-cause mortality and CVD and respiratory hospitalizations (Tables 3 and 4). This may have been partially related to the poor spatial representation in the CSN data sets of Ni exposures in the populations. In the 21 cities where monitor-to-monitor temporal correlations of PM_{2.5} components among multiple monitors could be examined (Figure 16), Ni showed one of the poorest monitor-to-monitor correlations (i.e., the poorest among the components analyzed in the health effects analyses). Poor monitor-to-monitor correlations imply that Ni was far more spatially variable than other components and that Ni exposure misalignment was greater than that for other components. Arsenic (As) is another component with very poor monitor-to-monitor correlations, as shown in Figure 16. Like Ni, As showed few associations with the health outcomes examined in this analysis.

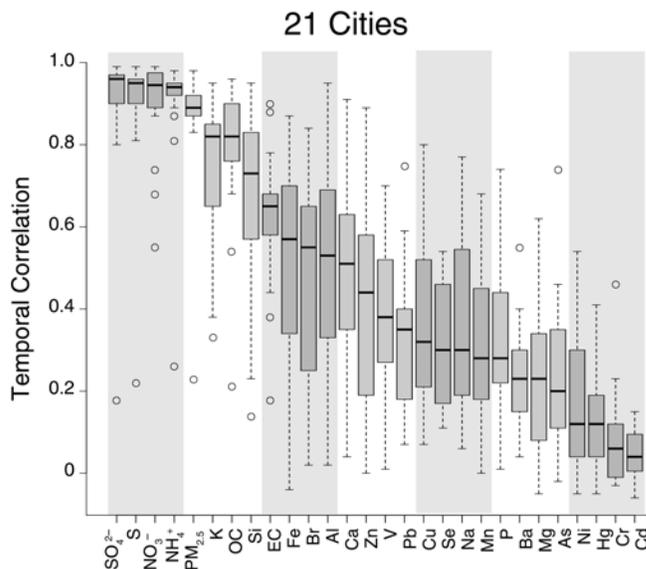


Figure 16. Distribution of median monitor-to-monitor temporal correlations for the key PM_{2.5} chemical components in 21 cities. The cities are those in which data from multiple monitors were available. Each city contributes the median value of its monitor-to-monitor correlations to the distribution (when a city has only two monitors, the correlation of those monitors is contributed). The bars show the median and the IQR, the dotted lines represent 1.5 times the IQR, and the circles are outliers.

POTENTIAL INFLUENCE OF OTHER DATA CHARACTERISTICS AND THE QUALITY OF CHEMICAL SPECIATION DATA

In addition to the differential spatial distributions of concentrations of the components mentioned above (i.e., different degrees of exposure misalignment), other differences in data characteristics across components also complicate the interpretation of their relative health effects. These data characteristics include indicators of data quality such as the percentage of values below the detection limit (Appendix Table B.5) and the percentage of zeros (Appendix Table B.6). While some of the components whose data were of poor quality according to these indicators were excluded from analysis at the initial stage, differential quality across the key components remained. For example, 60% or more of the values for As, Pb, Se, Ni, and V for the entire CSN data set were below the detection limit, whereas the values for the components associated with the secondary aerosols, SO_4^{2-} , NO_3^- , OC, were all nearly 100% above detection limit. This contrast in detectability percentages does not necessarily explain the pattern of associations with health effects (e.g., V showed more significant associations than NO_3^-), but one also needs to be cautious about interpreting the lack of associations for the components with poorer quality data (e.g., As). It should be noted that, although the poorer data quality of some components may attenuate their potential underlying associations with health outcomes in short-term time-series analyses, this may not be the case for the second-stage analysis, or for that considered in Study 4 of this report, in which the long-term average values were considered.

The difference in distributional characteristics across the components also complicates the comparison of the risk estimates. The more skewed the distribution of concentrations for a component, the smaller the relative standard error of the health effect regression coefficient (and the estimate of effect) becomes. As a result, its 95% confidence band appears narrower when risk estimates are computed for the IQR. For example, in Figure 7, the 95% confidence bands computed for the Ni (the distribution for which was highly skewed) risk estimates are much narrower than those for OC (the distribution for which was much less skewed), even though the number of observations was the same. Although the skewed distributions also tend to make the effect estimates smaller, the narrower confidence bands (which appear to represent more precision) for the highly skewed components can be misleading, as these components tend to be the ones with higher percentages of zeros and greater numbers of values below the detection limit, which is a condition equivalent

to having fewer observations at mid-to-high concentration ranges. This problem also may contribute to the general appearance of the figures with risk estimates for multiple pollutants: The risk estimates associated with $\text{PM}_{2.5}$ mass and gaseous pollutants tend to appear larger than those of $\text{PM}_{2.5}$ components because their distributions tend to be less skewed. This limitation, whose extent varies across the components, needs to be acknowledged in this analysis.

NEGATIVE ASSOCIATIONS

An overwhelming majority of the significant associations found in this analysis were positive associations (i.e., higher concentrations were associated with increased adverse risk). However, there were some significantly negative (i.e., “protective,” if causally interpreted) associations whose lag structures were often peculiar and worth mentioning. These include negative associations: (1) between 1- and 2-day-lag $\text{PM}_{2.5}$ and cerebrovascular hospitalizations (Figure 5); (2) between the 1-day-lag Metals source category and all-cause mortality (Figure 8); (3) between 1-day lag NO_2 and CVD hospitalizations (Figure 9); (4) between 1- and 2-day-lag NO_2 and respiratory hospitalizations; (5) between 0- and 2-day-lag Na and respiratory hospitalizations; (6) between 1-day lag Fe and respiratory hospitalizations; and (7) between 2-day-lag Pb and respiratory hospitalizations. (See the results in Figure 11 for numbers 4 through 7 in the list.) The negative associations for the 1-day-lag NO_2 are peculiar because 0-day-lag NO_2 in these cases was positively (and strongly significantly so in the case of CVD hospitalizations) associated with the outcomes. Note also that most of these negative associations occurred in the hospitalization analyses (in six of the seven examples mentioned above, four of which are for respiratory hospitalizations). In Peng et al.’s (2008) analysis of Medicare respiratory and CVD hospitalization data, the investigators also reported a significantly negative association between $\text{PM}_{2.5}$ mass concentrations and cerebrovascular hospitalizations at 1-day lag (2-day lag was also nearly significant) when the association was significantly positive at 0-day lag. We did not further investigate the cause of these occasional negative associations. However, because hospitalization time series — even when they are for emergency admissions, as they were in this study — have a strong day-of-week pattern (Ito et al. 2011) with lower admissions on the weekend, the negative association could be due to negative autocorrelation. Also, the interaction between the day-of-week pattern of the pollutant concentrations and the day-of-week pattern of the outcomes may induce such positive and negative associations when the pollution and the outcome are associated. Performing a time-stratified case-crossover analysis (i.e., comparing the pollution levels

for the same day of the week in the same month of the same year for the case and referent days) may be helpful in investigating this issue.

TEMPORALITY OF ASSOCIATIONS

In this analysis, PM_{2.5} mass was most significantly associated with all-cause mortality at 0-day lag, and the associations were mainly in the warm season (Table 4; Figures 4 and 7; Appendix Figures B.4 [first panel] and B.5 [first panel]). The 0-day-lag association between PM_{2.5} mass and all-cause mortality is in part consistent with Peng and colleagues' (2005) multicity analysis of PM₁₀ mass and all-cause mortality, in which the investigators found that the nationwide combined risk estimate was most significant at 0-day lag in the spring but at 1-day lag in the summer and in the all-year analysis. It should be noted that the associations between all-cause mortality and some of the gaseous pollutants and components were more frequently seen with lags (Tables 3 and 4), except in the cases of the 0-day-lag associations with NO₃⁻ and SO₄²⁻ in the warm season (Figure 7 and Appendix Figure B.4 [first panel]; though they are not statistically significant), which may have driven the 0-day-lag association for PM_{2.5} in the warm season. Thus, the simplest interpretation of these results is that the warm-season association between PM_{2.5} mass and all-cause mortality was driven by SO₄²⁻ and NO₃⁻, which are major mass components of PM_{2.5}. However, the lag structure of associations of these pollutants can also be influenced by their correlation with other covariates in the model, and therefore, this simple interpretation may have its limits.

The associations with pollutants for both CVD and respiratory hospitalizations most clearly appeared at 0-day lag, especially in the cold season (respiratory hospitalizations showed distributed lagged associations for OC and SO₄²⁻ in the warm season). These 0-day-lag associations were also observed in other multicity analyses of elderly hospitalizations (Dominici et al. 2006; Peng et al. 2008). What is peculiar about the 0-day-lag associations is that the 1-day-lag association for the same pollutants was usually either null or negative, significantly for some pollutants, as though the lags were negatively autocorrelated. The results from the analysis of PM_{2.5} mass and cause-specific CVD hospitalizations (Figure 5) show that this pattern was most prominent in the results for hospitalizations for dysrhythmias and cerebrovascular disease. As noted earlier, Peng et al. (2008) also reported a significantly negative association between PM_{2.5} and cerebrovascular hospitalizations at 1-day lag (when the association was significantly positive at 0-day lag). This is inconsistent with our expectation in terms of biological plausibility —

we expect distributed lag associations because the lengths of time for individuals to respond to the effects of air pollution are heterogeneous. However, as also mentioned in the section above, this may have been due to the day-of-week pattern in the hospitalizations (even though the admissions were all emergencies) and day-of-week pattern in the pollution series. This is one potential limitation of the use of administrative data. In any case, the immediate association between the air pollution and these outcomes suggests that, if causal, the nature of the association was such that the pollutants were not contributing to the *development* of the health condition (which would take time) but rather to the *worsening* of the condition.

INFLUENCE OF BOTH LOCAL AND REGIONAL POLLUTANTS

The gaseous pollutants, PM_{2.5} components associated with traffic, and the Traffic source category all showed positive associations with all-cause mortality, CVD hospitalizations, and respiratory hospitalizations (Tables 3 and 4). Also, the weekday excess PM_{2.5} mass concentration was an important predictor in the second-stage analysis of risk estimates for all of these outcomes associated with PM_{2.5} exposure (Figure 14). To the extent that Si and the Soil source category (which was a significant predictor of all-cause mortality) showed a day-of-week pattern (Appendix Figures G.12 and G.13), Si and the Soil source category's associations may also have reflected local pollution activities including traffic. V, which is a likely tracer of residual oil combustions, also showed associations with all-cause mortality and CVD hospitalizations (Tables 3 and 4). However, regional pollutants also showed associations with these outcomes. SO₄²⁻ showed a nearly significant association with all-cause mortality in the warm season (Figure 7), a significant association with CVD hospitalizations in the cold season (Table 4), and significant associations at multiple lags with respiratory hospitalizations in the warm season (Table 4). NO₃⁻ was a significant predictor of CVD hospitalizations in the warm season (Table 4). Also, OC, which should include a large fraction of secondary organic compounds, was associated with all of the health outcomes (Tables 3 and 4). Thus, both local and regional pollutants had impacts on mortality and hospitalizations.

USEFULNESS AND LIMITATIONS OF FACTOR ANALYSIS

The factor scores examined in the health effects models showed that the Traffic category displayed the most consistent associations across the health outcomes examined (Table 3). However, the Traffic category was also correlated with the highest number of PM_{2.5} components and gases

(Figure 2). The Traffic and other source categories also did not yield risk estimates that were more significant than those of individual pollutants associated with the category. Thus, these source categories did not prove to be better predictors of the health effects than individual pollutants. This may be because these categories consisted of linear combinations of individual pollutants, and in this process, the components that had large exposure errors may have caused the quality of the category to deteriorate as an alternative exposure index. For example, while V showed associations with both CVD and respiratory hospitalizations, the Residual Oil Combustion category did not, perhaps because the contribution of Ni to this category increased the exposure error associated with this component.

We note that, while the components associated with the factor scores in the nationwide factor analysis (Figure 2) generally support our naming of the source categories, such as Traffic and Soil, naming these source categories is inherently limited, as some of the components associated with these source categories are not always exclusive to them. Thus, the identification of source categories through factor analysis (following the paradigm described in the Methods section) conducted for individual cities (Figure 3) likely resulted in misidentifications in some cities. However, more elaborate source-apportionment methods typically applied for individual cities would not have been feasible for a multicity analysis like this.

Overall, the factor analysis used in this study served as a useful summary indicator (and reduced dimensionality) of source-associated multiple pollutants, but also identified the issue just described.

ADVANTAGES AND DISADVANTAGES OF THE METHODS APPLIED AND RECOMMENDATION FOR FURTHER RESEARCH

We conducted multicity time-series analyses of mortality and of hospitalizations of older adults, considering $PM_{2.5}$ mass, its components, and gaseous criteria pollutants. Alternative exposure indices based on factor analysis were also used in the health effects analyses, to incorporate both gaseous criteria pollutants and components of $PM_{2.5}$. We believe that these methods provide an advantage over

single- and multipollutant models of correlated pollutants, and also provide a source-oriented means of assessing multiple pollutants that will be needed in future policy decisions. However, one major shortcoming of the analysis was that it did not quantitatively incorporate available pollutant emissions data from national and local emission inventories and source profiles. Although conducting detailed source apportionment in each of the cities we analyzed would not have been feasible, we believe that developing a systematic means of including emissions data in the automated factor analysis may be possible in future research. Budget and time constraints, however, did not allow us to develop such methods, but future research could explore this area further.

CONCLUSIONS

Through characterizations of the components of $PM_{2.5}$, factor analysis, multicity analysis of both mortality and hospitalizations, and a second-stage evaluation of the city-to-city variation in the estimated health effects associated with $PM_{2.5}$ across cities, we provided a source-oriented assessment of multipollutant effects on mortality and morbidity outcomes. This study used source-apportionment methods to reduce the dimensionality of multiple pollutant atmospheres and suggests that a major fraction of variation in multiple pollutants could be attributable to traffic-related sources and that the temporal variations in traffic are also associated with temporal variations in daily counts of all-cause mortality and CVD and respiratory hospitalizations. The results for individual $PM_{2.5}$ components and gaseous criteria pollutants are generally consistent with the results from the factor-based approach. However, this study also found that the secondary aerosols, SO_4^{2-} , NO_3^- , and OC, which were excluded from the source apportionment, were associated with these outcomes.

APPENDIX B. Additional Results and Supporting Information

See page 193 at the end of the Investigators' Report.

NPACT Study 4. Mortality and Long-Term Exposure to PM_{2.5} and Its Components in the American Cancer Society's Cancer Prevention Study II Cohort

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ABSTRACT

INTRODUCTION

Epidemiologic studies conducted over recent decades have shown that long-term exposure to elevated ambient levels of PM_{2.5}* is associated with increased risk of death, especially from ischemic heart disease (IHD) and lung cancer. The earlier analyses of the American Cancer Society's (ACS) Cancer Prevention Study II (CPS-II) cohort (Pope et al. 1995, 2002, 2004), the largest prospective cohort study, found that mortality from all causes and from cardiopulmonary diseases increased in positive association with the level of ambient PM_{2.5}. However, the components in PM_{2.5} (e.g., ions, trace metals, organic compounds) and the emission sources of the particles (e.g., coal-fired power plants, residual oil combustion, traffic, soil) that are most closely associated with the increased risk of mortality have yet to be determined.

SPECIFIC AIMS

We investigated the associations of specific chemical components of PM_{2.5} and their possible sources with human mortality using the nationwide CPS-II cohort and the recent U.S. EPA CSN data for PM_{2.5} mass, elements, and other components.

This Investigators' Report is one part of Health Effects Institute Research Report 177, which includes Investigators' Reports of three other studies, a Commentary by the NPACT Review Panel, an HEI Statement about the research project, and a Synthesis of the NPACT Initiative relating this report to Research Report 178. Correspondence concerning the Investigators' Report may be addressed to Dr. George Thurston, New York University Medical Center, 57 Old Forge Road, Tuxedo, NY 10987. george.thurston@nyu.edu.

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* A list of abbreviations and other terms appears at the end of the Investigators' Report.

METHODS

Data from the Chemical Speciation Network (CSN) for PM_{2.5} mass and trace element components were collected; factor analysis and source-apportionment methods were applied to identify source categories of PM_{2.5}; and cohort exposures to PM_{2.5} were estimated. Standard and multi-level random effects Cox proportional hazards models (proposed by Sir David Cox in 1972) were used to assess the risk of death from all causes, IHD, respiratory disease, and lung cancer in relation to exposures to PM_{2.5} and its components. In addition, a new risk metric, total risk index (TRI), was developed to evaluate the relative influence of different pollutants, in a multiple-pollutant model, on the air pollution-mortality risk.

RESULTS

Overall, our modeling results indicated that long-term exposures to PM_{2.5} from a few key sources (identified by their elemental tracers) explained most of the PM_{2.5}-mortality associations found in past studies of the CPS-II cohort. In particular, the Coal Combustion source category was most consistently associated with increased risk of IHD mortality across the four models (i.e., the standard Cox model and the random effects Cox model, each one with and without contextual variables). The tracers for the Traffic and Salt source categories (EC and Cl, respectively) also showed significant associations with IHD mortality in most models. Associations between PM_{2.5} and lung cancer mortality were displayed for the Coal Combustion source category but not for other categories. Soil and Biomass Combustion source categories were consistently not associated with any causes of mortality in all models.

The TRI analysis found weak evidence of an association of mortality with any of the three gaseous pollutants over and above any association with PM_{2.5} mass and its components. However, overall risk based on either factor scores or source-apportioned mass (and especially with the secondary aerosols [sulfates, nitrates, and OC] included) were generally larger than the risk estimates based on PM_{2.5}

mass alone. This suggests that the source-specific information allows for a more accurate exposure and risk estimate, and that past estimates using non-specific PM_{2.5} mass alone may have provided underestimates of the total effect of PM_{2.5} exposure on mortality.

CONCLUSIONS

Long-term exposure to PM_{2.5} and its key components from combustion sources, especially the Coal Combustion source category, explains most associations between PM_{2.5} mass and increased risk of mortality from all causes, IHD, and lung cancer found in earlier studies of this cohort.

INTRODUCTION

Epidemiologic studies conducted over recent decades have indicated that long-term exposures to elevated ambient levels of PM_{2.5} are associated with increased risk of death. Most notably, two U.S. cohort studies — the Harvard Six Cities study (Dockery et al. 1993), a 20-year prospective cohort study, and the CPS-II study (Pope et al. 1995), a large prospective cohort study involving 151 cities — have found that all-cause and cardiopulmonary mortality increased in association with an increase in exposure to PM_{2.5}.

The CPS-II investigators undertook a subsequent analysis, which nearly doubled the study follow-up time to more than 16 years and tripled the number of deaths that could be analyzed, that confirmed these associations more definitively (Pope et al. 2002). For that study, exposure data were expanded to include gaseous co-pollutants and new PM_{2.5} data that had been collected since 1999 as a result of the National Ambient Air Quality Standard for PM_{2.5} enacted in 1997 (U.S. EPA 1997). Advances in statistical modeling were also incorporated, in particular the use of random effects (i.e., a relaxation of the assumption that observations are independent) and control for spatial autocorrelation. Results from this 2002 analysis by Pope and colleagues provided the strongest evidence to date that long-term exposure to PM_{2.5} is an important risk factor for deaths from lung cancer and cardiopulmonary disease. For each 10- $\mu\text{g}/\text{m}^3$ increase in long-term average PM_{2.5} ambient concentrations, the associated risks of death from all causes, cardiopulmonary disease, and lung cancer increased by approximately 4%, 6%, and 8%, respectively. Pope and colleagues (2004) further examined disease subcategories to identify potential pathways by which inhaled PM_{2.5} may increase deaths from cardiopulmonary disease. Of the associations between PM_{2.5} exposure and mortality from any cardiopulmonary disease, the

effect estimate was largest for IHD, which suggests a mechanism of inflammation and accelerated atherosclerosis.

In addition to the various analyses and insights from the CPS-II and Harvard Six Cities studies (Dockery et al. 1993), other cohort studies have investigated the effects of PM on mortality. The Women's Health Initiative gathered data for 65,893 postmenopausal women with no history of cardiovascular disease (CVD) who lived in 36 U.S. metropolitan areas from 1994 to 1998 (Miller et al. 2007). Analyses showed that each increase of 10 $\mu\text{g}/\text{m}^3$ of PM_{2.5} was associated with a 24% increase in the risk of a cardiovascular event (hazard ratio = 1.24; 95% CI = 1.09–1.41) and a 76% increase in the risk of death from CVD (hazard ratio = 1.76; 95% CI = 1.25–2.47). These findings further strengthened the evidence linking long-term exposure to PM_{2.5} with mortality and demonstrate that the magnitude of the PM_{2.5}–mortality association is larger than previously estimated.

The Adventist Health Study on Smog (AHSMOG) cohort has provided some limited evidence for associations between long-term exposure to PM_{2.5} and mortality. The original analyses of the AHSMOG cohort found positive associations between long-term exposure to PM₁₀ and mortality, at 15 years of follow-up, due to natural causes and lung cancer (Abbey et al. 1999). McDonnell and associates (2000) reanalyzed these data and concluded that the previously observed association of long-term ambient PM₁₀ concentrations with mortality for males were best explained by a relationship of mortality with PM_{2.5} rather than with PM₁₀. In addition, the Netherlands Cohort Study (Brunekreef et al. 2009) estimated the effects of traffic-related air pollution on cause-specific mortality in a cohort of approximately 120,000 subjects of age 55 to 69 years at enrollment. For a 10- $\mu\text{g}/\text{m}^3$ increase in PM_{2.5} concentration, the relative risk for natural-cause mortality in the full cohort increased by 6% (relative risk = 1.06; 95% CI = 0.97–1.16), which is similar in magnitude to the results reported earlier by the ACS.

Overall, past studies indicate that long-term exposure to ambient outdoor PM_{2.5} air pollution is significantly associated with an increased mortality risk, especially for deaths from IHD and lung cancer. However, neither the components (e.g., trace metals) nor the emission sources (e.g., PM_{2.5} attributable to traffic, coal combustion, or oil combustion), that may be most associated with the increased risk of death have been determined.

SPECIFIC AIMS

The overall goal of this project's research was to investigate the associations between long-term exposures to source-related chemical components of PM_{2.5} air pollution and mortality in the United States. We built upon our research team's earlier work in which we definitively confirmed that long-term exposure to PM_{2.5} air pollution is associated with increased risk of cardiopulmonary and lung cancer mortality (Pope et al. 2002, 2004; Jerrett et al. 2005; Krewski et al. 2009).

This study included data from 445,860 enrollees in the ACS CPS-II study that began in 1982. To more fully address which of the PM_{2.5} components (e.g., ions or metals or organic compounds) and sources are most associated with the increased mortality risk, we needed more recent data. Therefore, we extended the CPS-II cohort's follow-up years through 2004, thereby increasing statistical power and encompassing the time span of the data from the new EPA CSN (2000–2005).

The specific aims were to apply recently developed source-apportionment methods to the newly available CSN PM_{2.5} component data and combine them with the extended CPS-II cohort data to test the following overall NPACT hypotheses, restated in terms of mortality as a response to air pollution exposure.

1. Associations between mortality and PM_{2.5} exposure are stronger with certain components of PM_{2.5} than with others.

PM_{2.5} has a wide variety of components that vary from place to place; logically, the variation in composition should affect the biological toxicity of PM_{2.5}. For example, the OC fraction may contain numerous mutagenic compounds and metals, such as Ni and As, that are known to be toxic and have been identified as potential carcinogens. We analyzed the available data for components (i.e., elements and ions) of PM_{2.5} in the CPS-II cities to test whether any specific PM_{2.5} components have stronger associations than others with mortality from certain causes.

2. Associations between mortality and PM_{2.5} exposure are stronger with some pollution sources than with others.

It is well documented that different air pollution sources produce different PM_{2.5} compositions (e.g., higher concentrations or proportions of Se and As from coal combustion, and V and Ni from residual oil combustion). To estimate source contributions to PM_{2.5} exposures in each city, we applied state-of-the-art multivariate source-apportionment methods (which are built upon compositional differences)

to the CSN data obtained through the HEI-funded Atmospheric and Environmental Research (HEI–AER) database (HEI Air Quality Database for Health Effects Studies 2005) of trace elements, which also included data for ambient gases. The results of source-apportionment analyses were then used to test whether certain source-related categories were more closely associated than others with increased risks of death from certain causes.

These specific objectives are directly responsive to pressing requests for PM_{2.5} research, most notably those outlined in reports issued by the National Academy of Sciences (NAS) Committee on Research Priorities for Airborne Particulate Matter (NAS 1998, 1999, 2001). These reports clearly requested that epidemiologic studies of the effects of long-term exposure to particle components should be undertaken (NAS 1999). Although the association between trace components and mortality has been investigated in smaller cohorts (e.g., Ostro et al. 2010), the question of trace components in source-specific PM_{2.5} has not been investigated in a large prospective cohort study.

METHODS AND STUDY DESIGN

AIR POLLUTION DATA

The U.S. EPA Air Quality System provides routine air monitoring measurements for PM_{2.5} mass, PM_{2.5} anions (sulfates [shown here as SO₄²⁻] and nitrates [shown here as NO₃⁻]) and cations (ammonium [NH₄⁺], Na, and K), trace elements reliably determined via x-ray fluorescence (i.e., some elements between Na and Pb on the periodic table), total carbon (including OC and EC), and gaseous criteria pollutants (CO, NO₂, SO₂, and O₃). These data are aggregated and maintained in the HEI–AER database (HEI Air Quality Database 2005).

For this work, PM_{2.5} speciation data including EC, OC, SO₄²⁻, NO₃⁻, NH₄⁺, plus NO₂, SO₂, and CO gaseous pollutant data gathered from 273 CSN and nearby gaseous monitoring sites across the United States during 2000–2005 were downloaded from the HEI–AER database (see Thurston et al. 2011).

CSN Data and Data Quality Control

Data from all available CSN monitoring sites (273) in the contiguous continental United States (i.e., excluding Hawaii, Alaska, and Puerto Rico) from February 9, 2000 through December 31, 2005 were downloaded from the HEI–AER database. Quality control steps were applied to those data to remove sites or observations that did not meet the study's

requirements, as described below and summarized in Table 1. These nationwide PM_{2.5} mass and speciation data have been previously characterized (Bell et al. 2007).

A minimum of 60 reported observations for each pollutant during 2000–2005 (equivalent to at least 6 months of measurements taken every third day) was required for data from a CSN site to be used. Daily data outliers for the included sites were also identified for possible exclusion; we computed the 6-year means and standard deviations for each pollutant at each site, and flagged the extreme outliers when an individual day’s measured value was more than 12 SDs from the mean for that element at that site. Also, observations influenced by January 1st or July 4th fireworks were removed from the dataset to avoid the influence of firework events (which are associated with spikes in K, usually an important tracer of biomass combustion); these events were not considered to be among the ubiquitous air pollution sources that produce major contributions throughout the year. Because of a known positive artifact for OC (Chow et al. 2010), we also applied the seasonal regression intercept adjustment approach described by Watson and associates (2009).

Since we were ultimately focused on calculating long-term (rather than daily) average impacts on mortality, no individual values were eliminated or adjusted when below

the limit of detection in order to achieve an unbiased overall estimate of the mean.

After quality control requirements were applied and outliers treated, 64,941 observations from 243 monitoring sites were available (Table 1). After we merged the CSN PM speciation data with NO₂ data from nearest-neighbor monitors, 46,478 observations from 212 monitoring sites were available for further analysis.

Gaseous Pollutant Data

Gaseous NO₂ is not routinely measured at CSN sites, so 24-hour average concentrations from the HEI–AER database were supplied from the NO₂ sampler nearest to each CSN site (nearest-neighbor monitors). We incorporated NO₂ data in the factor analysis and source-apportionment models because it is considered to be, in combination with EC, a marker of emissions from traffic; the NO₂ data aided in separating the Traffic source category from other local combustion sources that also emit OC (e.g., Biomass Combustion). Other gases were investigated, but limitations in the monitoring sites or the data at or near CSN monitors would have resulted in excluding otherwise valid monitoring sites and cities; thus the only gaseous pollutant included in the factor analysis was NO₂.

Table 1. Characteristics of Data Sets and Analyses

Data Set	Daily Observations	Sites	MSAs	Analysis
All available CSN data for 2/9/2000–12/31/2005 downloaded from the HEI–AER database	71,667	273		
After quality control and data requirements applied and outliers treated	64,941	243		
CSN PM speciation and nearest-neighbor NO ₂ data (within MSA not specified)	46,478	212		Factor Analysis
CSN PM speciation and nearest-neighbor NO ₂ data (within MSA only)	29,837	114	51	NO ₂ and Traffic Factor
CSN PM speciation and nearest-neighbor NO ₂ data (outside MSA only)	16,641	98	49	NO ₂ and Traffic Factor
CSN + NO ₂ data from sites with at least 30 winter and 30 summer observations	39,849	167	100	Source Apportionment
CPS-II Mortality Analyses — Pollutant site averages for each source category	39,849	167	100	Mortality Analyses

Issue of NO₂ Data from Nearest-Neighbor Monitors

Subsequent to the source-apportionment analysis, which included NO₂ data (discussed below), we discovered that approximately one-third of the NO₂ data downloaded from the AER database for the nearest-neighbor sites (16,641 of 46,478 observations) were from sites outside the metropolitan statistical area (MSA) where the matched CSN site was located. We subsequently evaluated what aspects of the results were affected by this, and reestimated the source categories and associations after addressing this NO₂ data issue.

AIR POLLUTION ASSESSMENT

PM speciation plus nearest-neighbor NO₂ data were available for 212 CSN monitoring sites across the United States. The 2000–2005 mean concentrations of mass for PM_{2.5} and each of its components for each site were compiled from the HEI–AER database. The means (\pm SEs) across all 212 sites are summarized by region and season in Table 2.

The multivariate factor analysis and subsequent source apportionment work proceeded in two overall stages. First, we applied factor analysis to the entire CSN database (212 sites) to identify the source-related groupings (factors or source categories) of PM_{2.5} components. Second, we apportioned the mass of PM_{2.5} and each of its components to each of the factors; factors were further analyzed to estimate PM_{2.5} mass contributions from the source categories assigned to factors by applying absolute principal component analysis (APCA; Thurston and Spengler 1985). In this analysis, a variation of APCA was applied, however, in that mass was also ascribed to separate secondary aerosol components (i.e., SO₄²⁻, NO₃⁻, and OC) in the mass regression step, allowing a fuller and clearer accounting of the PM_{2.5} mass by source category.

Factor Analysis and Source-Apportionment Modeling

The PM_{2.5} mass and component data were analyzed using the APCA PM_{2.5} mass source-apportionment method developed to analyze similar data for the Harvard Six Cities study's PM_{2.5} and trace element database (Thurston and Spengler 1985). This APCA method involved: (1) a multivariate factor analysis of the element data; (2) an identification of source-related categories for each multivariate factor based on prior understanding of the relationship between source emissions and distinct tracer elements for each factor (U.S. EPA 2003); (3) an adjustment of the normalized factor scores into absolute factor scores; and (4) a regression of the PM_{2.5} mass data on the APCA scores to apportion PM_{2.5} mass to each source category at each site. (In this application of APCA we added the three secondary aerosol components

to the mass regression to yield a more complete mass apportionment.) The results of these analyses are provided in more detail in Thurston and colleagues (2011).

Factor Analysis and Source Identification

Seventeen input variables were included in the initial nationwide factor analysis of the PM component data from 212 sites; these were daily values at each site for each of the following components of PM_{2.5}: As, Ca, Cu, Cl, Fe, Pb, Mn, Ni, Se, V, Si, Zn, K, Na, Mg, EC, and the gaseous pollutant NO₂. These specific elements were chosen to be consistent with earlier source-apportionment studies (U.S. EPA 2003), because they are usually well measured by the x-ray fluorescence elemental analysis and the ion chromatography methods used by the CSN, and because they were deemed of key relevance as potential tracers of pollution source categories. The factor analysis and source apportionment was conducted using statistical software SAS (version 9.1; SAS Institute, Cary, NC).

The initial factor analysis intentionally did not include PM_{2.5} components associated with secondary aerosols (SO₄²⁻, NO₃⁻, and OC) because when they have been included in source-apportionment models in the past, they typically aligned numerous source tracers on one combined secondary aerosols factor, which confounded the subsequent process of apportioning the mass into unique individual source categories. In this analysis, components that are markers of secondary aerosols (such as S) were therefore not included in the initial factor analysis, but were instead incorporated in the subsequent two-stage mass regression procedure (described in detail below). In that procedure, PM_{2.5} mass was first apportioned to the source categories identified by factor analysis and the remaining unexplained mass was apportioned to the secondary aerosols. However, it should be noted that the mass of the secondary aerosols was apportioned as a multiyear average value and thus did not reflect the known seasonal differences in secondary aerosol concentrations. Because of this, we have also conducted sensitivity analyses of both a year-round model and a regional–seasonal model.

Unlike previous nationwide factor and source-apportionment analyses, this work used the daily measured values of PM_{2.5} components rather than quarterly or annual average estimates. Therefore, both the day-to-day variations in elemental concentrations, as well as the site-to-site variations, were used to derive distinct source categories in the initial factor analysis. The APCA model is data-driven, rather than model-driven (like the chemical mass balance [CMB] model). In this approach, the factor scoring coefficients of the model are estimated using all available data. That is, the

Table 2. Concentrations of PM_{2.5} Mass and Its Components by Region and Season^a

Component	Season ^b							
	Overall	Winter	Summer	Northeast	Southeast	Southwest	Northwest	California
PM _{2.5} (µg/m ³)	14.2 ± 0.2	13.7 ± 0.2	14.6 ± 0.3	14.7 ± 0.2	15.0 ± 0.1	11.2 ± 0.2	10.8 ± 0.2	18.9 ± 0.3
As	1.3 ± 0.0	1.4 ± 0.0	1.3 ± 0.1	1.5 ± 0.1	1.4 ± 0.0	0.9 ± 0.0	1.1 ± 0.0	1.1 ± 0.0
Ca	54.9 ± 2.5	47.6 ± 2.4	62.6 ± 2.8	45.4 ± 1.6	50.8 ± 2.9	73.4 ± 2.8	69.7 ± 2.6	74.1 ± 3
Cu	3.9 ± 0.2	4.0 ± 0.2	3.8 ± 0.2	4.0 ± 0.2	3.8 ± 0.2	3.0 ± 0.1	3.7 ± 0.2	7.5 ± 0.2
Cl	29.0 ± 3.3	41.0 ± 4.4	18.6 ± 2.9	30.6 ± 3.2	16.6 ± 2.2	42.9 ± 5.6	32 ± 2.3	50.6 ± 1.4
Fe	87.3 ± 4.4	82.3 ± 4.9	93.7 ± 4.3	97.3 ± 5.6	73.6 ± 3.3	88.3 ± 3.5	74.3 ± 2.3	134.9 ± 4.2
Pb	4.7 ± 0.3	4.8 ± 0.3	4.6 ± 0.4	6.0 ± 0.3	4.5 ± 0.4	2.6 ± 0.1	3.5 ± 0.1	3.9 ± 0.1
Mn	3.6 ± 0.7	3.7 ± 0.8	3.5 ± 0.7	3.6 ± 0.3	4.5 ± 1.3	2 ± 0.1	3.2 ± 0.2	2.8 ± 0.1
Ni	1.7 ± 0.2	1.8 ± 0.2	1.5 ± 0.1	2.4 ± 0.2	1.0 ± 0.0	0.9 ± 0	1.2 ± 0.1	2.5 ± 0.1
Se	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.8 ± 0.1	1.4 ± 0.0	0.6 ± 0	0.7 ± 0	0.9 ± 0
V	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.2	1.7 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	3.3 ± 0.1
Si	104.3 ± 4.1	72.2 ± 3.2	137.5 ± 5.6	75.0 ± 2.1	96.5 ± 2.6	189.2 ± 6.1	108.7 ± 2.9	152.8 ± 3.7
Zn	16.2 ± 1.7	18.3 ± 1.7	14.4 ± 1.8	19.6 ± 1.1	17.1 ± 2.8	9.7 ± 0.4	11.5 ± 0.4	12.5 ± 0.6
K	68.2 ± 2.2	70.5 ± 2.9	65.5 ± 1.8	61.5 ± 2.6	70.2 ± 1.5	74.1 ± 1.6	71.8 ± 2.7	91.6 ± 1.6
Na	65.9 ± 3.1	62.7 ± 2.7	68.6 ± 3.8	51.0 ± 1.5	62.6 ± 2.4	92.2 ± 4.5	69.1 ± 3.7	154.1 ± 4.1
Mg	9.0 ± 0.4	6.3 ± 0.3	11.8 ± 0.5	7.5 ± 0.3	7.3 ± 0.3	15.3 ± 0.6	9.1 ± 0.3	15.6 ± 0.3
EC	646.0 ± 22.2	724.4 ± 26.0	568.5 ± 20.2	678.0 ± 24.5	623.5 ± 19.2	521.3 ± 20.3	637.1 ± 17.6	1030.5 ± 21.5
SO ₄ ²⁻ (µg/m ³)	1.2 ± 0.0	0.9 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.0 ± 0.0	0.5 ± 0.0	0.8 ± 0.0
OC (µg/m ³)	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	3.9 ± 0.1	4.6 ± 0.1	3.6 ± 0.1	4.3 ± 0.1	6.4 ± 0.1
NO ₃ ⁻ (µg/m ³)	1.7 ± 0.1	2.5 ± 0.1	1.0 ± 0.1	2.0 ± 0.0	1.1 ± 0.0	0.9 ± 0.0	1.8 ± 0.1	5.6 ± 0.2
NO ₂ (ppb)	14.1 ± 0.5	16.4 ± 0.5	12.5 ± 0.4	17.2 ± 0.4	10.7 ± 0.4	11.2 ± 0.5	14.3 ± 0.4	20.4 ± 0.4

^a Means ± SEs of site averages (2000–2005) for the 212 sites with both speciation and NO₂ data used in the initial factor analysis. Averages cover 6 months to 6 years depending on the site. Data are ng/m³ unless specified.

^b For this analysis, we did not restrict the number of observations in summer and winter, as we did with source apportionment and mortality analyses.

pollutant profiles for the various factors were determined using data from all 212 sites. The estimation of contributions of mass from the source categories is analogous to the CMB model in that the same pollutant profile for a source category is assumed to be consistent for the entire country; however, the advantage of the APCA model is that it uses multiple sites to determine the source category profile, unlike the CMB model, which usually relies on source profiles estimated at a single site. As discussed in the results that follow, the factor loadings (or correlations of the components with each factor) were then used to identify which source category a factor represented (e.g., high loadings of Se and As indicate a factor related to Coal Combustion, V and Ni indicate a factor related to Residual Oil Combustion) and the key tracers (i.e., components with the highest loadings) for each factor.

Computation of APCA Scores for Mass Apportionment

Although conventional factor analysis and principal component analysis are useful for identifying underlying source categories based on component concentrations that contribute to the PM_{2.5} mass, they do not directly provide an apportionment of mass. However, the individual output values from a factor analysis (i.e., the factor scores) can be applied to provide a quantitative solution that can be used to apportion mass. Briefly, this approach uses the factor scores from a zero-pollution observation day to define baseline values for each factor's scores, which are then subtracted from the observed factor scores to derive estimates of the APCA absolute factor scores. These provide positive indices of attributable emissions upon which PM_{2.5} mass concentrations can be regressed to achieve a source apportionment of PM_{2.5} mass to the source categories derived from the factor analysis. This approach produces apportionment results comparable to other multivariate PM_{2.5} mass source-apportionment approaches (Thurston et al. 2005; Hopke et al. 2006). More details are provided in Thurston and Spengler (1985).

Source-Apportionment Mass Regression Models

Based on the results from the factor analysis, we conducted a mass apportionment using the APCA approach, in which a linear mixed model was used for the regression analysis of the APCA factor scores. The linear mixed model was applied to 39,849 daily observations from 167 CSN monitoring sites for which at least 30 observations in the warm season and 30 in the cold season were available. The source category-to-mass relationships were modeled as fixed terms, with a random intercept by monitoring

site (I_j), thereby providing an overall estimate across all sites (e.g., PM_{2.5} mass per absolute factor score).

As noted above, the initial factor analysis explicitly did not include SO₄²⁻, NO₃⁻, and OC in the model, so we used a two-stage process to include them in the mass apportionment. Thus, before the overall PM_{2.5} mass-apportionment regression models were applied, regressions were conducted to ascribe the PM_{2.5} mass to SO₄²⁻, NO₃⁻, and OC in order to account for the secondary mass that would be otherwise unexplained ($I_j + e_{ij}$) by the absolute factor scores, APC_k:

$$\begin{aligned} \text{SO}_4^{2-}{}_{ij} &= \text{APC}_{1ij} + \dots + \text{APC}_{kij} + I_j + e_{ij} \\ \text{OC}_{ij} &= \text{APC}_{1ij} + \dots + \text{APC}_{kij} + I_j + e_{ij}, \text{ and} \\ \text{NO}_3^{-}{}_{ij} &= \text{APC}_{1ij} + \dots + \text{APC}_{kij} + I_j + e_{ij}. \end{aligned}$$

Therefore, for each secondary aerosol, a portion of the mass not accounted for by the identified source categories could then be quantified on a daily basis and included in the final PM_{2.5} mass regression step to ascertain a complete mass apportionment.

Absolute Principal Component Mass Regression Model

In this second mass regression step, the total PM_{2.5} mass was regressed onto the APCA scores and the three secondary mass terms derived above. The model was set up for the k PM_{2.5} APCs and the secondary aerosols such that

$$\begin{aligned} \text{PM}_{ij} &= \beta_1 * \text{APC}_{1ij} + \dots \\ &+ \beta_k * \text{APC}_{kij} + \beta_{(k+1)} * \text{SO}_4^{2-}{}_{ij} \\ &+ \beta_{(k+2)} * \text{OC}'_{ij} \\ &+ \beta_{(k+3)} * \text{NO}_3^{-}{}_{ij} + I_j. \end{aligned}$$

By deriving fixed coefficients in this linear mixed regression model, absolute factor score-to-mass conversions were estimated. This analysis thereby provided an overall estimate of the year-round source category-to-mass relationships.

As part of a sensitivity analysis, we applied separate regression models for each regional-seasonal subset of monitor sites, using the same mass source-apportionment method (as shown above) to test whether the interpretation of results would be influenced by U.S. regions and seasons. We performed ten separate regional-seasonal regression sensitivity analyses for five regions in the United States (Northeast, Southeast, Southwest, Northwest, and California) and two seasons (winter = October–March, summer = April–September) for each region. Sites were assigned to one of the five regions based on their latitude and longitude. Latitude > 39° N and longitude > -92° E were designated North and East, respectively, except for California, which was defined

as a region separate from the Northwest or Southwest. Results from the regional–seasonal models were then compared with the national model to evaluate any differences in the predicted contributions of source-apportioned mass to PM_{2.5}. (Further details of the application of this APCA method to the U.S. PM_{2.5} and trace element database for these U.S. metropolitan areas, as well as comparisons with a past source apportionment of the Inhaled Particles Network [IPN] PM_{2.5} data in the early 1980s, are provided in Thurston et al. 2011.)

Evaluation of NO₂ Data from Nearest-Neighbor Monitors

Approximately one-third of the nearest-neighbor NO₂ observations provided by the HEI–AER database were from sites outside the MSAs where the matched CSN sites were located. To evaluate the importance of this to the initial factor analysis and subsequent source apportionment, we repeated the factor analysis twice: (1) for the CSN data set with only element data (i.e., excluding all nearest-neighbor NO₂ data; $n = 46,478$ observations from 212 sites); and (2) for the subset of sites that had NO₂ data from within the same MSA as the matched CSN site ($n = 29,837$ observations from 114 sites in 51 MSAs).

Computing Spatial Representativeness of Source Categories, Factors, and Components

In order to assess whether the CSN central-site monitors spatially represent the factors, source categories, and component concentrations, we compared all monitors located within 20 miles of one another (mean separation distance = 9.9 miles; $n = 53$ unique site pairs). Using this subset of monitoring data, we calculated the difference in site concentration means for all pairs of sites. From these means, we calculated the percent coefficient of variation (%CV; $[\{\text{standard error}/\text{mean}\} \times 100]$) of the mean difference for PM_{2.5} mass, the mass apportioned to each source category, and the mass of each source category's key component tracer.

ANALYSIS OF MORTALITY IN THE ACS CPS-II COHORT

This study extended the follow-up period of the largest cohort study of the association between PM_{2.5} exposure and mortality. Earlier studies of this cohort linked individual risk factors and vital status data with national ambient air pollution data (Pope et al. 1995, 2002, 2004). For the current analysis, we expanded the subject follow-up time from 16 years to 22 years, which increased by more than one-third the number of deaths available for analyses (compared with earlier analyses of the CPS-II cohort and air pollution; e.g., Pope et al. 2002).

For the CPS-II mortality analyses, sufficient air pollution data were available for at least one monitoring site in each of the 100 MSAs that had CPS-II cohort members in residence. For each pollutant, an MSA's overall 2000–2005 average concentration — calculated from all available CSN monitoring data within each MSA over the period — was used as a summary measure of exposure and assigned to each subject within the MSA. Thus, all subjects residing in an MSA were assigned the same exposure value in the mortality analyses. Of the 100 MSAs used, data from 76 had also been used in earlier analyses of CPS-II data (Pope et al. 1995, 2002, 2004); we added 24 MSAs that have new EPA CSN monitoring sites.

Study Population

The health effects analyses are based on 445,860 men and women drawn from the CPS-II cohort, an ongoing prospective mortality study of approximately 1.2 million adults (Calle and Terrell 1993; Krewski et al. 2009). Participants were enrolled by ACS volunteers in the fall of 1982; they resided in all 50 states, the District of Columbia, and Puerto Rico; and they were generally friends, neighbors, or acquaintances of the ACS volunteers. Enrollment was restricted to persons who were at least 30 years of age and who were members of households with at least one individual 45 years of age or older. Participants completed a confidential questionnaire that included questions about age, sex, weight, height, smoking history, alcohol use, occupational exposures, diet, education, and marital status.

Deaths that occurred between September 1, 1982, and December 31, 2004, were ascertained through personal inquiries by volunteers in September 1984, September 1986, and September 1988, and subsequently through linkage with the National Death Index. Cause of death was coded according to the International Classification of Diseases, 9th Revision (ICD-9) until the end of 1999, and the 10th Revision (ICD-10) after that (NCHS 2008, 2010). Death certificates or codes for cause of death were obtained for over 98% of all known deaths. Based on the participants' addresses and 3-digit Zip Code areas (ZCAs) at the time of enrollment, each participant was assigned a metropolitan area of residence. The analytic cohort included all participants with adequate questionnaire and cause-of-death data who resided — at the start of the CPS-II study — in one of the 100 MSAs that later had CSN PM_{2.5} component air pollution data available for the follow-up period (445,860 total subjects).

Statistical Methods and Data Analysis

Standard and multilevel random effects Cox proportional hazards models were used to assess mortality hazard ratios

— the change in the risk of death — related to exposure to the interquartile range (IQR) of a pollutant's concentration, as determined for various indices of air pollution (e.g., for the respective tracer elements and source categories). The baseline analysis estimated adjusted mortality hazard ratios, including an MSA-based random effects component. The time variable used in the models was survival time from date of enrollment. Survival times of participants who were still living at the end of the follow-up period in 2004 were censored. All models were stratified by 1-year age categories, gender, and race (white versus other), which allowed each age-sex-race category to have its own baseline risk.

Individual-level covariates were used to control for characteristics of the subjects that might confound or modify the association between air pollution and time of death; they were based largely on past analyses of CPS-II data in order to allow the most consistent comparisons with earlier results. The data had been collected on the CPS-II questionnaire and were thought to be of potential importance on the basis of previous studies. Descriptive statistics of the CPS-II cohort at baseline (1982) are provided in Table 3, both overall and as a function of the PM_{2.5} exposure level (i.e., quartile of PM_{2.5} mass). Covariates included tobacco smoking, education, marital status, body-mass index, alcohol consumption, occupational exposure, and diet (accounting for fat consumption and consumption of vegetables, citrus, and high-fiber grains). Specifically, we included data from the CPS-II cohort questionnaires for 42 individual-level covariates:

- six variables to represent active smoking habits, including two nonlinear terms for cigarettes per day and number of years smoked;
- six variables to characterize former smoking habits;
- one variable to indicate pipe or cigar smoker only;
- one variable for exposure to passive smoke;
- two variables to represent marital status;
- two variables to characterize level of education;
- two variables to represent linear and squared indices of body-mass index;
- six variables to characterize consumption of beer, wine, and other alcohol;
- eight variables to represent diet;
- seven variables to characterize the subject's main lifetime occupation and his or her possible exposure to PM in the workplace; and
- one variable for self-reported exposure to dust and fumes in the workplace.

We also examined six ecologic covariates to represent social and economic variables measured at the ZCA scale (see Table 4). Data were obtained for ZCAs from the 1990 United States Census (Jerrett et al. 2009):

- median household income;
- percentage of persons over 16 years of age who were unemployed;
- percentage of adults with a post-secondary education;
- income disparity (a measure of the inequality of income or wealth distribution within a neighborhood or city; range from 0 to 1, with 0 indicating an equal distribution of income and 1 indicating that one person has all the income and everyone else has no income; Willis et al. 2003);
- percentage of population who were black; and
- percentage of population who were Hispanic.

These covariates were included as (1) the average for the MSA and (2) the difference between the average for the ZCA and the average for the MSA.

The standard Cox model we applied for our base model analyses assumes that all observations are statistically independent, an assumption that was relaxed in the subsequent analyses that addressed random effects (see next section).

Our base model analyses used a variety of PM_{2.5} pollution exposure indices, including PM_{2.5} mass, source category-related contributions to PM_{2.5} mass, and concentrations of elemental components. Later we tested how sensitive the association between a PM_{2.5} component and mortality would be to different modeling approaches and assumptions by using standard Cox models and random effects Cox models, each with and without the ecologic covariates.

Random Effects Modeling

Ma and coworkers (2003) developed a modification of the standard Cox model that incorporates random effects at the community level to represent spatial patterns in the data (the random effects Cox model) and established large-sample properties of the maximum likelihood estimates of the model parameters. This model allowed us to take into account residual variation in mortality among communities (Ma et al. 2003). The baseline hazard function was modulated by a community-specific random variable representing the residual risk of death for subjects in that community after individual-level and ecologic covariates had been controlled for. The standard Cox model assumes that the survival times for individuals are statistically independent. Our earlier analyses of the CPS-II cohort (Krewski et al. 2000) found evidence of responses clustering at the MSA level, which needed to be addressed in the current analyses.

Table 3. Descriptive Characteristics of the CPS-II Cohort at Baseline in 1982^a

Variable	Entire Cohort (N = 445,860)	Subjects in Each PM _{2.5} Quartile			
		n = 109,313	n = 109,684	n = 101,775	n = 125,088
Concentration range for PM _{2.5} quartile (µg/m ³)		8.60–13.35	13.36–15.16	15.17–16.49	16.50–26.93
Number of MSAs	100	26	31	23	20
Age (years)	56.6 ± 10.5	57.4 ± 10.6	56.5 ± 10.4	56.3 ± 10.6	56.5 ± 10.4
Male sex (%)	43.6	43.9	43.7	43.5	43.3
White race (%)	94.1	96.3	94.5	92.2	93.3
Education (%)					
Less than high school	12.5	11.8	13.5	11.7	13.0
High school	31.3	31.3	32.8	29.3	31.6
More than high school	56.2	56.9	53.7	59.0	55.4
Smoking Status					
Current smokers					
Subjects (%)	21.6	20.0	22.1	22.0	22.3
Cigarettes/day (n)	22.1 ± 12.5	22.1 ± 12.6	22.2 ± 12.5	21.9 ± 12.5	22.2 ± 12.6
Duration (years)	33.3 ± 11.1	33.6 ± 11.0	33.2 ± 10.9	33.3 ± 11.2	33.2 ± 11.1
Started smoking < 18 years (%)	41.0	40.2	41.6	40.9	41.2
Former smokers					
Subjects (%)	25.3	25.4	25.2	25.8	24.8
Cigarettes/day (n)	21.3 ± 14.9	21.3 ± 14.7	21.6 ± 15.0	21.1 ± 14.9	21.3 ± 14.9
Duration (years)	22.1 ± 12.6	21.9 ± 12.6	22.2 ± 12.5	22.0 ± 12.6	22.2 ± 12.7
Started smoking < 18 years (%)	38.3	37.1	39.2	39.2	37.9
Exposure to smoke (hours/day) ^b	3.2 ± 4.4	2.9 ± 4.3	3.3 ± 4.5	3.3 ± 4.4	3.3 ± 4.5
Pipe or cigar smoker only (%)	9.7	9.4	9.8	10.0	9.6
Marital status (%)					
Married	83.7	84.9	83.6	83.8	83.7
Single	3.5	3.0	3.5	4.2	3.4
Other	12.8	12.1	12.9	13.0	13.3
Body-mass index	25.1 ± 4.1	25.1 ± 4.0	25.3 ± 4.1	25.0 ± 4.1	25.2 ± 4.1
Occupational dirtiness index (%) ^c					
Level 0	50.4	50.6	49.5	50.4	51.1
Level 1	13.0	12.6	13.5	13.5	12.6
Level 2	11.2	11.9	10.5	11.6	10.8
Level 3	4.7	4.8	4.9	4.5	4.7
Level 4	6.4	6.7	7.3	6.0	5.7
Level 5	4.3	4.4	4.6	3.8	4.2
Level 6	1.2	1.0	1.2	1.0	1.4
Not able to ascertain	8.8	8.0	8.5	9.2	9.5
Industrial exposure reported (%)	19.7	19.5	20.2	19.0	19.9
Dietary fat consumption (% in quintile) ^d					
1st	14.5	13.4	14.7	14.9	14.8
2nd	15.9	15.4	15.9	15.9	16.2
3rd	17.3	17.4	17.1	17.3	17.5
4th	21.1	21.6	21.1	20.7	21.1
5th	31.2	32.2	31.2	31.2	30.4

Table continues next page

^a Data with ± values are means ± SDs. Percentages are % of applicable cohort.

^b Subjects exposed to second-hand tobacco smoke.

^c Dirtiness increases with increasing number.

^d Dietary fat or fiber consumption increases with each quintile.

Table 3 (Continued). Descriptive Characteristics of the CPS-II Cohort at Baseline in 1982^a

Variable	Entire Cohort (N = 445,860)	Subjects in Each PM _{2.5} Quartile			
		n = 109,313	n = 109,684	n = 101,775	n = 125,088
Dietary fiber consumption (% in quintile) ^d					
1st	17.1	16.2	18.1	16.5	17.2
2nd	20.1	19.5	20.8	19.8	20.2
3rd	18.9	18.8	18.9	19.2	18.7
4th	22.6	23.0	22.0	22.9	22.5
5th	21.3	22.5	20.2	21.6	21.1
Beer consumption (%)					
Yes	22.4	22.4	22.8	22.4	22.0
No	9.5	9.5	9.2	9.4	9.7
Missing	68.1	68.1	68.0	68.2	68.3
Liquor consumption (%)					
Yes	26.7	28.2	26.1	27.3	25.5
No	8.8	8.6	8.6	8.6	9.1
Missing	64.5	63.2	65.3	64.1	65.4
Wine consumption (%)					
Yes	22.0	21.8	21.7	22.5	21.9
No	8.9	9.1	8.8	8.8	9.1
Missing	69.1	69.1	69.5	68.7	69.0
Region (%)					
East	80.4	61.2	91.2	95.3	75.6
West	19.6	38.8	8.8	4.7	24.5

^a Data with \pm values are means \pm SDs. Percentages are % of applicable cohort.

^b Subjects exposed to second-hand tobacco smoke.

^c Dirtiness increases with increasing number.

^d Dietary fat or fiber consumption increases with each quintile.

Table 4. Ecologic Risk Factors for CPS-II Cohort in 1990^a

Variable	Cohort with Ecologic Covariate Information (N = 445,860)	Subjects in Each PM _{2.5} Quartile			
		n = 109,313	n = 109,684	n = 101,775	n = 125,088
Concentration ranges for PM _{2.5} quartiles ($\mu\text{g}/\text{m}^3$)					
		8.60–13.35	13.36–15.16	15.17–16.49	16.50–26.93
Black (%)	10.5 \pm 18.0	5.9 \pm 12.3	11.9 \pm 19.3	14.3 \pm 20.2	10.4 \pm 18.1
Hispanic (%)	5.2 \pm 10.2	6.2 \pm 11.2	4.4 \pm 8.4	3.4 \pm 5.6	6.6 \pm 12.9
High-school education or greater (%)	33.8 \pm 12.6	35.7 \pm 11.6	31.4 \pm 11.5	35.4 \pm 13.3	33.0 \pm 13.4
Unemployment level ^b (%)	5.4 \pm 3.0	5.3 \pm 2.4	5.6 \pm 3.1	4.9 \pm 3.0	5.6 \pm 3.4
Income disparity ^c	0.39 \pm 0.05	0.39 \pm 0.05	0.39 \pm 0.05	0.39 \pm 0.05	0.39 \pm 0.04
Annual household income (thousands of dollars)	36.5 \pm 13.9	34.7 \pm 12.2	35.9 \pm 14.0	39.2 \pm 15.4	36.5 \pm 13.5

^a Census data were first available at the ZCA level in 1990. This cohort includes only those subjects with ecologic covariate data available. Data are means \pm SDs.

^b Percentage of subjects over the age of 16 years and unemployed.

^c A measure of the inequality of income or wealth distribution within neighborhoods and cities. Reported as the Gini coefficient.

Here, we describe a Cox model with a single level of spatially correlated (e.g., within an MSA) random effects. Suppose that the cohort of interest is composed of m spatially correlated clusters indexed by i . Within the i th cluster, there are J_i spatially correlated subclusters indexed by (ij) . Specifically, we assume that the cluster-level random effects U_1, \dots, U_m are positive random effects with expectation and covariance $E(U_i) = 1$, $\text{Var}(U_i) = \sigma^2$, and $\text{Cov}(U_i, U_j) = 0$ for $i \neq j$, where σ^2 is the MSA-level variance of the random effects, $0 < r_1 < 1$, and $d(s, i)$ indicates the distance between the independent clusters indexed by s and i , which is defined as $d_1(s, i) = \infty$. We also assume that, given the cluster-level random effects $U^* = u^* = (u_1, \dots, u_m)$, the subcluster-level random effects U_{11}, \dots, U_{mJ_m} are positive and spatially dependent.

Furthermore, within each subcluster (ij) there are n_{ij} individuals. Suppose that the cohort is stratified on the basis of one or more relevant covariates and these strata are indexed by $s = 1, 2, \dots, a$. Given the random effects, we assume that the individual hazard functions are conditionally independent, with:

$$\lambda_{ik}^{(s)}(t) = \lambda_o^{(s)}(t) u_i \exp\left(\beta^T X_{ik}^{(s)}\right)$$

where $\lambda_{ik}^{(s)}(t)$ is the hazard function for the k th subject in the i th MSA in strata s ; $\lambda_o^{(s)}(t)$ is the baseline hazard function common to all subjects in strata s ; u_i is the realization of the random effect for the i th MSA; and β is a vector of unknown regression parameters linking the design vector $X_{ik}^{(s)}$ to the hazard function. Note that in this model formulation, the survival times, either observed or censored, are spatially correlated. The distribution of random effects is assumed to not depend on the regression parameter. A detailed description of the random effects mortality modeling methods and approach is provided elsewhere (Krewski et al. 2009).

RESULTS

AIR POLLUTION ESTIMATES

The study's full air pollution data set included 46,478 daily observations from 212 CSN and nearest-neighbor NO₂ monitoring sites distributed throughout the United States. These sites produced an average of 220 observations per site (or almost two years of data over the 6-year

period per site at the common network data collection rate of every-third-day sampling). Table 2 provides an overall summary of PM_{2.5} and its components used in the factor analysis along with averages by season and by the five U.S. regions used in the sensitivity analyses.

Factor Analysis Results: Identification of Major Source Categories and Their Key Tracers

An eight-factor solution using varimax rotation was chosen as optimum based on an examination of both the factor eigenvalues (i.e., the data variance explained by the component) and the source-related interpretability of the factors. The oblique solution was found to provide similar results to the orthogonal solution. The latter was chosen because it required the factor scores to be uncorrelated (i.e., independent of one another), which is desirable in the subsequent source-apportionment regression steps.

Table 5 provides the factor loadings (i.e., the correlations between the factor scores and observable input variables, such as element concentrations) that aid in the interpretation of the factors. The physical identification of the factors (as previously documented for major U.S. source classes [Cooper and Watson 1980; Gordon 1980; Thurston and Spengler 1985; U.S. EPA 2003]) was possible by comparing elements that have the highest loading (correlation) with each factor against the elements emitted in relatively large amounts or concentrations by known source categories (in comparison with emissions of other elements, or from other sources).

However, this and all other source-apportionment analyses are limited in that key tracer elements known to be associated with a particular source category are also emitted by other sources that had not been identified for the analysis. Those contributions can be mistakenly attributed to the most similar source category being analyzed, which can then overestimate the contributions of a specific source category. Therefore, each source category described here can be interpreted as including the named source (e.g., Steel Industry) plus any other sources that emit the same key tracer elements (Fe and Mn) associated with the named source.

On the basis of this information, Table 5 shows which elements (based on their correlation with sources) could be used to identify the factor for a sample of particles. For example, in the first column, a factor with high loadings for (i.e., correlations with) the common earth crustal elements Ca and Si, which are most commonly found together in soil-derived particles (e.g., wind-blown dust), would indicate Soil as the factor.

Table 5. Factor Loadings for Each Element in Each Factor^a

	Soil	Metals	Traffic	Salt	Residual Oil Combustion	Steel Industry	Coal Combustion	Biomass Combustion
As	0.11	0.58	0.07	-0.08	0.01	-0.08	0.49	0.05
Ca	0.75	0.06	0.15	0.01	0.05	0.04	0.01	0.16
Cu	0.10	0.27	0.73	0.05	-0.10	0.14	-0.10	-0.15
Cl	-0.12	0.00	0.09	0.58	0.02	0.01	0.34	0.44
Fe	0.46	0.14	0.29	0.02	0.12	0.64	0.10	0.16
Pb	0.04	0.87	0.09	0.02	0.01	0.06	0.11	-0.01
Mn	-0.01	0.13	-0.02	0.02	0.02	0.93	0.02	0.02
Ni	-0.01	0.02	0.15	0.02	0.82	0.08	0.03	-0.09
Se	0.00	0.04	0.09	0.01	0.01	0.07	0.87	-0.03
V	0.07	0.03	0.08	0.08	0.82	-0.01	-0.01	0.09
Si	0.85	-0.01	-0.03	0.12	0.00	0.06	0.02	0.07
Zn	-0.02	0.75	0.09	0.13	0.04	0.22	-0.12	0.09
K	0.33	0.09	0.11	0.03	-0.01	0.10	-0.06	0.78
Na	0.01	0.11	-0.05	0.86	0.10	0.02	-0.08	0.09
Mg	0.43	-0.02	0.00	0.72	-0.03	0.03	-0.04	-0.24
EC	0.14	0.09	0.68	0.02	0.22	0.03	0.29	0.30
NO ₂	-0.03	-0.05	0.71	-0.09	0.34	-0.04	0.10	0.13
PM _{2.5} ^b	0.17	0.13	0.34	0.01	0.13	0.06	0.25	0.26

^a Correlation between each component and the factor. Values are dimensionless. Key tracers for each factor are shown in bold.

^b PM_{2.5} was not included in the factor analysis.

The Metals factor has high loadings for Pb and Zn. Historically, these elements were also related to refuse burning (Pb) and automobile emissions (Zn); but phasing out both open refuse burning and the use of leaded gasoline has essentially eliminated those two sources in ambient air, which allows these elements to now be used as tracers for emissions from processing non-ferrous metals.

The motor vehicle Traffic factor has high loadings for both EC and NO₂. Although each of these two pollutants individually has a variety of sources, the factor most associated with both EC and NO₂ emissions is Traffic. Cu was also found to be associated with Traffic and has been attributed to traffic-related brake wear in past studies (Schauer et al. 2006). Unfortunately, although EC is present in much higher quantities in diesel emissions than in gasoline emissions (Schauer et al. 2006), a unique tracer to separate gasoline- from diesel-fueled vehicles was not available in this dataset. Therefore, a clear-cut interpretation of this Traffic factor is difficult, and identification may vary spatially as the mix of diesel and gasoline vehicles changes.

The Salt factor is based upon its loadings for both Na and Cl and would include contributions from marine aerosols (along the coasts) and road salt (in the northern states).

The Residual Oil Combustion factor is based upon its high loadings for both V and Ni, two elements that are commonly found in high concentrations in heavier fuel oils, such as residual fuel oil Number 6 (burned in the past by power plants and large buildings in New York City) and cargo ship bunker fuels.

The Steel Industry factor is based on its loadings for both Fe and Mn, elements found in high concentrations in emissions from ferromanganese steel furnaces.

The Coal Combustion factor is based on its high loadings for both As and Se. These are two elements that are also individually emitted by metals operations (As) and smelters (Se), but Coal Combustion produces both elements together (see Helble 2000). Therefore, this factor, which is loaded for both of these elements, is identified as being related to the combustion of coal (about 94% of which is burned in coal-fired electric power plants in the

United States [U.S. Energy Information Administration 2010]).

The Biomass Combustion factor (which is mostly wood burning and forest fires) is based primarily on its high loading for K, an element emitted during vegetative burning (U.S. EPA 2003).

U.S. maps of the estimated MSA averages of PM_{2.5} mass contributions from each source category support the above interpretations of the factor analysis and source apportionment (see Figures 1 and 2). The contributions of mass from the Traffic source category were elevated throughout the United States and were highest in Southern California; Soil contributions were high in the desert Southwest; Steel Industry contributions were highest in cities with major steel works (e.g., Detroit, MI; Birmingham, AL); Coal Combustion contributions were highest in the Ohio Valley region (e.g., Pittsburgh, PA); Residual Oil Combustion contributions were highest in northeastern cities that use residual oil for heating and generating electricity (e.g., New York City, NY; Providence, RI; and New Haven, CT), as well as in cities with major seaports (e.g., Los Angeles and Long Beach, CA; Savannah, GA; Seattle, WA; and Newark, NJ), which is consistent with a major contribution from cargo ships burning bunker fuel in port cities; Salt contributions were high in coastal locations; and Biomass Combustion was highest in the Northwestern U.S., as expected.

As a further verification of the source categories, correlations of factor scores with air pollution variables not included in the factor analysis were also examined. For example, correlations were tested for daily observations of gaseous pollutants, such as SO₂ and CO, at sites near the CSN monitors for the subsets of days for which gas data were available. SO₂ ($n = 42,093$ observations) was most correlated over space and time with Residual Oil Combustion ($r = 0.32$, $P < 0.001$) and Coal Combustion ($r = 0.22$, $P < 0.001$). In addition, Coal Combustion had the highest factor score correlation with S (measured in PM_{2.5}), a tracer for the SO₄²⁻ secondary aerosol, that largely results from coal-burning emissions of SO₂, especially in the Eastern United States ($r = 0.25$, $P < 0.001$, in the daily dataset). As also expected, CO ($n = 39,324$ observations) was most correlated with Traffic ($r = 0.38$, $P < 0.001$).

Similarly, we investigated the factor score correlations with Hg, an elemental tracer primarily emitted into the air by coal combustion (U.S. EPA 2005). As expected, we found that the Coal Combustion factor scores had the highest correlation with Hg observations from the same monitoring sites ($r = 0.19$, P value < 0.001). The correlation of Se, As, and Hg (predominantly from coal combustion in the U.S.; U.S. EPA 2005) further supports the interpretation that the factor that contains Se and As is an index of coal combustion emissions.

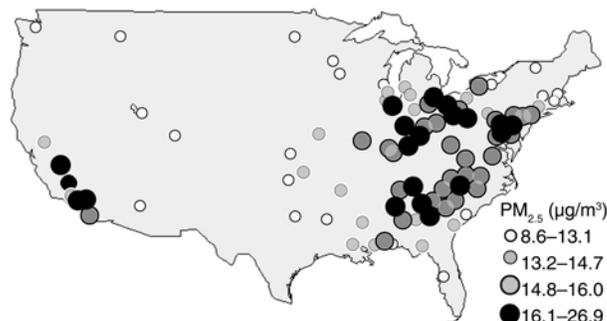


Figure 1. CSN sites ($n = 167$; 100 MSAs) with speciation and NO₂ data. Each circle radius is proportional to the overall mean PM_{2.5} concentration for each site (relative to a maximum value of 26.5 µg/m³).

Correction for NO₂ Data from Nearest-Neighbor Monitors

To evaluate the importance to the initial factor analysis, and to the subsequent source apportionment, of the fact that about one-third of the nearest-neighbor NO₂ data used were from sites outside the MSA in which the matched CSN site was located, we repeated the factor analysis twice: (1) for the CSN data set with only element data (i.e., excluding all NO₂ data; $n = 46,478$ observations; 212 sites); and (2) for the subset of the CSN data set for which NO₂ data were available from within the same MSA as the matched CSN site (local NO₂ data; $n = 29,837$ observations; 114 sites; 51 MSAs).

In the first repetition, all factor scores except those for Traffic were reproduced with negligible changes, producing correlations to the initial factor scores as follows: Soil $r = 1.00$; Metals $r = 0.99$; Salt $r = 1.00$; Residual Oil Combustion $r = 0.98$; Steel Industry $r = 1.00$; Coal Combustion $r = 0.99$; and Biomass Combustion $r = 0.98$. However, the Traffic factor scores from the first repetition (without the NO₂ data) had a distinctly lower correlation with the initial Traffic factor scores ($r = 0.86$), which had included NO₂ as a variable. The Traffic factor was most notably affected by the elimination of the NO₂ data because it was the factor with the largest loading for NO₂ in the initial factor analysis. Without the inclusion of NO₂ in the first repetition, it was not possible for the factor analysis to separate out a distinct Traffic factor.

In the second repetition (for the subset of MSAs that had local NO₂ data; $n = 29,837$ observations; 114 sites; 51 MSAs), we found that the Traffic factor was again identified and the factor scores were well correlated ($r = 0.95$) with the initial Traffic factor scores for this subset of MSAs. This indicates that the initial identification of the Traffic factor was not an artifact of the NO₂ data from the

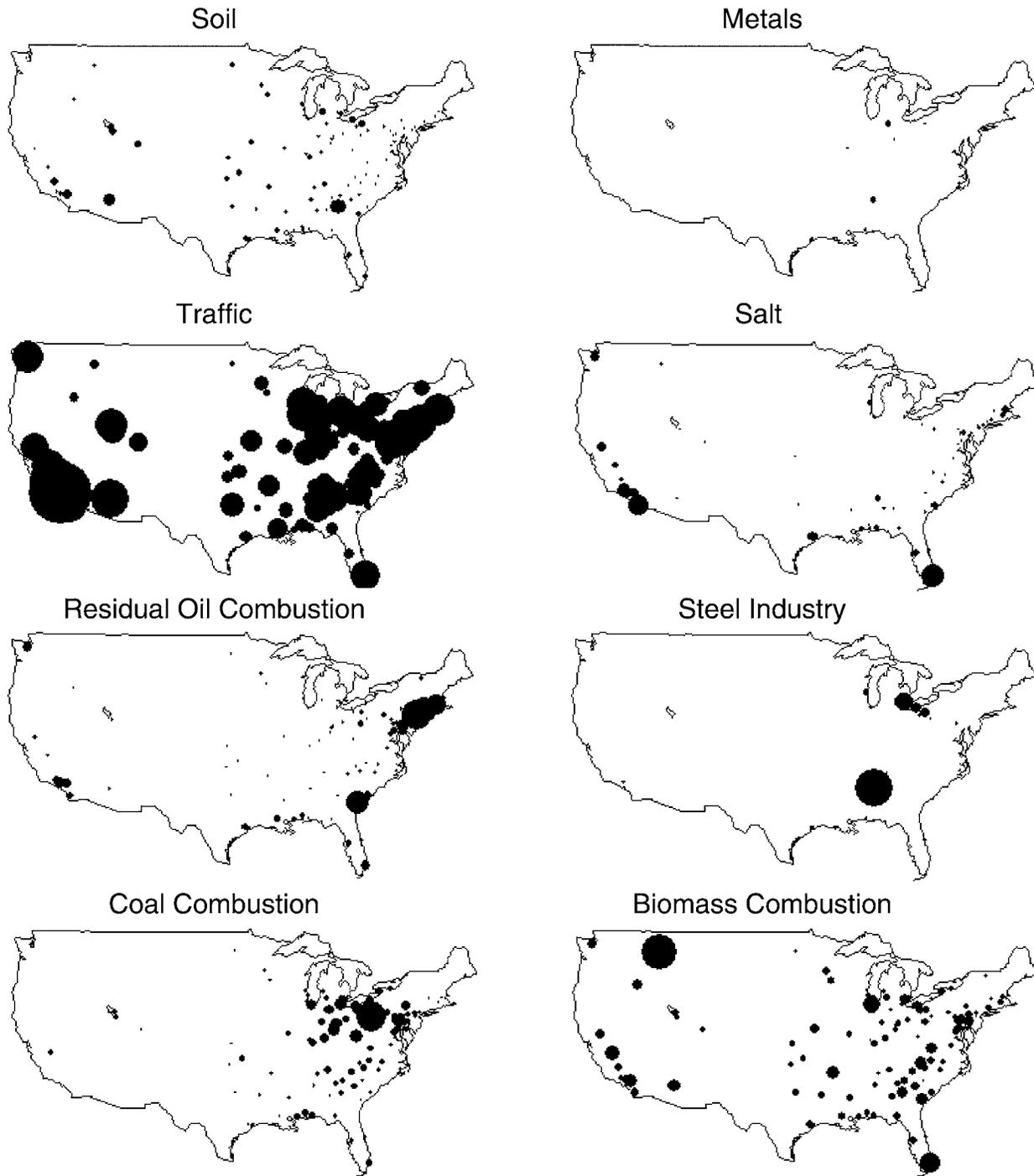


Figure 2. Spatial plots of PM_{2.5} mass for each source category based on 39,849 observations at 167 monitors in 100 MSAs. Each circle radius is proportional to the annual average of all available data (for up to 6 years) of the source category's contribution for each MSA (relative to a maximum value of 11.8 $\mu\text{g}/\text{m}^3$; except for the Salt and Steel Industry source categories, which are relative to a maximum value of 2.4 $\mu\text{g}/\text{m}^3$).

outside-MSA monitors, but that it was a valid representation of the Traffic-related factor.

Based on the above findings, using the initial factor model's scoring coefficients, we reestimated the factor scores for all the factors that had not been affected by the NO₂ data (i.e., all but Traffic) using the full data set. Then we recomputed the Traffic factor scores based only on the CSN element data (excluding all NO₂ data) for the MSAs without local NO₂ data (16,641 observations; 98 sites; 49 MSAs). This was accomplished by first regressing the initial Traffic factor scores for the 29,837 observations (114 monitors; 51 MSAs) that had local NO₂ data on the CSN element concentrations for those same observations. This provided a predictive equation for estimating the Traffic factor scores that is based upon only the CSN element data (i.e., excluding all NO₂ data). This regression resulted in the relationship shown in Figure 3, with a slope of 1.0 and an $R^2 = 0.89$. This indicates that, once the factor vectors were defined by the initial factor analysis, we were able to reliably estimate the Traffic factor scores using only the element data.

The differences between the initial and recomputed Traffic factor scores were negligible. Furthermore, Traffic factors calculated using the data sets with all nearest-neighbor NO₂ data, with only local MSA NO₂ data, and without NO₂ data, when applied to mortality analyses (not shown) produced nearly identical risk estimates. Therefore, Traffic factor estimates based on elemental data with NO₂ data measured in the same MSA were used for the mortality analyses.

Source-Related Mass Estimates

The average mass estimates for the eight source categories and three secondary aerosols were based on averaging across the 167 monitoring sites (out of 212) that were located inside an MSA and had at least 30 observations in both summer and winter.

Regional–seasonal averages were derived from the season-specific site averages for the sites within each of the five regions (Northeast, Southeast, Northwest, Southwest, California).

From the CSN site-specific averages, MSA averages for the PM_{2.5} contributions from each source category were also computed for the 100 MSAs used in the mortality analyses. These allowed comparisons with historical MSA-based estimates for similar sources identified using data from the EPA's IPN (which operated between 1979 and 1982 [see supplement to Thurston et al. 2011]).

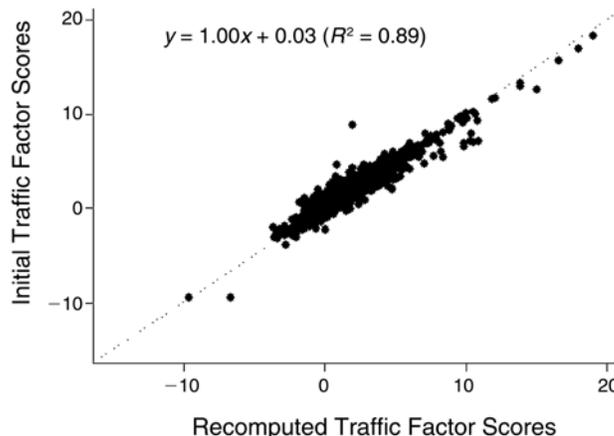


Figure 3. Initial Traffic factor scores (based on 46,478 observations including CSN element and all NO₂ data; 212 sites) compared with recomputed Traffic factor scores using only CSN element data for the MSAs that had no local NO₂ data (16,641 observations; 98 sites; 49 MSAs).

Regression Model for Source Category Tracer Mass and Its Results

For the simpler mass regression model for a single tracer, the key tracers designated to represent each of the eight source categories were Si for Soil, Zn for Metals, EC for Traffic, Cl for Salt, Ni for Residual Oil Combustion, Fe for Steel Industry, Se for Coal Combustion, and K for Biomass Combustion. These characteristic source category tracers were chosen based on their high correlations with each of the factor scores. Therefore, the mass-apportionment regression was set up for the eight source category tracers and the three secondary aerosols, such that:

$$PM_{ij} = \beta_1 * Si_{ij} + \beta_2 * Se_{ij} + \beta_3 * Ni_{ij} + \beta_4 * K_{ij} + \beta_5 * Fe_{ij} + \beta_6 * Cl_{ij} + \beta_7 * Zn_{ij} + \beta_8 * EC_{ij} + \beta_9 * SO_4^{2-}{}_{ij} + \beta_{10} * OC'_{ij} + \beta_{11} * NO_3^{-}{}_{ij} + I_j$$

Therefore, the β_k coefficients provide the tracer mass-to-source mass scaling factor. For example, β_1 is the Si mass-to-Soil mass scaling factor. Table 6 provides the estimates of PM_{2.5} mass for each source category and the percentage of mass attributed to the source category that is comprised of its key tracer component.

APCA Mass Regression Model and Results

The mass regression model using the APCA was set up for the eight source categories and the three secondary aerosols, such that:

Table 6. Nationwide Results from the Single-Tracer Mass Source-Appportionment Method^a

	Soil (Si)	Metals (Zn)	Traffic (EC)	Salt (Cl)	Residual				Other ^b		
					Oil Combustion (Ni)	Steel Industry (Fe)	Coal Combustion (Se)	Biomass Combustion (K)	SO ₄ ²⁻	NO ₃ ⁻	
PM _{2.5} (µg/m ³) ^c	0.2 ± 0.1	0.1 ± 0.1	4.3 ± 2.1	0.1 ± 0.2	0.2 ± 0.3	0.1 ± 0.1	0.8 ± 0.6	1.5 ± 0.8	4.3 ± 2.0	0.9 ± 1.5	2.6 ± 1.0
Tracer Mass (%) ^d	42.3 ± 3.6	21.6 ± 1.8	15.3 ± 0.1	23.6 ± 1.9	0.9 ± 0.0	91.4 ± 19.3	0.2 ± 0.0	4.3 ± 0.1	20.3 ± 0.1	79.4 ± 1.0	79.4 ± 0.6

^a Values are nationwide means ± SEs; based on 39,849 observations at 167 sites in 100 MSAs.

^b SO₄²⁻, OC, and NO₃⁻ mass not included with another component or source category; values were estimated via regression.

^c Contribution of PM_{2.5} from each source category.

^d Tracer element mass as percentage of source category mass.

Table 7. Average PM_{2.5} Mass Contributions Apportioned to Each Source Category for Each Region and Season Based on Full-Year Mass Data^a

	Soil	Metals	Traffic	Salt	Residual				SO ₄ ²⁻	OC	NO ₃ ⁻	Predicted PM _{2.5} Mass	Total PM _{2.5} Mass
					Oil Combustion	Steel Industry	Coal Combustion	Biomass Combustion					
Full Year	0.7 ± 0.0	0.1 ± 0.0	3.8 ± 0.2	0.1 ± 0.0	0.9 ± 0.1	0.1 ± 0.0	1.1 ± 0.1	1.3 ± 0.1	4.4 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	14.2 ± 0.3	14.3 ± 0.2
Winter													
NE	0.4 ± 0.0	0.1 ± 0.0	4.3 ± 0.3	0.1 ± 0.0	1.3 ± 0.2	0.1 ± 0.0	1.6 ± 0.2	1.5 ± 0.2	2.9 ± 0.1	0.2 ± 0.0	1.5 ± 0.1	13.9 ± 0.1	13.7 ± 0.3
SE	0.5 ± 0.1	0.2 ± 0.0	3.3 ± 0.3	0.1 ± 0.0	0.7 ± 0.1	0.1 ± 0.1	1.1 ± 0.1	1.5 ± 0.1	3.9 ± 0.1	1.9 ± 0.1	0.5 ± 0.1	13.7 ± 0.0	13.3 ± 0.2
SW	0.9 ± 0.2	0.0 ± 0.0	4.8 ± 1.0	0.1 ± 0.0	0.6 ± 0.1	0.0 ± 0.0	0.5 ± 0.1	1.7 ± 0.3	1.8 ± 0.4	0.3 ± 0.1	0.7 ± 0.3	11.4 ± 0.3	11.4 ± 0.6
NW	0.6 ± 0.1	0.1 ± 0.0	4.7 ± 0.6	0.1 ± 0.0	0.7 ± 0.2	0.0 ± 0.0	0.8 ± 0.1	2.2 ± 0.4	0.8 ± 0.2	0.5 ± 0.2	2.2 ± 0.4	12.8 ± 0.2	12.9 ± 0.6
Summer													
NE	0.7 ± 0.0	0.2 ± 0.0	4.0 ± 0.2	0.1 ± 0.0	1.0 ± 0.1	0.1 ± 0.0	1.4 ± 0.1	0.8 ± 0.1	6.6 ± 0.2	0.2 ± 0.0	0.2 ± 0.1	15.2 ± 0.0	15.7 ± 0.3
SE	1.0 ± 0.1	0.2 ± 0.1	2.5 ± 0.2	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	7.6 ± 0.3	1.6 ± 0.1	0.1 ± 0.0	15.8 ± 0.0	16.6 ± 0.3
SW	1.6 ± 0.2	0.1 ± 0.0	2.8 ± 0.5	0.1 ± 0.0	0.6 ± 0.1	0.0 ± 0.0	0.5 ± 0.1	1.6 ± 0.2	3.8 ± 0.8	0.3 ± 0.1	0.1 ± 0.1	11.5 ± 0.2	12.1 ± 0.9
NW	1.3 ± 0.1	0.1 ± 0.0	2.8 ± 0.4	0.1 ± 0.0	0.8 ± 0.2	0.0 ± 0.0	0.5 ± 0.1	1.3 ± 0.2	1.1 ± 0.3	0.7 ± 0.2	0.3 ± 0.1	9.1 ± 0.1	8.7 ± 0.5

^a Values are means ± SEs (µg/m³); based on 39,489 observations at 167 monitoring sites that had at least 30 winter and 30 summer observations during 2000–2005.

$$\begin{aligned} \text{PM}_{ij} = & \beta_1 * \text{Soil}_{ij} + \beta_2 * \text{Coal}_{ij} + \beta_3 * \text{Oil}_{ij} \\ & + \beta_4 * \text{Biomass}_{ij} + \beta_5 * \text{Steel}_{ij} + \beta_6 * \text{Salt}_{ij} \\ & + \beta_7 * \text{Metals}_{ij} + \beta_8 * \text{Traffic}_{ij} + \beta_9 * \text{SO}_4^{2-'}_{ij} \\ & + \beta_{10} * \text{OC}'_{ij} + \beta_{11} * \text{NO}_3^{-}_{ij} + I_j \end{aligned}$$

Therefore, the product of the β_k and the absolute factor scores provides the mass contributions associated with each source category.

The model β coefficients β_9 , β_{10} , and β_{11} for SO_4^{2-} , NO_3^- , and OC were 5.09, 1.22, and 1.25, respectively. These estimates compare favorably with known molar conversion factors for elemental S to ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]^-$ (4.125), OC to organic mass (1.4), and NO_3^- to ammonium nitrate $[\text{NH}_4^+\text{NO}_3]^-$ (1.29) (Malm et al. 1994); these values thus support the validity of this mass regression approach.

Compositional profiles for source categories were estimated for both of the mass regression approaches we applied. In the tracer-mass regression model, the reciprocal of the β coefficients ($1/\beta_k$) provided estimates of the compositional profile percentages for each of the source category tracers. For the APCA mass regression model, source profiles were estimated by regressing each variable (e.g., daily trace element concentrations) onto the APCA's daily mass contributions for all eight source categories in a linear model with an intercept term. We similarly assessed the source profile fractions of OC, NH_4 , NO_3^- , and SO_4^{2-} , although these were not incorporated in the factor analysis model. These source profiles yielded estimates of the compositional mix of the source category mass, as well as providing an aid to the interpretation of their respective factors. As shown in Figure 4, the source composition profiles indicate that the majority of the source-related mass is usually explained by the mass of the secondary aerosols associated with the individual sources. These percentages of elements and metals associated with each source category are consistent with the identifications of the source category for each (Thurston et al. 2011).

Table 7 provides the overall and the regional–seasonal average estimates (\pm SE) for PM_{2.5} mass contributions from the eight source categories and three secondary aerosols. For a better understanding of the variations across the 167 monitoring sites, we averaged source category–related mass contributions for the U.S. regions by season. Figure 5 depicts boxplots by source category for the variation in contributions of apportioned PM_{2.5} mass ($\mu\text{g}/\text{m}^3$) across the 100 MSAs. Some source categories — Traffic, Soil, and Biomass Combustion — were found to be widespread and appeared in nearly all MSAs. Other source categories —

Steel Industry and Metals — were found in a very limited number of sites.

Spatial Representativeness of Source Category Contributions

The %CVs of the mean between-site differences in mass were calculated for all monitoring sites within 20 miles of each other for: (1) PM_{2.5} mass associated with each of the source categories; and (2) concentration of the respective key source category tracers. The estimated mass %CV values from lowest to highest were: PM_{2.5} = 11.1%; Traffic = 11.4%; Salt = 14.4%; Residual Oil Combustion = 14.7%; Soil = 16.8%; Biomass Combustion = 21.5%; Metals = 25.5%; Coal Combustion = 30.9%; and Steel Industry = 54.6%. Among the key tracers, the %CV values from lowest to highest were: OC = 11.6%; SO_4^{2-} = 12.2%; Si = 13.9; EC = 16.3%; Ni = 19%; Fe = 20.7%; Se = 28.6%; Cl = 24.9%; K = 28.1%; Zn = 32.2%; and Mn = 52.0%. These results show, as expected, that PM_{2.5} mass and two of the secondary aerosols (SO_4^{2-} and OC) were most spatially homogeneous among sites (indicated by having the lowest %CV between paired sites), and therefore the most spatially representative measurements. Among the source categories, Traffic and its tracer EC were the most spatially representative. The estimates for PM_{2.5} from the Metals and Steel Industry source categories and their associated tracers (e.g., Zn, Mn) were generally among the least spatially representative of nearby monitors (having the largest %CVs).

MORTALITY ANALYSES

For each cause of death, the PM_{2.5}–mortality hazard ratios (and 95% CIs) from the standard Cox modeling approach for the various PM_{2.5} components, factor scores, and the source-apportioned mass (i.e., mass apportioned to each source category) are presented in Figures 6 through 17 along with analogous results for the random effects Cox model. In each figure, the results of analyses without contextual ecologic covariates are marked with an open circle (○), and those with ecologic covariates are designated with a solid circle (●).

In general, after controlling for individual-level covariates, such as smoking, education, and marital status, the inclusion of ZCA-level ecologic covariates generally had only modest influence on the estimated mortality associations with PM_{2.5} or one of its components. However, although introducing random effects to the model also had little effect on the mortality hazard ratios, it generally resulted in larger standard errors of the various estimates and, therefore, somewhat wider 95% CIs. The models that included both the contextual ecologic covariates and the

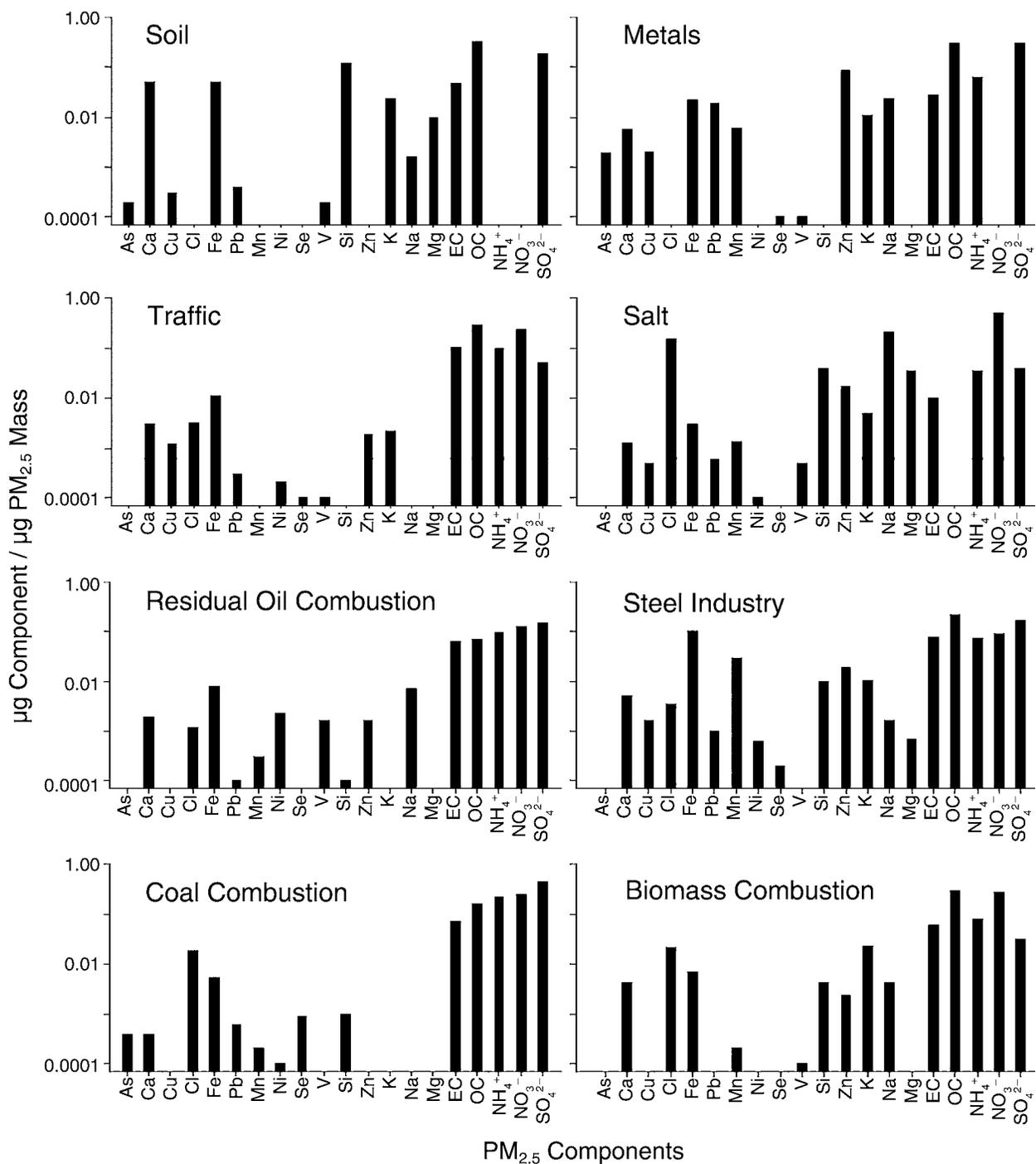


Figure 4. Estimated mass for each component in each source category compared with total PM_{2.5} mass shown as component mass/total PM_{2.5} mass (µg/µg) on a log scale.

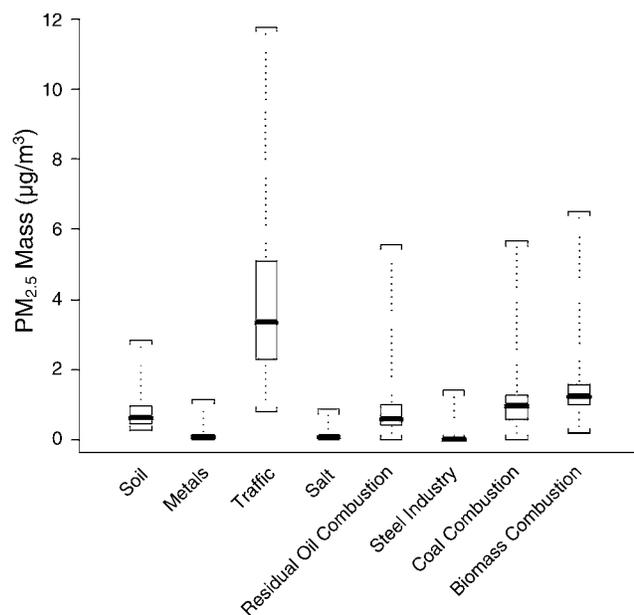


Figure 5. Distribution of average PM_{2.5} mass estimates (µg/m³) by source category for 100 MSAs (39,849 observations; 167 sites).

random effects assumption were generally the most conservative estimates (usually resulting in somewhat lower estimates with larger confidence intervals); but they are, conversely, the least parsimonious and therefore are most susceptible to possible over-specification. In our discussion, we have given the greatest weight to results that are least affected by choice of model (i.e., results that are most consistent across all four models).

All-Cause Mortality

As displayed in Figure 6, all-cause mortality was consistently (i.e., in all four models) significantly associated with PM_{2.5} mass and three of the elements (As, Se, and S). Consistent with these results, in the analysis of factors (Figure 7) only the Coal Combustion factor displayed significant associations across the four models (note that As and Se are the key tracers for Coal Combustion). In the analysis of source-apportioned PM_{2.5} mass (Figure 8), again only Coal Combustion was consistently associated with all-cause mortality irrespective of the model chosen.

Overall, the results were largely consistent among analyses for all-cause mortality with PM_{2.5} components, factors, and source-apportioned PM_{2.5} mass.

IHD Mortality

Many more, and stronger, associations with PM_{2.5} and its components were found for IHD mortality than for all-cause mortality. As shown in Figure 9, although most elements displayed a positive hazard ratio, IHD mortality was consistently (i.e., in all four models) significantly associated with PM_{2.5} mass and the elements As, Cl, Pb, and Se; IHD mortality with EC was also significant in three of the four models. (Note that EC and Cl are the respective elemental tracers for the Traffic and Salt source categories.) However, IHD was consistently not associated with several other PM components (i.e., Mn, Si, K, and OC).

In the analysis of IHD mortality and factors (Figure 10), only Coal Combustion displayed significant associations with IHD mortality across all models. Traffic and Salt factors showed some significant associations with IHD mortality in some models. Similarly, in the analysis of source-apportioned PM_{2.5} mass (Figure 11), Coal Combustion was significantly associated with IHD mortality, irrespective of the model chosen.

Overall, PM_{2.5} components from most industrial [i.e., Metals and Steel Industry] and fossil fuel combustion [i.e., Residual Oil, Coal Combustion, and Traffic] source categories had hazard ratios above 1.0 for IHD deaths (Figure 9).

In the analyses with factors and with source-apportioned mass, associations with mortality were largely consistent with those from the analysis with individual components; the metals most strongly associated with IHD (As and Se) are key tracers of the Coal Combustion source category, which was also most strongly related in the analysis with factors (Figure 10) and in the analysis with source-apportioned mass (Figure 11).

Although Coal Combustion was most consistently associated with and, of the statistically significant estimates, had the highest hazard ratio per µg/m³ of source-apportioned PM_{2.5} mass (Figure 11), EC — a key elemental tracer of diesel vehicles (Traffic source category) — was also associated with IHD in some models shown in Figures 9–11. Although the models to estimate PM_{2.5} or Traffic with IHD mortality tended to yield hazard ratios per unit mass that were much lower than those for Coal Combustion, Traffic had a much higher estimated PM_{2.5} mass apportionment than Coal Combustion (Traffic mean PM_{2.5} of 3.8 µg/m³ compared with 0.7 µg/m³ for Coal Combustion [see Figure 11]).

PM_{2.5} mass originating from wind-blown Soil or from Biomass Combustion were generally not associated with increased risk of IHD mortality in this cohort, and other source categories were more equivocal in their associations across models.

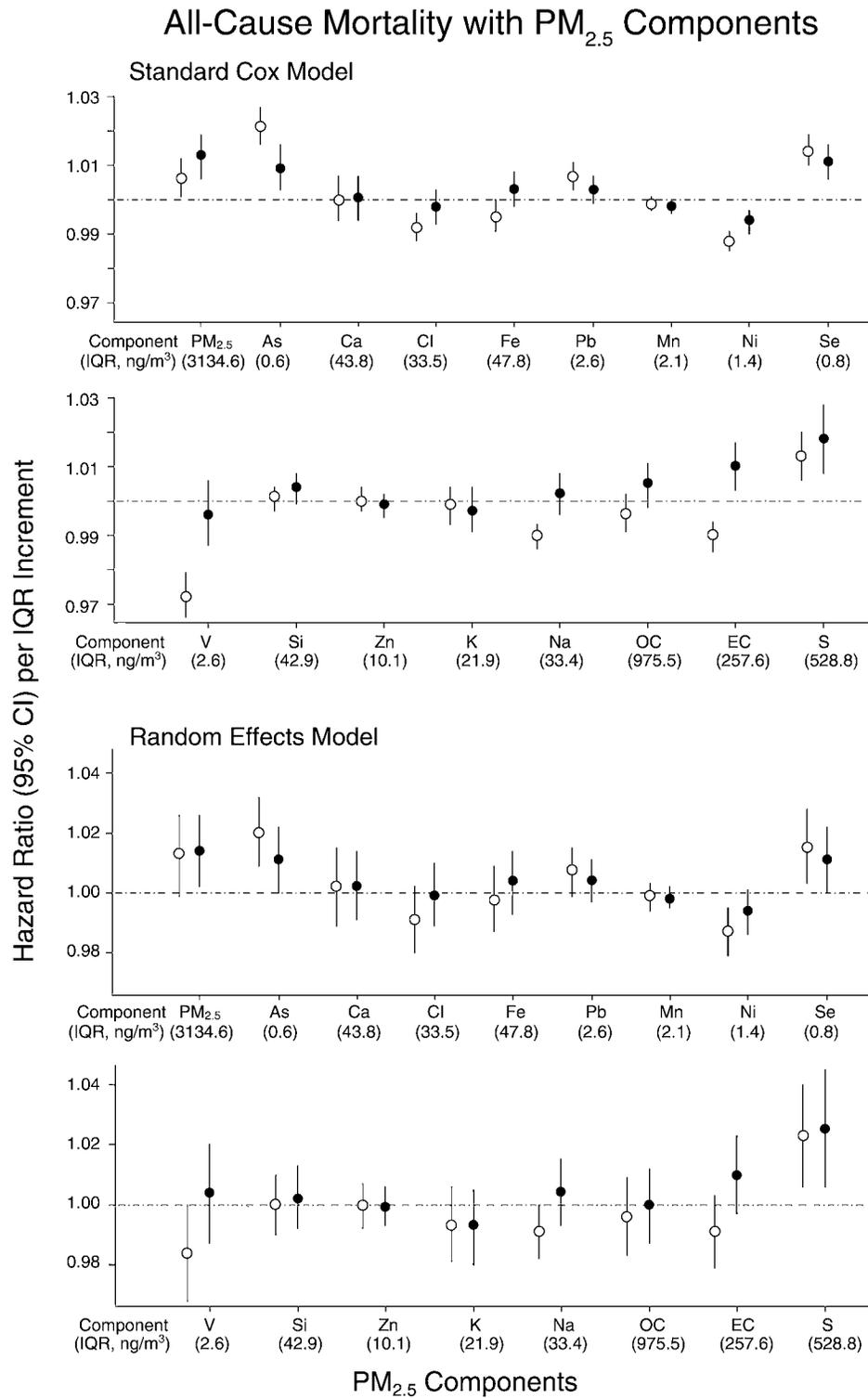


Figure 6. Associations between all-cause mortality and mass of PM_{2.5} and its components. PM_{2.5}, As, and Se were significantly associated with all-cause mortality in all four models. Note that As and Se are the tracers for Coal Combustion, shown in Figures 7 and 8. Data are hazard ratios with 95% CIs per IQR increment given in ng/m³ on the x axis. ○ indicates models without ecologic covariates; ● indicates models with ecologic covariates. Note that the y axis scales differ.

All-Cause Mortality with Factors

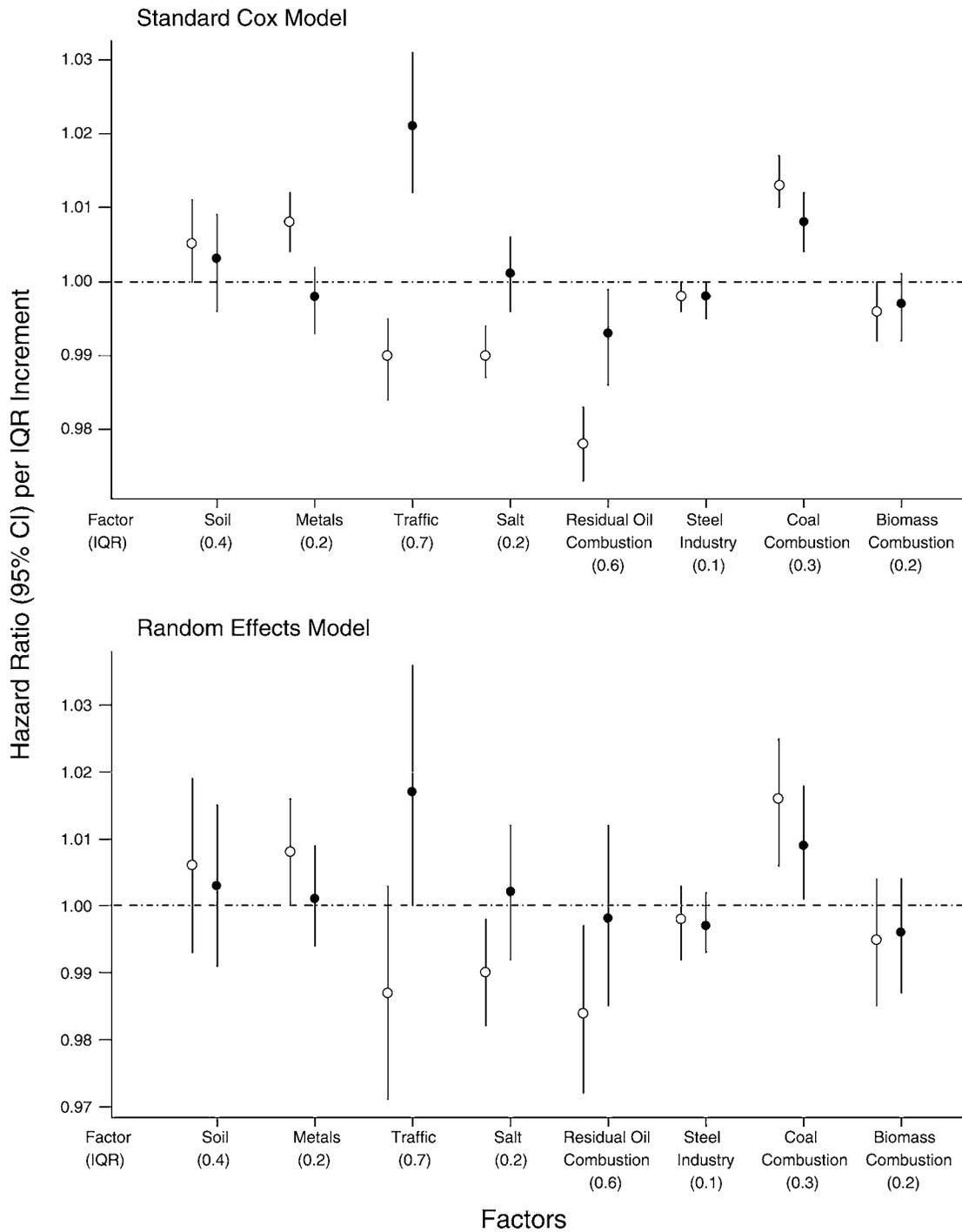


Figure 7. Associations between all-cause mortality and factors. Only Coal Combustion was significantly associated with all-cause mortality in all four models. Data are hazard ratios with 95% CIs per IQR increment (unitless). ○ indicates models without ecologic covariates; ● indicates models with ecologic covariates.

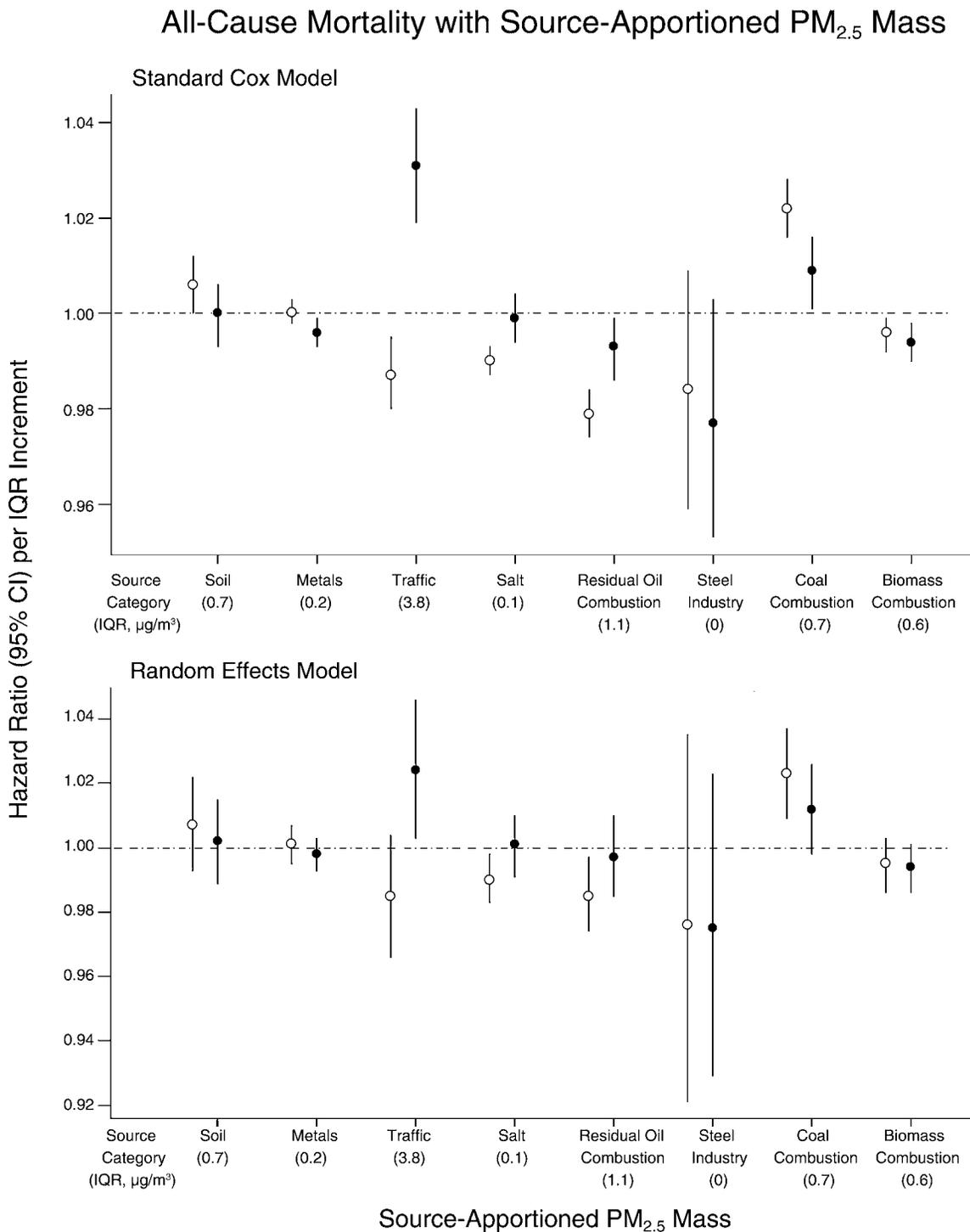


Figure 8. Associations between all-cause mortality and source-apportioned PM_{2.5} mass. Only Coal Combustion was consistently associated with all-cause mortality. Data are hazard ratios with 95% CIs per IQR increment given in $\mu\text{g}/\text{m}^3$ on the x axis. o indicates models without ecologic covariates; • indicates models with ecologic covariates. Note that the y axis scales differ.

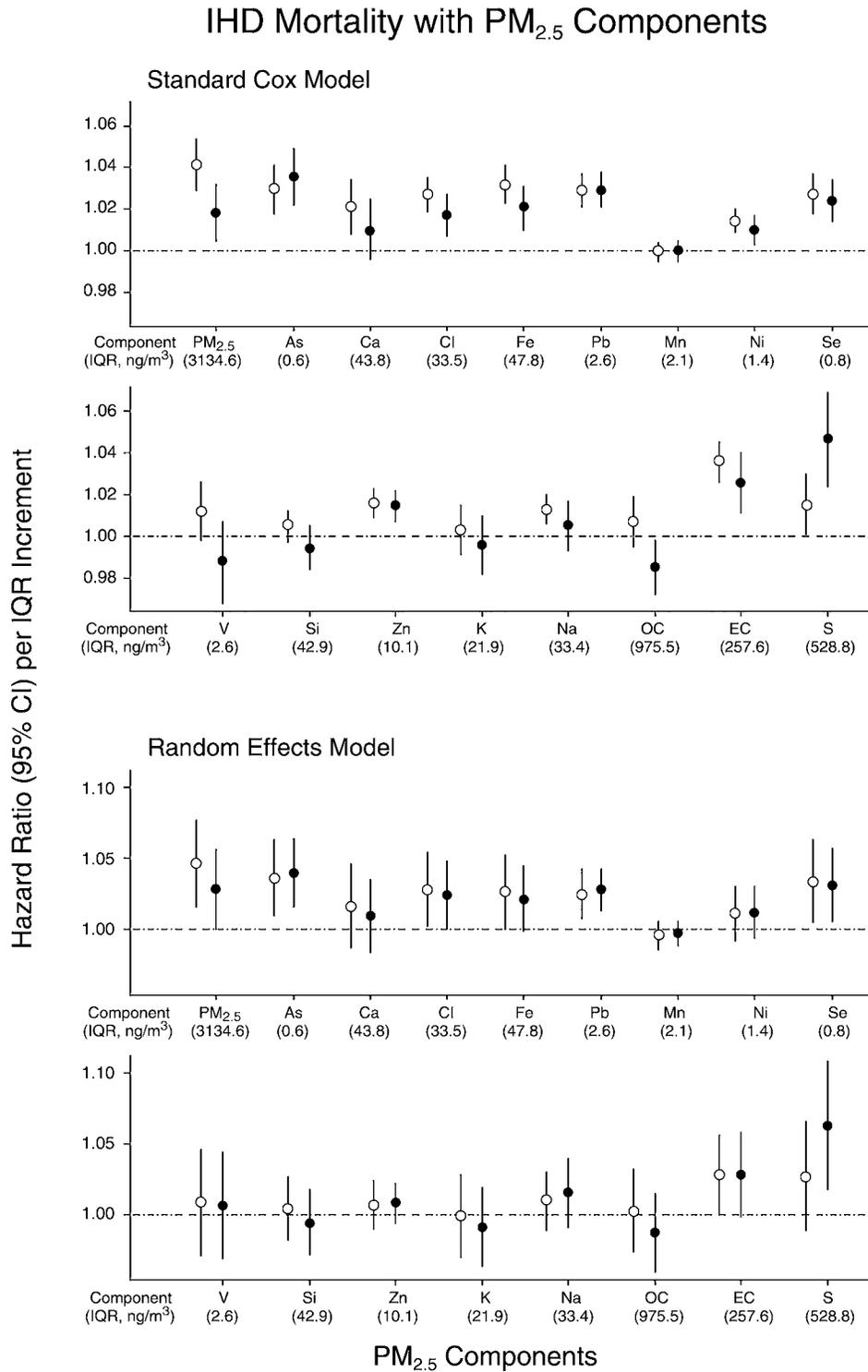


Figure 9. Associations between IHD mortality and mass of PM_{2.5} and its components. PM_{2.5}, As, Cl, Pb, and Se were significantly associated in all models, and EC was significantly associated in three of the four. Data are hazard ratios with 95% CIs per IQR increment given in ng/m³ on the x axis. **o** indicates models without ecologic covariates; **•** indicates models with ecologic covariates. Note that the y axis scales differ.

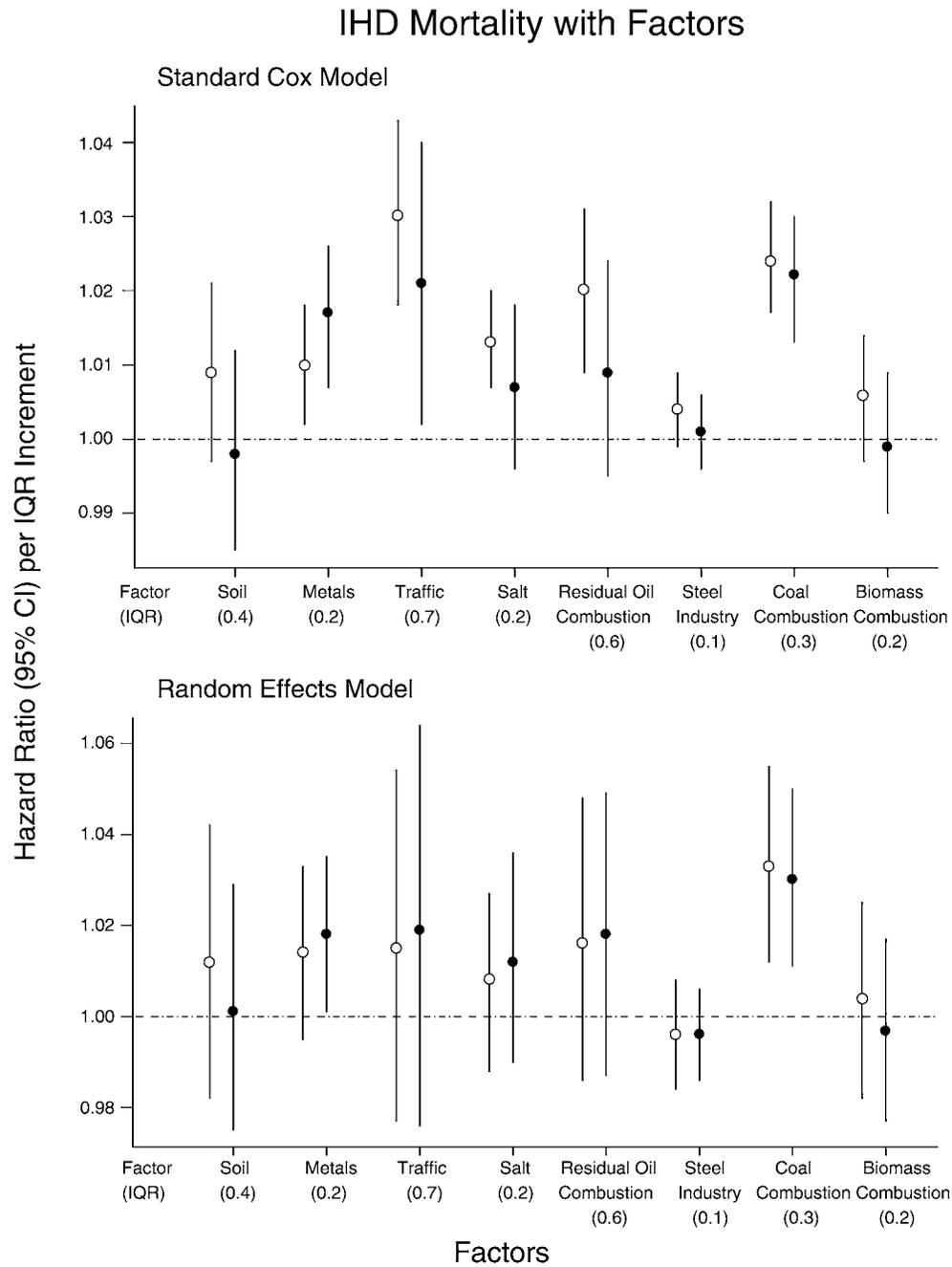


Figure 10. Associations between IHD mortality and factors. Only Coal Combustion was significantly associated with all-cause mortality in all four models. Data are hazard ratios with 95% CIs per IQR increment (unitless). ○ indicates models without ecologic covariates; ● indicates models with ecologic covariates. Note that the y axis scales differ.

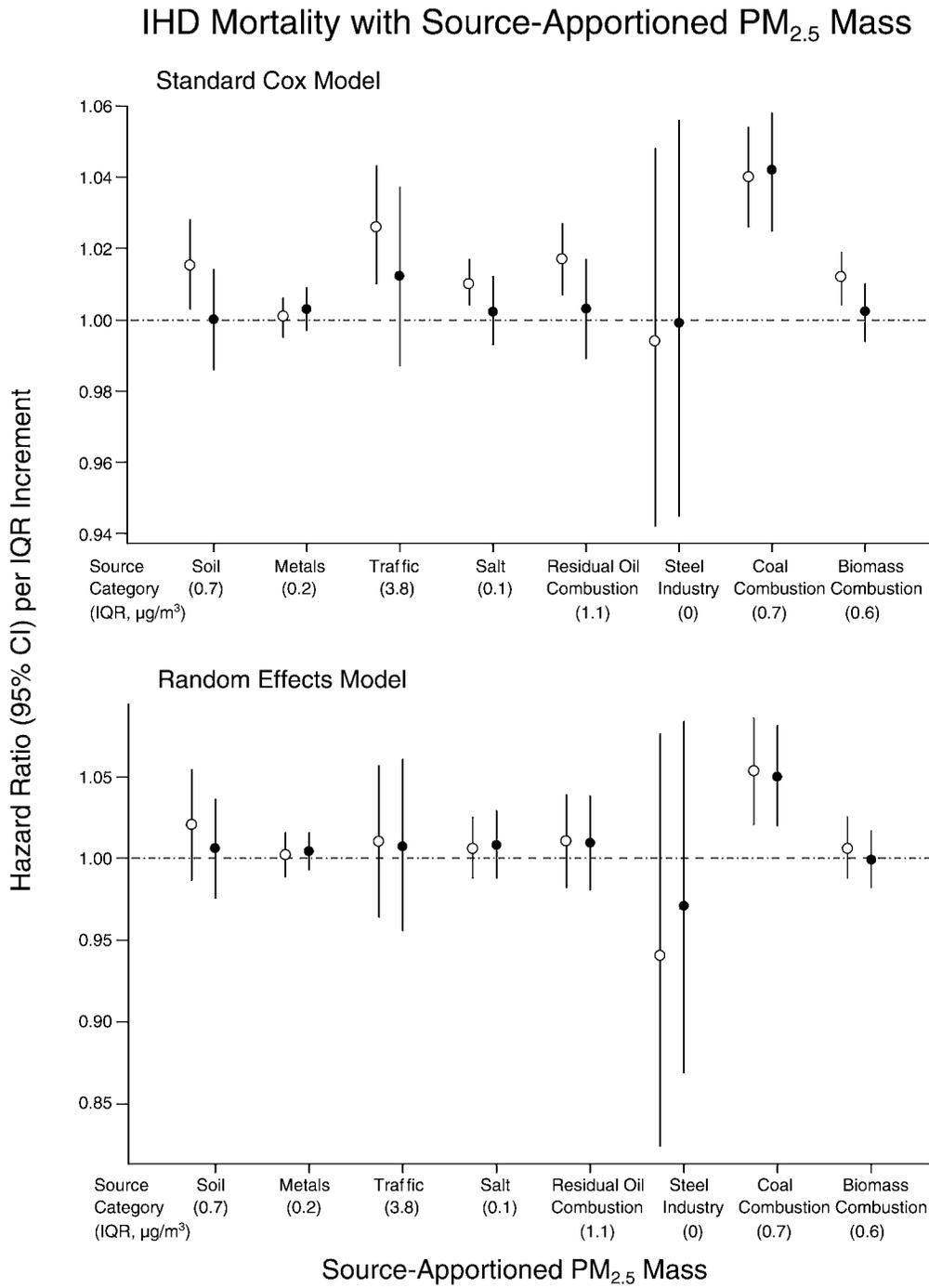


Figure 11. Associations between IHD mortality and source-appORTioned PM_{2.5} mass. Coal Combustion was significantly associated with all-cause mortality in all four models. Data are hazard ratios with 95% CIs per IQR increment given in $\mu\text{g}/\text{m}^3$ on the x axis. **o** indicates models without ecologic covariates; **•** indicates models with ecologic covariates. Note that the y axis scales differ.

Respiratory Mortality

The $PM_{2.5}$ mass and component associations shown in Figure 12 indicate that the mass for only $PM_{2.5}$ and the secondary aerosol OC are significantly associated with respiratory mortality across all models, and the key tracers for the Soil source category (Si and Ca) were at or near statistical significance in all models. The respiratory hazard ratio estimates provided for $PM_{2.5}$ are higher and more significant than those reported in another recent analysis of the CPS-II cohort (Jerrett et al. 2009), which estimated the hazard ratio as 1.031 (95% CI 0.955–1.113) per $10\text{-}\mu\text{g}/\text{m}^3$ increase in $PM_{2.5}$. However, our analyses covered a larger number of MSAs (100 vs. 89) and 4 more years of follow-up; these differences provided significantly more statistical power to detect pollutant–mortality associations than most earlier analyses of the CPS-II cohort. The finding of a significant association between respiratory mortality and $PM_{2.5}$ exposure is also consistent with results from an extended follow-up study of the Harvard Six Cities cohort (Laden et al. 2006).

Consistent with the respiratory mortality associations with elements, the analysis with factors shown in Figure 13 indicates that Soil (for which Si and Ca are tracers) is significantly associated with respiratory mortality in the standard Cox models; and both models that included the ecologic covariates show significant associations with Traffic. In Figure 14, however, most associations of source-apportioned mass with respiratory mortality were not significant. Thus, although $PM_{2.5}$ mass is associated with increased risk of respiratory mortality (Figure 12), in this dataset it is less consistently clear which of the $PM_{2.5}$ components are most involved in this association.

Lung Cancer Mortality

Although the hazard ratio estimates for $PM_{2.5}$ mass with lung cancer mortality are consistently higher than 1.0 across models (Figure 15), they are not quite statistically significant in each case. For example, the random effects model with ecologic covariates has a hazard ratio of 1.019 (95% CI = 0.995–1.043 per IQR of $3.134\ \mu\text{g}/\text{m}^3\ PM_{2.5}$). Despite the lack of statistical significance at the 95% confidence level, the lung cancer hazard ratio estimate found here (1.019), when recalculated for an increase of $10\ \mu\text{g}/\text{m}^3\ PM_{2.5}$, the hazard ratio of 1.06 (95% CI = 0.98–1.14) is not statistically different from the previously published estimates from this cohort (e.g., in Pope et al. 2002; lung cancer hazard ratio = 1.08, 95% CI = 1.01–1.16 per $10\text{-}\mu\text{g}/\text{m}^3$ increase in $PM_{2.5}$).

Se, a Coal Combustion tracer, is close to significance in all models but is significant in only one. Only S is clearly significant in all four models.

Both Figures 16 and 17 show that Coal Combustion stands out as most consistently associated with lung cancer mortality — significant in two models and nearly significant in the other two in Figure 16 (for factors), and significant in all four models in Figure 17 (for source-apportioned mass). These results are consistent with the hypothesis that some types of particulate matter are more carcinogenic than others, as would be expected based on their different compositions.

TOTAL RISK INDEX MEASURES TO ASSESS EFFECTS OF MULTIPLE PARTICULATE AND GASEOUS AIR POLLUTANTS

A fundamental requirement of multiple-pollutant analyses to evaluate the relative importance of various mixtures of air pollutants is to recognize the substantial and often complex correlation structures among the various pollutant compounds, gases, and sources. Even efforts to construct a mix of pollutants emitted by specific known sources do not necessarily result in a mix of $PM_{2.5}$ components and sources that are fully orthogonal (i.e., distinct and uncorrelated) because of conditions (meteorologic, for example) that affect all sources similarly. Given the correlation between some pollutants, it is even difficult to confidently interpret the regression coefficient from a single-pollutant model because this single coefficient may reflect the incomplete but combined effects of multiple, correlated pollutants. These complex correlations among many pollutants make it even more difficult to interpret regression coefficients from multiple-pollutant models. Including several pollutants in a model can result in various outcomes: (1) coefficients that are mostly unaffected by others (suggesting independent effects); (2) coefficients that retain the same sign (positive or negative) but are each smaller in size (suggesting that they share the effect or are both imperfect indicators of a true risk estimate); or (3) coefficients that are highly unstable, some becoming inflated and others becoming null or changing signs. Estimates of the standard errors of coefficients may also be inflated due to multicollinearity. In short, the individual risk coefficients of correlated variables (such as multiple pollutants) are not estimable in an unbiased way, but the linear combination of the coefficients *can* be reliably estimated, even if the individual variables are correlated with each other.

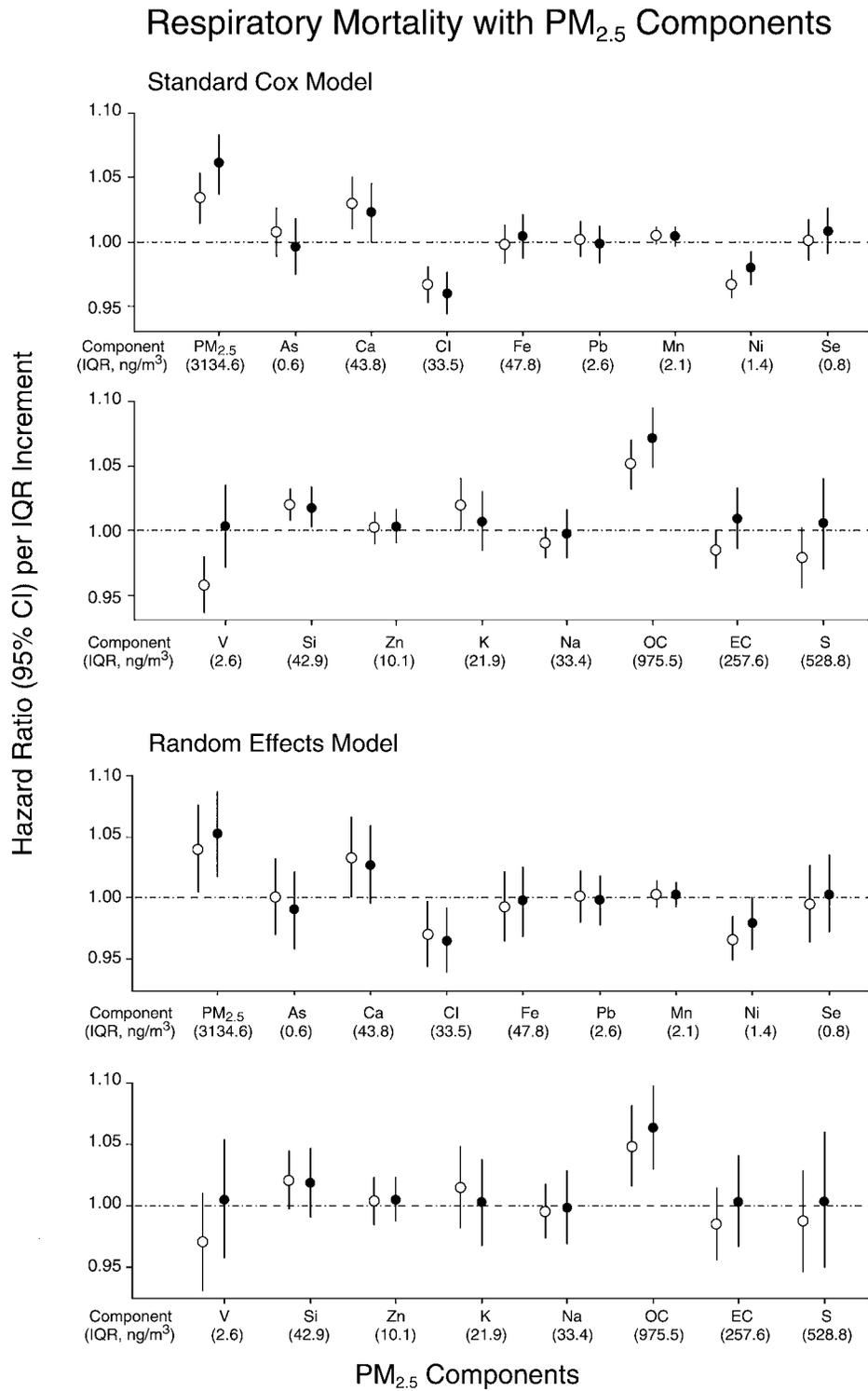


Figure 12. Associations between respiratory mortality and mass of PM_{2.5} and its components. PM_{2.5} and OC were significantly associated in all models, and the key tracers for the Soil source category (Si and Ca) were at or near significance. Data are hazard ratios with 95% CIs per IQR increment given in ng/m³ on the x axis. ○ indicates models without ecologic covariates; ● indicates models with ecologic covariates.

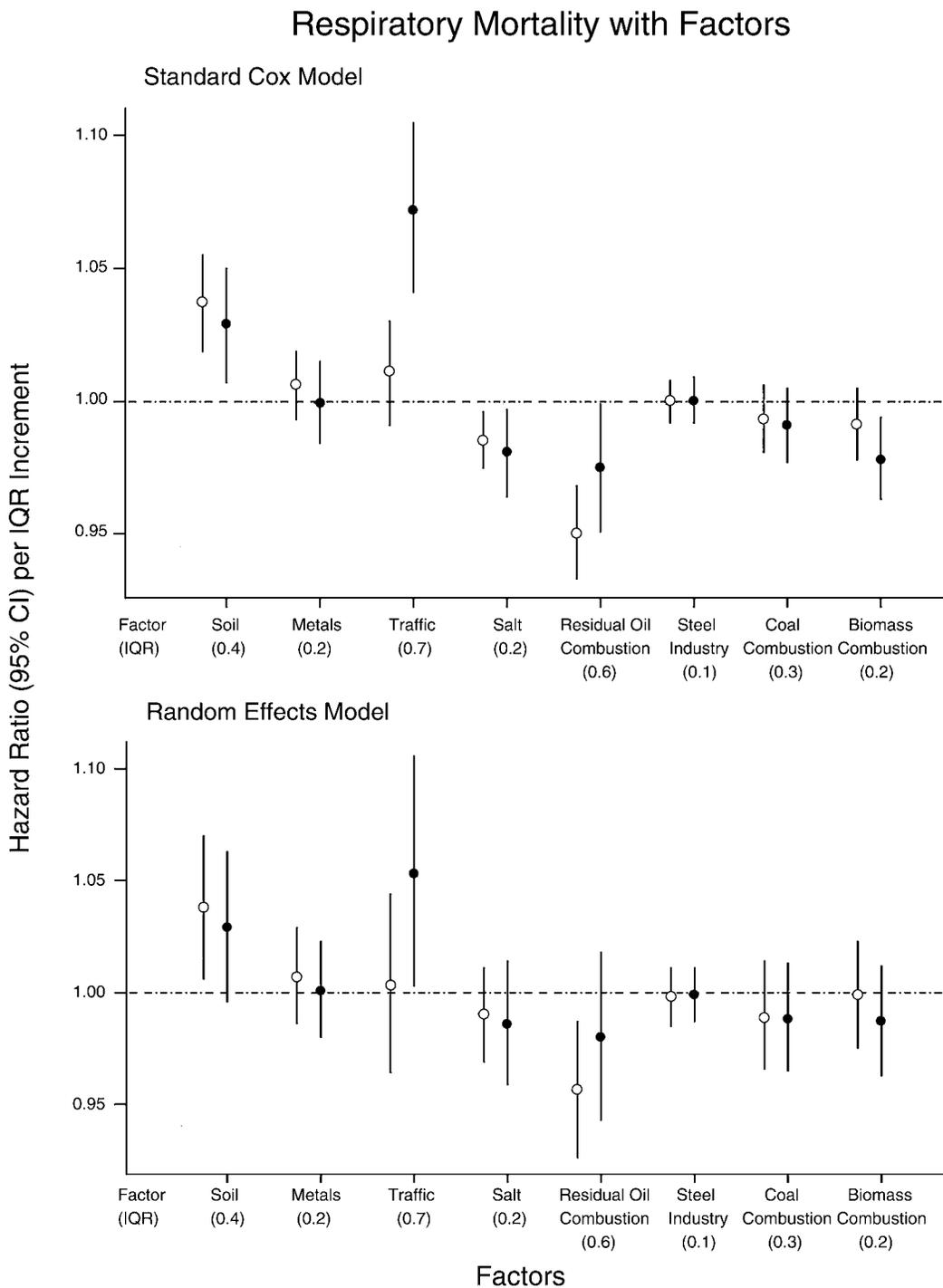


Figure 13. Associations between respiratory mortality and factors. Soil was significantly associated with respiratory mortality in the standard Cox models, and Traffic showed significant associations in the models with ecologic covariates. Data are hazard ratios with 95% CIs per IQR increment (unitless). **o** indicates models without ecologic covariates; **•** indicates models with ecologic covariates.

Respiratory Mortality with Source-AppORTioned PM_{2.5} Mass

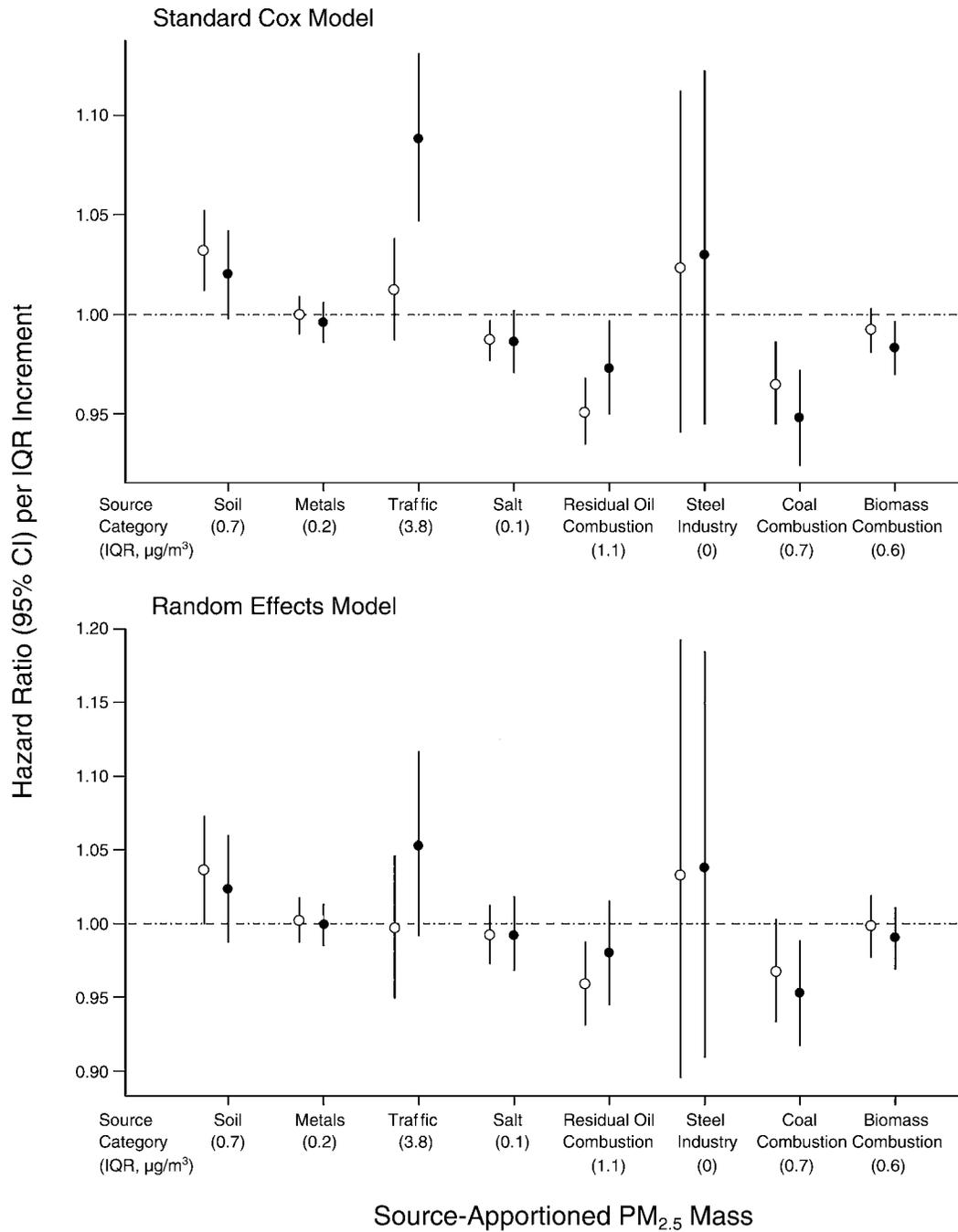


Figure 14. Associations between respiratory mortality and source-apportioned PM_{2.5} mass. The standard Cox model with ecologic covariates showed a significant association for Traffic. Most other associations were not significant. Data are hazard ratios with 95% CIs per IQR increment given in $\mu\text{g}/\text{m}^3$ on the x axis. **o** indicates models without ecologic covariates; **•** indicates models with ecologic covariates. Note that the y axis scales differ.

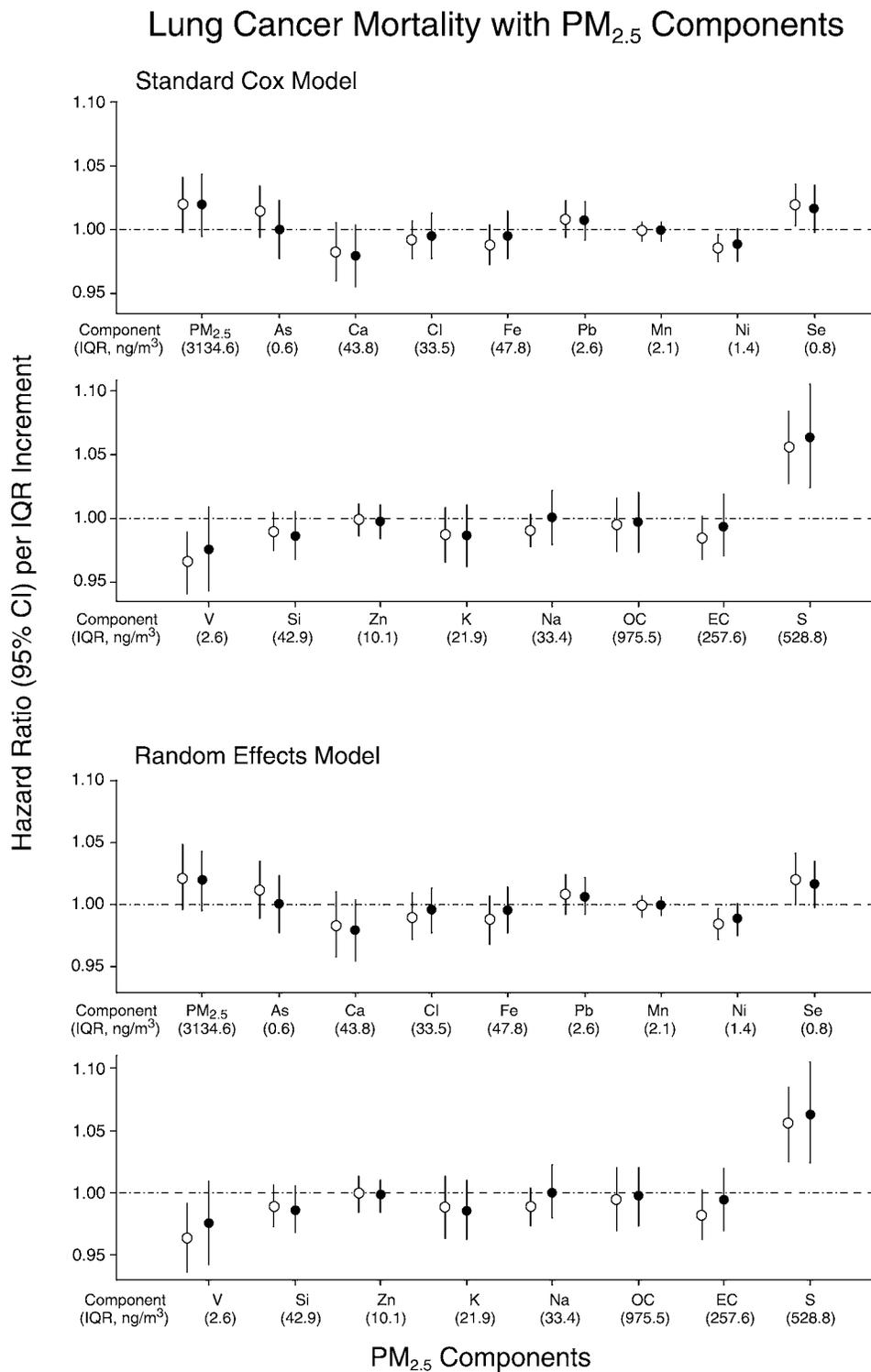


Figure 15. Associations between lung cancer mortality and mass of PM_{2.5} and its components. Se, a Coal Combustion tracer, is close to significance in all models, but is significant in only one; only S is clearly significant in all four models. Data are hazard ratios with 95% CIs per IQR increment given in ng/m³ on the x axis. ○ indicates models without ecologic covariates; ● indicates models with ecologic covariates.

Lung Cancer Mortality with Factors

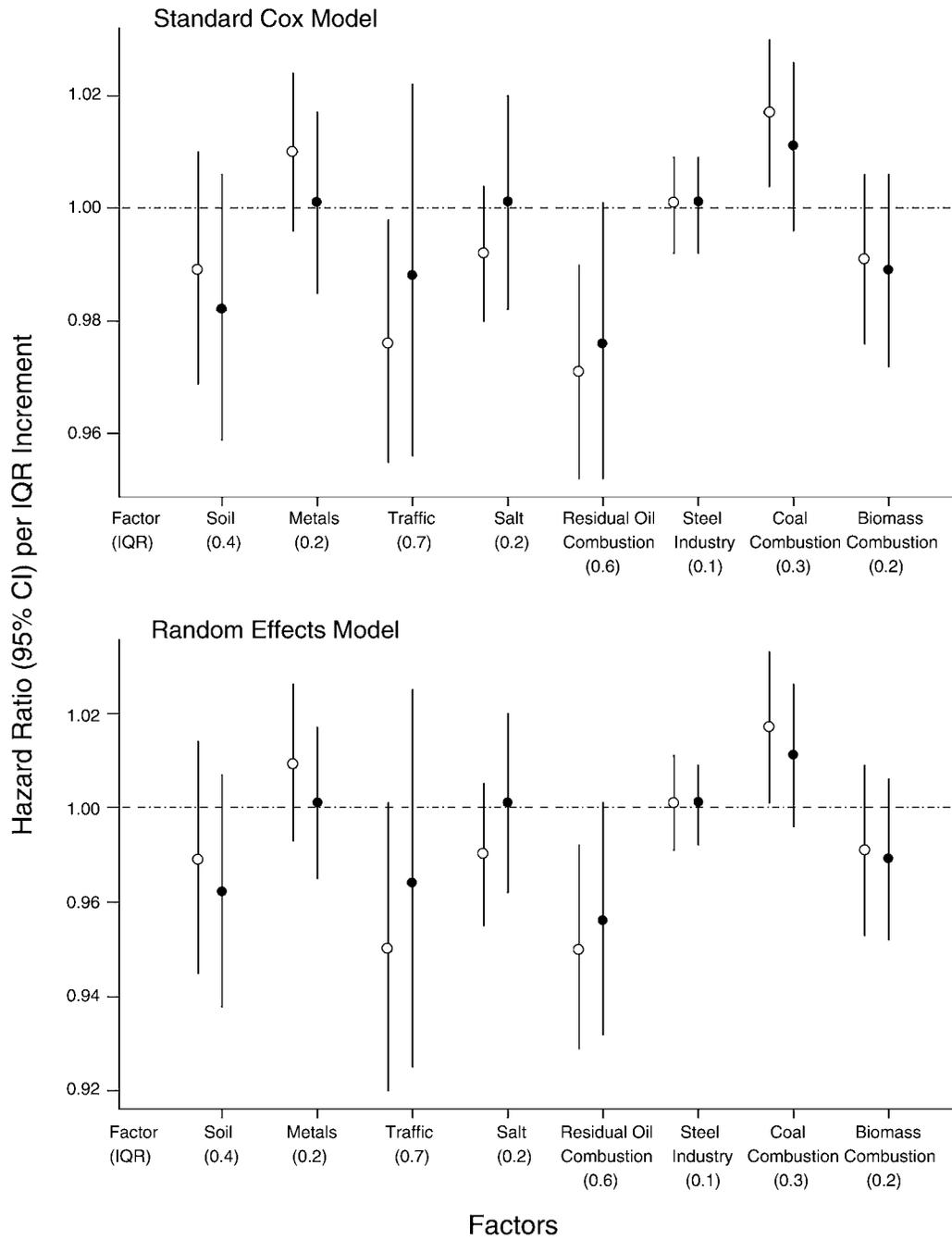


Figure 16. Associations between lung cancer mortality and factors. The two models for Coal Combustion with no ecologic covariates showed significant associations. Data are hazard ratios with 95% CIs per IQR increment (unitless). **o** indicates models without ecologic covariates; **•** indicates models with ecologic covariates. Note that the y axis scales differ.

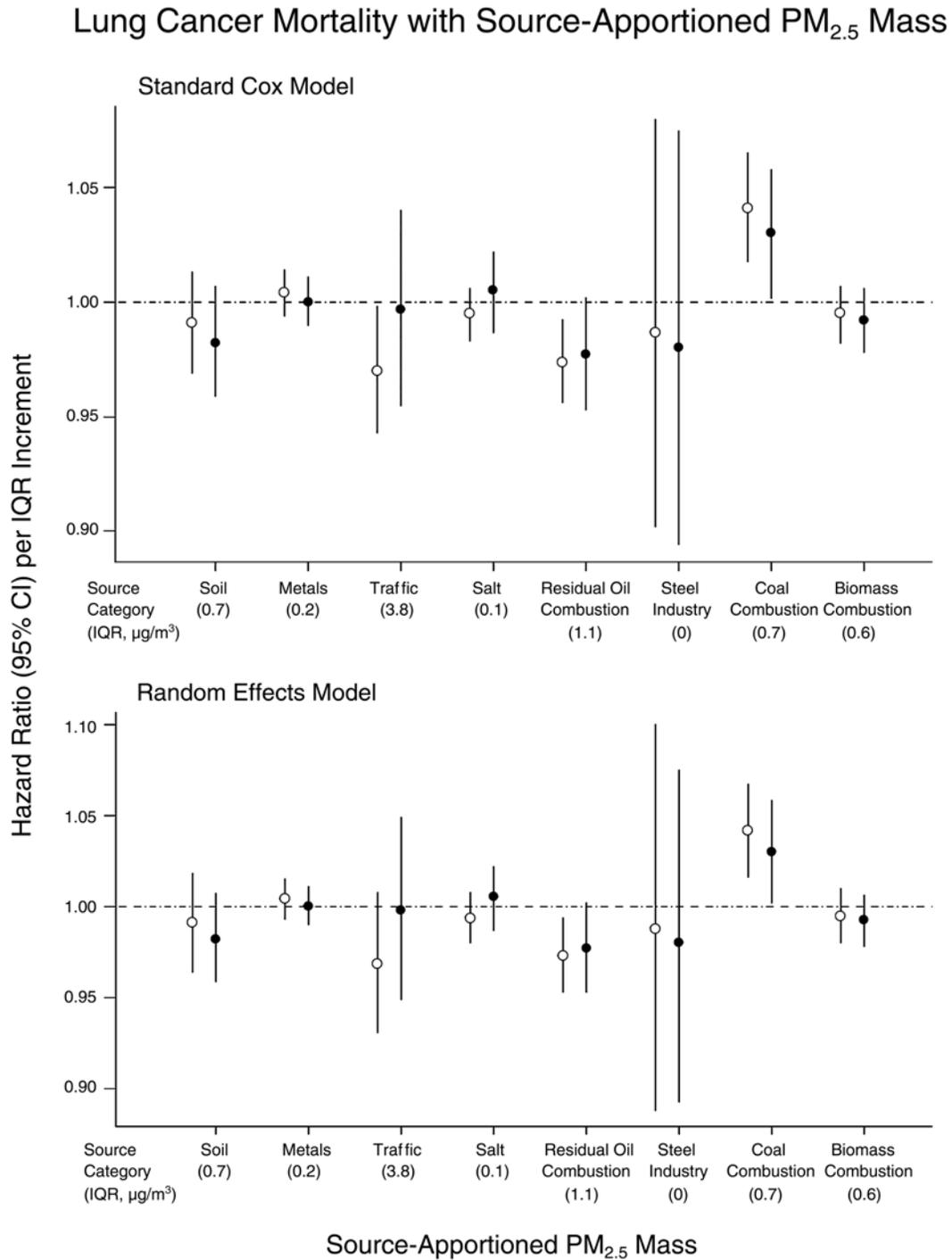


Figure 17. Associations between lung cancer mortality and source-appORTioned PM_{2.5} mass. The Coal Combustion source category showed significant associations in all four models. Data are hazard ratios with 95% CIs per IQR increment given in $\mu\text{g}/\text{m}^3$ on the x axis. ○ indicates models without ecologic covariates; ● indicates models with ecologic covariates.

In the TRI analysis, we compared the combined hazard ratios for different groups of pollutants, or the TRIs for different model specifications (e.g., for a model with two pollutants compared with a model with one pollutant) in order to assess the change in effect produced by the addition of different pollutant variables to the model.

Thus, although coefficients from multiple-pollutant models cannot provide reliable effect estimates for individual pollutants, we can test whether or not models with various *combinations* of pollutants provide significantly different estimates of the overall pollution-related TRI of mortality, as compared with the same model with only a single index of pollution. We can also conduct focused exploratory analyses regarding the combination of variables that significantly contribute to estimates of the TRI.

In this section, we briefly describe our TRI modeling and present results for selected analyses. Please see Appendix I (available on the HEI Web site) for a more complete description of the TRI methods, data, and a full set of health analyses results.

STATISTICAL APPROACH

Using the standard Cox model and our random effects Cox model as described in the previous section, we estimated the association between mortality from various causes and each pollutant, or set of pollutant variables, simultaneously. As in the single-pollutant mortality analyses, the model evaluated the survival data of CPS-II cohort members from 1982 through 2004; however, instead of estimating parameters for a single pollutant, the Cox models estimated vector parameters for the entire matrix of pollutants used to formulate the TRI.

To do this, we first defined the combined relative risk of a single pollutant or a set of pollutants, evaluated at their respective IQRs, denoted by the vector \tilde{x} . Let $\hat{\beta}$ represent the vector of estimated log-hazard ratios within the random effects Cox model structure associated with the multiple-pollutant variables contained in the model, and let \tilde{x} be a vector of the IQRs of the pollutant concentrations. The TRI of the set of pollutants is defined as: $TRI = \exp(\hat{\beta}'\tilde{x})$, where the 95% CIs are given by $\exp\left[\hat{\beta}'\tilde{x} \pm 1.96\sqrt{\tilde{x}'Cov(\hat{\beta})\tilde{x}}\right]$.

DATA SETS

Cohort Mortality Data

Mortality data and individual-level covariate data were obtained from the American Cancer Society for their CPS-II cohort. (Please see Tables 3 and 4 for detailed descriptive information on this cohort.) For the TRI, we analyzed

mortality from all-causes, cardiopulmonary disease, CVD, IHD, and lung cancer. In this brief section, we focus on the IHD analysis.

Exposure Variables in TRI Formulation

The initial analysis of mortality associated with components, factors, and source-apportioned mass used data on PM_{2.5} components derived from all monitoring sites that had both PM_{2.5} component and nearest-neighbor NO₂ data that included 30 winter and summer observations ($n = 167$ sites; 100 MSAs). These data and the factor analysis and source apportionment methods used to construct the factor and source category variables are described in the previous section of this report.

In the TRI analysis, we used the same data but created three formulations for the TRI that were intended to represent complex atmospheres without duplicating the exposure data. First, we computed TRIs for each of the health outcomes using PM_{2.5} mass alone in order to compare our TRI results to conventional analyses and to other TRI formulations. Second, because our CSN data consisted of measurements of the components of PM_{2.5} mass, we included a set of Components in a TRI formulation. These analyses used all of the following pollutants in the TRI formulation: PM_{2.5} components known to be associated with specific source categories or their key tracers (i.e., Si for Soil, Zn for Metals, EC for Traffic, Cl for Salt, Ni for Residual Oil Combustion, Fe for Steel Industry, Se for Coal Combustion, and K for Biomass Combustion); other PM_{2.5} components such as individual metals (As, Ca, Cu, Cl, Fe, Pb, Mn, Ni, Se, V, Si, Zn, K, and Na); and OC and EC. We also estimated TRIs for source categories in the forms of both factor scores (set of Factors) and source-apportioned PM_{2.5} mass (set of Source Mass).

To complete the characterization of the atmospheres for TRI analyses, we included the estimates of residual mass for SO₄²⁻, OC, and NO₃⁻ (estimated via the APCA analysis described in the previous section) as variables in the TRI formulations. This set of Secondary Aerosols was included along with the variable sets for Source Mass, Factors, or Components in the mortality models. (We used normalized versions of mass for the three secondary aerosols when they were added to a TRI formulation with the set of Factors.) Because the secondary aerosols are known to often comprise the bulk of PM_{2.5} mass, adding them as a set to the model allowed us to more directly compare the multiple-pollutant TRI with a TRI based on PM_{2.5} mass alone.

Gases were also considered in the TRI analyses. They were not part of the TRI formulations, but were included as separate random variables in the Cox models; thus we calculated separate effect estimates. The results of these analyses are not included in this brief overview, but are presented in Appendix I.

RESULTS OF TRI ANALYSES

Summary results detailing hazard ratios from the random effects Cox model with ecologic covariates for all-cause, cardiopulmonary, CVD, and IHD mortality are presented in Figure 18. Numerical results of various TRI formulations and IHD mortality are presented in Table 8. Full detailed numerical results from all analyzed TRI formulations, including analyses with gaseous pollutant data, are available in Appendix I.

Comparison Between Standard Cox and Random Effects Cox Models With and Without Ecologic Covariates

For all causes of death, CIs tended to be larger for the random effects model with ecologic covariates than those for the standard Cox model with or without ecologic covariates. The larger CIs were due primarily to the incorporation of spatial dependencies in the models. This result was also found in some of our earlier analyses of PM_{2.5} mass (Krewski et al. 2009), as well as in the main mortality analyses of this NPACT study. Differences in TRIs among model specifications, however, were not as consistent for cause-specific mortality analyses.

Interpretation of TRI Results for IHD Mortality

Patterns of TRI results for IHD mortality were similar to those for cardiopulmonary, cardiovascular, and all causes of death.

The TRIs for IHD deaths were generally higher than those for cardiovascular deaths, a pattern observed in previous analyses of this cohort (Pope et al. 2004; Krewski et al. 2009). The TRIs based on the set of Factors, and especially when the set of Secondary Aerosols was included, tended to be substantially larger than the TRIs based on PM_{2.5} mass alone. For example, in Table 8, the TRI estimate for IHD from the random effects model with ecologic covariates is 1.028 for PM_{2.5} mass alone, 1.135 for Factors, and 1.173 for Factors + Secondary Aerosols.

The TRI estimate for IHD from the random effects model with or ecologic covariates for NO₂ alone was significant (TRI = 1.046), which is consistent with an effect of Traffic-related pollution.

Distinguishing between the effects of data availability and the effects of a pollutant on the TRI estimate is challenging when different pollutants are available in different MSAs. Results from TRI analyses of the subset of 45 MSAs for which we had SO₂ data were generally much lower than those for the full 100 MSAs. This makes it difficult to interpret the joint impact of SO₂ and PM_{2.5} mass (TRI = 1.075).

Unexpectedly, SO₂ alone was positive and significantly related to IHD mortality (TRI = 1.072) in the subset of

45 MSAs, although the hazard ratio was smaller than those for the sets of Factors, Source Mass, or Components. This result is difficult to interpret, since no association with SO₂ was observed with cardiovascular mortality (TRI = 1.002; results shown in Appendix I), which implies a negative association between SO₂ and other cardiovascular deaths, the majority of which were due to stroke. However, in the initial mortality analyses, the Coal Combustion factor and source category, which were among the most strongly correlated with SO₂ (see Thurston et al. 2011), were also important predictors of IHD (see Figures 10 and 11); this may indicate that SO₂ could be acting as a surrogate for the Coal Combustion factor in the TRI analyses.

TRI Scaling

In Table 8, we present the TRI estimates based on the IQR of each set of variables that comprised a specific TRI formulation. We were particularly interested in comparing the TRI based on the different sets of variables (e.g. Components, Factors, or Source Mass) with that based on PM_{2.5} mass alone. However, we found that a TRI based on PM_{2.5} mass alone was consistently lower than the TRIs based on the sets Components, Source Mass, or Factors. Using the IQR for all pollutants in a TRI formulation may have overstated the magnitude of the TRI when the sum of the IQRs for the sets used to calculate the TRI was much higher than the IQR for PM_{2.5} mass alone, as we found in these TRI analyses. Thus, it is not certain which are the most appropriately scaled values (e.g., IQR or other) for the variables when the goal is to compare multiple-pollutant models using TRI formulations.

We therefore investigated an alternative method of evaluating TRIs, in which the TRI is determined based on each cohort subject's exposure values for each set of variables used in a TRI formulation. We hypothesized that, if a representation of the atmospheric mix appears to be toxic to human health, the distribution of subject-specific TRIs based on that representation (i.e., PM_{2.5} mass, or a set of Components, Source Mass, or Factors) will be more dispersed. That is, the TRIs associated with that representation would fall in a wider range, but one that is more representative of our cohort's exposure to multiple pollutants.

The results (presented in full in Appendix I) were consistent with the results from the mortality analyses with TRIs based on pollutant IQRs, but had hazard ratios and confidence intervals that were shifted to lower values. Both methods indicate higher TRIs for the various sets of Components and Source Mass than for PM_{2.5} mass alone. Thus, even considering the uncertainties around these estimates, it does appear that, for IHD, the association of the mixture yielded a larger impact on mortality than PM_{2.5} mass alone.

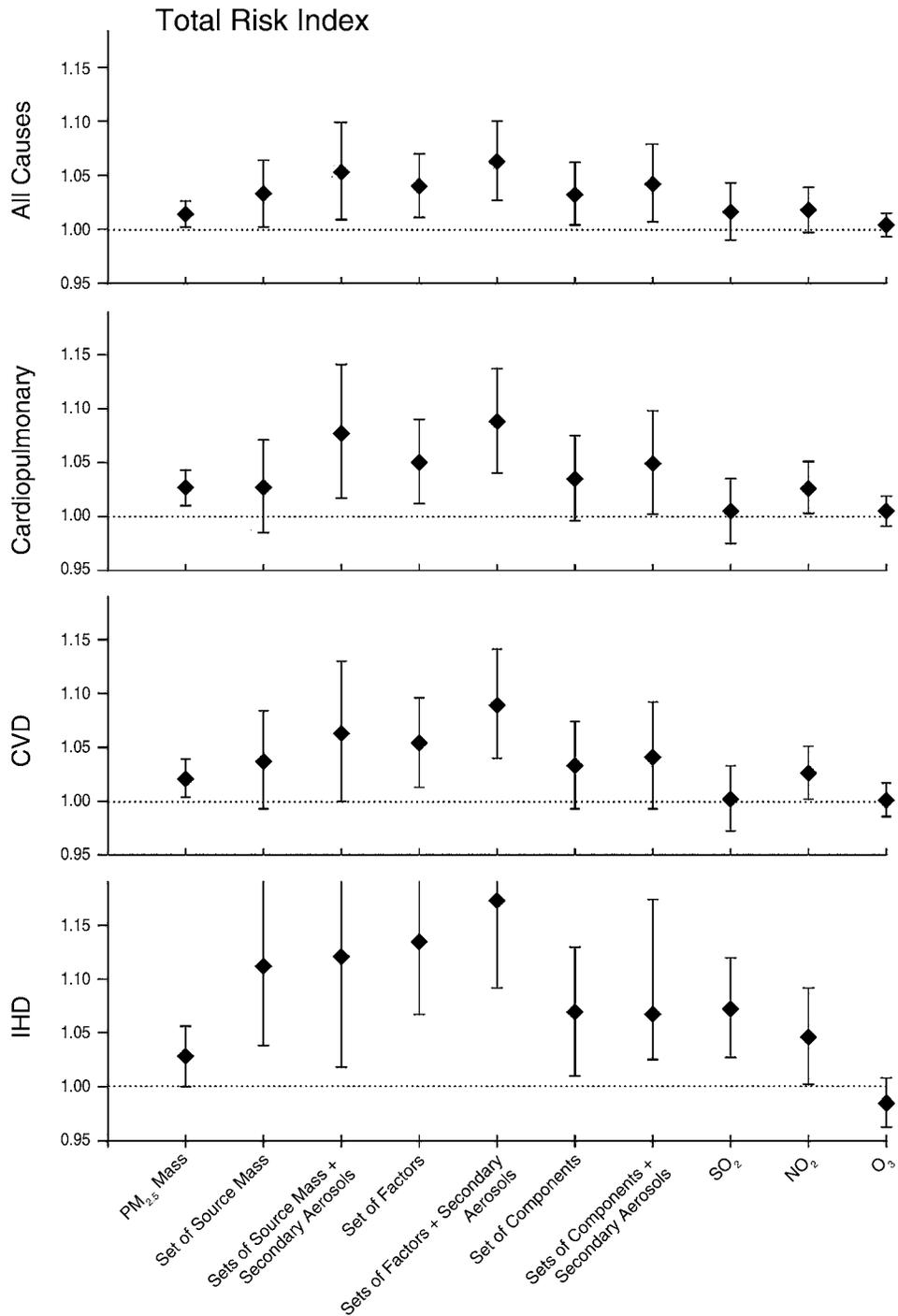


Figure 18. TRIs (with 95% CIs) at the IQR from the random effects Cox model including individual-level and ecologic covariates.

Table 8. TRIs for IHD Mortality for Combinations of PM_{2.5} Mass and Sets of Source Mass, Factors, Secondary Aerosols, and Components with Individual Gases Added^a

Sets of Variables in TRI Formulation	Standard Cox Model		Random Effects Cox Model	
	Without Ecologic Covariates	With Ecologic Covariates	Without Ecologic Covariates	With Ecologic Covariates
100 MSAs (445,860 CPS-II Subjects)				
PM _{2.5} mass	1.041 (1.029–1.054)	1.018 (1.005–1.032)	1.046 (1.016–1.077)	1.028 (1.000–1.056)
Source Mass	1.150 (1.116–1.186)	1.083 (1.044–1.124)	1.151 (1.073–1.234)	1.112 (1.038–1.192)
Factors	1.165 (1.132–1.199)	1.111 (1.072–1.152)	1.152 (1.082–1.226)	1.135 (1.067–1.208)
Components	1.085 (1.044–1.128)	1.048 (1.006–1.091)	1.101 (1.026–1.182)	1.069 (1.010–1.130)
Source Mass + Secondary Aerosols	1.178 (1.131–1.227)	1.078 (1.022–1.137)	1.173 (1.072–1.282)	1.121 (1.018–1.233)
Factors + Secondary Aerosols	1.194 (1.151–1.238)	1.143 (1.092–1.196)	1.181 (1.100–1.268)	1.173 (1.092–1.260)
Components + Secondary Aerosols	1.120 (1.068–1.174)	1.063 (1.010–1.118)	1.150 (1.063–1.245)	1.097 (1.025–1.174)
51 MSAs with NO₂ Data (289,522 CPS-II Subjects)				
NO ₂	1.027 (1.013–1.042)	1.039 (1.018–1.062)	1.013 (0.967–1.062)	1.046 (1.002–1.092)
PM _{2.5} mass	1.045 (1.031–1.059)	1.013 (0.995–1.031)	1.048 (1.010–1.087)	1.022 (0.987–1.059)
Source Mass	1.122 (1.065–1.182)	1.004 (0.933–1.081)	1.128 (0.989–1.286)	1.086 (0.957–1.232)
Factors	1.125 (1.072–1.181)	1.098 (1.034–1.165)	1.140 (1.028–1.264)	1.142 (1.044–1.249)
Components	0.987 (0.916–1.063)	0.927 (0.850–1.011)	1.011 (0.901–1.134)	0.927 (0.850–1.011)
PM _{2.5} mass + NO ₂	1.050 (1.034–1.068)	1.038 (1.015–1.063)	1.042 (0.992–1.096)	1.049 (1.002–1.098)
Source Mass + NO ₂	1.122 (1.065–1.182)	1.004 (0.932–1.082)	1.129 (0.993–1.285)	1.088 (0.965–1.227)
Factors + NO ₂	1.129 (1.075–1.186)	1.092 (1.027–1.160)	1.135 (1.023–1.260)	1.134 (1.036–1.241)
Components + NO ₂	0.926 (0.852–1.006)	0.945 (0.860–1.039)	0.963 (0.856–1.085)	0.946 (0.860–1.039)

Table continues next page

^a Data are presented by analytic model (standard Cox or random effects Cox) with or without ecologic covariates for subsets of MSAs. Models were adjusted for 42 individual-level covariates, stratifying the baseline hazard function by age (1-year groupings), gender, and race. Values show increase in risk per increase in exposure equal to the magnitude of the IQR (95% CIs). IQRs were calculated on the full data sets: 100 MSAs with CPS-II subjects in residence (full dataset for mortality analyses); 45 MSAs with SO₂ data; 51 MSAs with local NO₂ data; and 68 MSAs with O₃ data.

Table 8 (Continued). TRIs for IHD Mortality for Combinations of PM_{2.5} Mass and Sets of Source Mass, Factors, Secondary Aerosols, and Components with Individual Gases Added^a

Sets of Variables in TRI Formulation	Standard Cox Model		Random Effects Cox Model	
	Without Ecologic Covariates	With Ecologic Covariates	Without Ecologic Covariates	With Ecologic Covariates
45 MSAs with SO₂ Data (240,692 CPS-II Subjects)				
SO ₂	1.069 (1.048–1.090)	1.078 (1.046–1.112)	1.078 (1.022–1.138)	1.072 (1.027–1.120)
PM _{2.5} mass	1.054 (1.039–1.069)	1.012 (0.990–1.034)	1.067 (1.023–1.113)	1.024 (0.983–1.066)
Source Mass	1.167 (1.101–1.237)	0.988 (0.904–1.079)	1.208 (1.040–1.402)	1.018 (0.903–1.147)
Factors	1.074 (1.018–1.134)	1.020 (0.952–1.093)	1.138 (1.016–1.275)	1.020 (0.952–1.093)
Components	0.958 (0.885–1.037)	0.989 (0.903–1.085)	1.004 (0.905–1.115)	0.989 (0.903–1.085)
PM _{2.5} mass + SO ₂	1.115 (1.089–1.142)	1.076 (1.040–1.112)	1.129 (1.065–1.198)	1.075 (1.024–1.130)
Source Mass + SO ₂	1.215 (1.143–1.290)	0.994 (0.910–1.087)	1.244 (1.076–1.438)	1.005 (0.911–1.109)
Factors + SO ₂	1.081 (1.022–1.144)	1.030 (0.958–1.107)	1.159 (1.031–1.302)	1.030 (0.958–1.108)
Components + SO ₂	1.007 (0.923–1.099)	1.015 (0.920–1.119)	1.049 (0.943–1.167)	1.015 (0.920–1.119)

^a Data are presented by analytic model (standard Cox or random effects Cox) with or without ecologic covariates for subsets of MSAs. Models were adjusted for 42 individual-level covariates, stratifying the baseline hazard function by age (1-year groupings), gender, and race. Values show increase in risk per increase in exposure equal to the magnitude of the IQR (95% CIs). IQRs were calculated on the full data sets: 100 MSAs with CPS-II subjects in residence (full dataset for mortality analyses); 45 MSAs with SO₂ data; 51 MSAs with local NO₂ data; and 68 MSAs with O₃ data.

DISCUSSION AND CONCLUSIONS

This study addressed three of the overall NYU NPACT hypotheses about long-term human responses to cumulative exposures to PM_{2.5}.

1. Exposure to PM_{2.5} is capable of producing chronic health effects of public health concern, but the effects may differ according to the composition of the PM_{2.5}.

Our results demonstrate that (1) only one source category (Coal Combustion) and its elemental markers (As and Se) showed elevated (if not significant) associations with increased annual mortality from IHD and lung cancer; (2) the Traffic source category was less strongly associated with excess annual mortality; and (3) the other source categories were not associated with increased mortality in this cohort during the time period analyzed.

2. Long-term PM_{2.5} exposures are closely associated with chronic health effects.

The estimated increase in risk of annual mortality is clearly an important effect of long-term exposure.

3. The source-apportionment techniques that we have developed and refined in recent years provide a useful basis for identifying major PM_{2.5} air pollution source categories and specific chemical components that have the greatest impacts on a variety of chronic health effects.

The adverse effects of long-term PM_{2.5} exposure were most closely associated with specific source categories, especially Coal Combustion, for IHD.

MAJOR FINDINGS

Initial Mortality Analyses

Factor Analysis and Source Categories A factor analysis of the 2000–2005 nationwide U.S. EPA CSN data for PM_{2.5}

identified major elemental groupings, interpretable as being associated with specific pollution source categories at 212 sites across the United States. The major source categories identified and their key tracer elements or compounds were Soil (Ca, Si); Metals (Pb, Zn); Traffic (Cu, EC, NO₂); Salt (Na, Cl); Residual Oil Combustion (V, Ni); Steel Industry (Fe, Mn); Coal Combustion (As, Se); and Biomass Combustion (K).

Inspection of spatial plots of PM_{2.5} mass in the source categories confirmed the factor interpretations: contributions of mass from Traffic were highest in Southern California; from Soil were generally highest in the Southwest (e.g., Phoenix and surrounding cities); from Steel Industry were highest in cities with steel works (e.g., Detroit, MI; Birmingham, AL); from Coal Combustion were highest in the Ohio Valley region (e.g., Pittsburgh, PA); and from Residual Oil Combustion were highest in cities burning residual fuel oil in wintertime (e.g., New York City) or having deep ports (e.g., Los Angeles and Long Beach, CA; Savannah, GA; Newark, NJ; New York City, NY), which is consistent with emissions from ocean-going ships burning bunker fuel. The analysis revealed the same major U.S. source categories and spatial distribution as those previously reported for the 1979–1982 years of the U.S. EPA's Inhalable Particle Network (Özkaynak and Thurston 1987), albeit at lower levels than in the earlier period; this indicates a qualitative consistency in the spatial representativeness of these results over the past two decades.

CPS-II Cohort Individual risk data for 445,860 adults collected for the CPS-II cohort from 100 MSAs were linked with fine PM_{2.5} mass, component, and source category exposure data. The strongest associations were found with IHD. Lung cancer deaths showed associations above 1.0 with PM_{2.5} mass; although they were not statistically significant (possibly because fewer CSN sites were used than in earlier analyses), they are nevertheless consistent with past analyses of the CPS-II cohort.

All-Cause Mortality All-cause mortality analyses showed that PM_{2.5} mass and the tracer elements Se and As (which are associated with the Coal Combustion source category) were most significantly associated with increased risk of death. The analyses with factors and source-apportioned PM_{2.5} mass both confirmed that only the Coal Combustion source category was linked with all-cause mortality.

IHD Mortality Although PM_{2.5} mass from most industrial and fossil-fuel combustion source categories had hazard ratios above 1.0 for IHD deaths, PM_{2.5} mass apportioned to the Coal Combustion source category and its correlated tracer elements (e.g., As and Se) were most strongly and consistently associated with IHD mortality across all

of the various model specifications. PM_{2.5} mass from the Traffic source category, and especially its key elemental tracer (EC), showed elevated, if not significant, associations with IHD in most models. The finding that the risk estimate for EC with IHD was stronger than that for the Traffic source category would be consistent with diesel-powered vehicles having a greater role in air pollution than gasoline-powered vehicles. PM_{2.5} mass originating from wind-blown Soil or from Biomass Combustion categories were generally not associated with increased risk of IHD mortality in this cohort. Other source categories were more equivocal in their associations across models.

Respiratory Mortality Respiratory mortality was most significantly associated with long-term exposure to the secondary aerosol OC and to Soil (Ca and Si), but not with other specific sources.

Lung Cancer Mortality Only the Coal Combustion source category and its tracer element Se were significantly associated with increased risk of death from lung cancer.

Ecologic Covariates in the Models Adjustment for contextual ecologic covariates led to a substantial change in the association between Traffic (and EC) and all-cause mortality, and to a relevant change for Traffic with respiratory mortality. However, the ecologic covariates did not have much impact on the Coal Combustion source category (and its key tracers). The associations between Coal Combustion and each of the four types of mortality were more consistent than any others across the various models and especially with IHD.

TRI Mortality Analyses

The TRIs based on the sets of Components, Source Mass, or Factors (and especially when the set of Secondary Aerosols was included) were generally larger than the TRIs based on PM_{2.5} mass alone. This suggests that the source-specific information allows for a more accurate estimate of exposure and risk, and that past estimates using non-specific PM_{2.5} mass alone may have produced underestimates of the total effect of PM_{2.5} exposure on mortality.

The TRIs that included the set of Secondary Aerosols tended to be somewhat higher than the directly comparable TRIs that excluded the Secondary Aerosols. This provides evidence that secondary aerosols contribute to associations with mortality.

The evidence of an association between mortality and any of the three gaseous pollutants (SO₂, NO₂, and O₃), over and above any association with PM_{2.5} mass and its components, was generally weak, although inferences are

somewhat constrained by the limited number of MSAs with data for the gases.

SUMMARY

Overall, modeling results indicate that long-term exposure to PM_{2.5} from a few key source categories, and to the related elemental tracers, were most explanatory of the PM_{2.5}–mortality associations found in past CPS-II cohort studies. In particular, the Coal Combustion source category was most consistently associated with increased risk of IHD mortality across models (i.e., the standard Cox and random effects models with and without ecologic covariates). Traffic and Salt source categories, and especially their respective tracers EC and Cl, also showed significant associations with IHD mortality in many models.

Elevated (if not statistically significant) associations between PM_{2.5} and lung cancer mortality were also partially explained by associations with the Coal Combustion source category and its tracers, Se and As, but not with other components of PM_{2.5}.

However, Soil and Biomass Combustion source categories and their elemental tracers (Ca, Si, and K) were consistently not associated with mortality across all models.

LIMITATIONS

Although these analytic models use state-of-the-art exposure and cohort data, some limitations should be noted.

First, the PM_{2.5} and trace element data used for these analyses were available for only the last 5 of the 22 years of cohort follow-up. We assumed that these exposure patterns across sites were indicative of patterns earlier in the cohort period, but this assumption might have led to an overestimate of the mortality effect on a per microgram basis because the concentrations of PM_{2.5} were higher earlier in the follow-up period. An earlier analysis that compared mass data for two time periods found that the circa-1980 PM_{2.5} data and the circa-2000 PM_{2.5} data were strongly correlated and produced similar mortality associations (Pope et al. 2002).

Similarly, we compared the source-apportionment and component–exposure estimates from the current study with those from an earlier analysis of the 1979–1982 IPN data collected at the start of the CPS-II study (see Thurston et al. 2011 and Appendix H, which is available on the HEI Web site). That analysis indicated that the cities with the highest and lowest emissions of key tracer elements attributed to specific source categories remained consistent over time, even though the absolute levels of PM_{2.5} in nearly all of those cities have declined over recent decades. Therefore,

persons living in high-exposure cities would still have higher exposures than those in low-exposure cities over a 20-year period — assuming that cohort members remained in the city where they lived in 1982 when the cohort was formed. This assumption also reflects a limitation of the CPS-II cohort data.

Other studies suggest that the most recent years of exposure are most important to air pollution mortality effects (Roosli et al. 2005; Schwartz and Laden 2004) and that the majority of deaths occur in the later years of follow-up, during or near to the time when these PM_{2.5} and speciation data were collected (2000–2005). The extent to which past exposure levels (generally higher) might bias the effect estimates provided here, when viewed on a per microgram basis, may be lessened.

Second, the limited spatial resolution (within-city) of the exposure assessment represents an intrinsic challenge. For example, the lack of a consistent effect of the Traffic source category, as described above, does not allow one to draw conclusions about the spatial variations in exposures to traffic within MSAs. In our analyses, the Traffic source category accounted for only one part of the overall variability of traffic-related exposure because it captures only the between-city differences in traffic exposure. Also, the use of data from a limited number of sites per MSA (especially for the speciation data) can contribute to exposure misclassification.

Although it is true that people spend much of their time indoors, where they are exposed to additional sources of PM, the portion of their PM exposure of interest in these epidemiologic analyses is not their total PM exposure, but their exposure to the PM of outdoor origins, which is well represented by monitors located outdoors (Wilson et al. 2000). Also, any outdoor air pollution exposure misclassification is expected to more likely lead to underestimation of effects toward the null (Zeger et al. 2000), and so the estimates presented here are likely to be conservative estimates of the impacts of PM_{2.5} mass, components, and source categories. Overall, the limitations of this type of analysis have not significantly undermined the validity of the results presented.

IMPLICATIONS OF THE FINDINGS

Long-term exposure to PM_{2.5} and the key tracers of the Coal Combustion source category (As and Se) are most explanatory of past associations between PM_{2.5} mass and increased risk of all-cause, IHD, and lung cancer mortality. Controlling PM_{2.5} emissions from coal combustion and other sources that produce a similar mix of pollutants would influence the greatest mortality benefits.

Overall Summary and Conclusions

SUMMARY OF RESULTS

The NPACT Initiative was designed to explore the associations between the components of ambient PM and a variety of health-related responses in humans and laboratory animals. We focused on particle size ranges and various PM components to assess their roles in short- and long-term health outcomes.

We conducted epidemiologic time-series studies of short-term responses to inhaled PM_{2.5} and PM_{2.5} elemental components, ions, EC, and OC by populations residing in 150 U.S. MSAs, as well as to gaseous criteria pollutants and PM_{2.5} elemental components, ions, EC, and OC in 64 U.S. MSAs. The time-series studies examined the pollutants for associations with daily mortality and hospital admissions by cause.

We also conducted laboratory time-series studies to examine cardiac function using ApoE^{-/-} mice, a model of atherosclerosis. The mice were exposed to CAPs for 6 hours/day, 5 days/week, over 6 months at five U.S. sites. We assessed daily variations in HR and HRV in these mice using time-series analyses, and we assessed long-term changes in baseline cardiac function and in atherosclerotic plaque progression over the 6 months.

The cumulative PM_{2.5} exposures (concentration × duration) of the mice over 6 months were more or less comparable to those of the urban residents of the MSAs being studied in our acute time-series epidemiologic studies, as well as to those of the CPS-II cohort studied in the mortality analyses. This similarity provided a basis for relating the chronic effects (aortic plaque progression) in the mice exposed to CAPs and CAPs components to the longevity reductions in humans that were associated with long-term average concentrations of PM_{2.5} components in the CPS-II cohort communities for which CSN data were available.

Our epidemiologic time-series and CPS-II cohort studies were limited to studying the PM_{2.5} particle size fraction

(fine PM) because they depended on concentration data generated by the EPA's CSN. The same particle size limitation applied to the 6-month CAPs inhalation studies with ApoE^{-/-} mice because particles larger than 2.5 μm do not penetrate to the lower respiratory tract in mice, and such particles were therefore removed before the CAPs entered the exposure chambers. Thus, three of our four studies were limited to the fine fraction of ambient PM.

We recognized that health effects in humans have been associated with both larger and smaller particle size ranges. PM₁₀ penetrates beyond the upper respiratory tract and is efficiently deposited on the tracheobronchial airways within the human thorax, where it can cause airway irritation and exacerbate asthma. PM_{0.2}, which contributes little mass to PM_{2.5}, has also been a health concern because it dominates the particle number concentration and can cross epithelial membranes that are barriers to the penetration of larger particles. To begin to characterize the relative toxicities of these three PM size fractions, which differ in chemical composition and deposition sites, we collected high-volume samples of all three size fractions at the same five sites studied in our mouse inhalation studies. These samples were used in both in vitro human cellular exposures and in vivo aspiration exposures of FVB/N mice to study associations between acute responses and the three size fractions as well as specific elemental components.

In the discussion that follows, we first summarize the key findings of each of our four studies, with special emphasis on the associations between health responses and specific components or source categories or both. We then compare and contrast the associations with acute and chronic responses found across studies, taking into account the differences in species and endpoints.

At the end, we present our conclusions about the acute and chronic health effects — and the possible differences among those health responses — that can be attributed to specific components and source categories of PM_{2.5}.

TOXICOLOGIC STUDIES (CHEN STUDY 1 AND GORDON STUDY 2)

Subchronic CAPs Inhalation Exposures with Mice (Chen Study 1)

- Mean concentrations of PM_{2.5} mass varied substantially among the exposure chambers at the five sites. Two sites had lower concentrations (Seattle, 61 µg/m³; East Lansing, 68 µg/m³) than the three other sites, which had about twice as much mass (Manhattan, 123 µg/m³; Irvine, 138 µg/m³; and Tuxedo, 136 µg/m³).
- Some differences in mean concentrations of PM_{2.5} components were even greater among the exposure chambers at the five sites. For example: S ranged from 3.8 µg/m³ at Seattle to 11.3 µg/m³ at Tuxedo; BC ranged from 0.53 µg/m³ at East Lansing to 2.67 µg/m³ at Manhattan; Fe ranged from 0.30 µg/m³ at East Lansing to 1.88 µg/m³ at Manhattan; Mn ranged from 14.2 ng/m³ at Tuxedo to 107 ng/m³ at Manhattan; Zn ranged from 51.3 ng/m³ at East Lansing to 760 ng/m³ at Manhattan; Cu ranged from 5.4 ng/m³ at East Lansing to 100.5 ng/m³ at Irvine; Se ranged from 6.0 ng/m³ at Seattle to 24 ng/m³ at Irvine; V ranged from 17.1 ng/m³ at Tuxedo to 45.7 ng/m³ at Irvine; and Ni ranged from 6.6 ng/m³ at East Lansing to 69.9 ng/m³ at Manhattan. The substantial variation in component concentrations at these five sites made it possible to explore the contributions of individual components to a range of health effects.

Cardiac Function Associations of CAPs concentrations with three measures of cardiac function (HR, SDNN, and RMSSD) were analyzed for three different lag days and four different times of day. Statistically significant differences in the measures were found between CAPs- and filtered air-exposed mice: 56 such differences were found at Manhattan and 38 at Tuxedo, whereas only 6 were found at East Lansing, 5 at Irvine, and 3 at Seattle. The cardiac responses, if any, could be positive or negative because functional change could be accelerated or retarded depending on the CAPs concentration the mice were exposed to on a given lag day.

Numbers of Associations Across Sites

- The number of differences in cardiac function between CAPs- and filtered air-exposed mice was much higher at the sites in the northeastern United States (Manhattan and Tuxedo) than elsewhere. This suggests that components of the secondary aerosol that occur in the northeastern region, which were absent at Irvine and Seattle, and much lower at East Lansing, likely played an important role in producing short-term responses in cardiac function.

- More statistically significant differences in cardiac function between CAPs- and filtered-air-exposed mice were found at Irvine than at East Lansing and Seattle, and most of them (at these three sites) were at 2-day lag. By contrast, the much larger numbers of differences at Manhattan and Tuxedo were at 0- and 1-day lags. This is consistent with the hypothesis that higher levels of exposure result in earlier significant differences in responses. Nearly equal numbers of differences in cardiac function were found during the four time periods of the exposure day at all five sites, although fewer were found at East Lansing, Seattle, and Irvine than at Manhattan and Tuxedo.
- The number of components for which statistically significant associations with differences in cardiac function between CAPs- and filtered-air-exposed mice were observed was poorly correlated with the mass concentration of CAPs. For example, Manhattan had CAPs concentrations of 123 µg/m³ and 56 significant differences in cardiac function (39 positive and 17 negative) between CAPs- and filtered-air-exposed mice, whereas Irvine had CAPs concentrations of 138 µg/m³ and only 5 such differences (2 positive and 3 negative).

Which Components Showed Associations

- A number of individual PM_{2.5} components (BC, Al, Mg, Na, Ni, P, and V) were associated with many more differences in cardiac function between CAPs- and filtered-air-exposed mice at Manhattan than at Tuxedo; in contrast, OC, Cr, Cu, K, Mn, Pb, and Zn were associated with many more differences in cardiac function at Tuxedo than at Manhattan.
- Groupings of elements were found to be highly correlated at Manhattan and Tuxedo, the two sites that showed the most exposure-response associations for cardiac function. The groupings included Al, Si, and Ti, which are derived from Soil; and Br, Se, P, and S, which are derived from Coal Combustion. At Manhattan, Ni was closely correlated with V, S, and EC, which are derived from Residual Oil Combustion; but at Tuxedo, Ni was correlated with Cr and Fe, which, along with Ni, have been shown to be present in stack emissions from a distant upwind Ni refinery in Sudbury, Ontario.
- Associations between each of 21 single PM_{2.5} components and three measures of cardiac function (HR, SDNN, and RSSMD) were analyzed for three lag days and four times of day. The significant differences in the measures of cardiac function between CAPs- and filtered-air-exposed mice at Manhattan and Tuxedo were about three times as many as those seen at East

Lansing and Seattle, and more than two times as many as those at the Irvine site.

- Positive exposure–response associations with HR, SDNN, and RSSMD measures of cardiac function were observed for Ni ($r^2 = 0.96$), Al ($r^2 = 0.81$), EC ($r^2 = 0.79$), P ($r^2 = 0.77$), S ($r^2 = 0.65$), and V ($r^2 = 0.35$). Negative, less consistent exposure–response associations were observed for Zn ($r^2 = 0.27$), Se ($r^2 = 0.19$), and K ($r^2 = 0.13$). Some components (Cu, Si, Fe, Mg, Mn, and Pb) showed inconsistent findings: Fewer exposure–response associations that were both positive and negative for different lag days and times of day were observed.

Which Source Categories Showed Associations

- Relatively large numbers of associations with the HR, SDNN, and RSSMD measures were observed for at least one source category at each of the five sites.
- The source category that showed the largest number of associations with changes in cardiac function differed at each site. At Manhattan, large numbers of associations were with Residual Oil Combustion and Secondary Aerosols, as well as Salt and Traffic; at Tuxedo, a large number of associations were observed for Sulfur–Coal (and also Soil), and a moderate number of associations with a distant, upwind Ni refinery were observed. At Seattle, the largest number of associations were with Soil, and a moderate number of associations were with Residual Oil Combustion.
- The source categories associated with Ni were either the first or second strongest at Manhattan and Seattle, which had the highest Ni concentrations, and were less influential at East Lansing and Tuxedo, which had much lower Ni concentrations.
- Two source categories that are not generally considered as likely to be causally related to health outcomes were among those associated with cardiac function changes: Soil at Seattle and Tuxedo, and Salt at Seattle.

Atherosclerotic Plaque Progression

- Long-term plaque-volume progression, as measured by ultrasound biomicroscopy, varied by exposure site; substantial plaque-volume progression after 6 months of exposure was found in the BA of mice exposed to CAPs, as opposed to filtered air, at Manhattan and Tuxedo (which had much higher sulfate [Manhattan and Tuxedo] and Ni [Manhattan] concentrations than the other three sites had). Mice had less, but still significant, plaque-volume progression after 6 months at East Lansing, but not at Seattle or Irvine.

- Supplemental measurements of plaque surface area by quantitative image analysis in mice exposed to CAPs or filtered air and killed after 6 months at Irvine showed similar plaque surface areas in both groups of mice. Comparable measurements for mice killed after 6 months of exposure to CAPs or filtered air were made at Seattle — where the mice were considerably older at the start of exposure — and although the plaque surface areas were again similar in both groups, they were greater than those for the Irvine mice at 6 months.

In Vivo and In Vitro Exposures to Coarse, Fine, and Ultrafine PM Collected on Filters (Gordon Study 2)

- Particle size range, season, and site significantly influenced intracellular ROS levels in vitro.
- Coarse PM elicited the greatest PMN response in vivo regardless of site.
- In vitro ROS production did not predict in vitro lung inflammation.
- Metals associated with the Traffic, Coal Combustion, and Residual Oil Combustion source categories were significantly correlated with in vitro responses, and other components associated with Traffic, as well as endotoxin, showed significant associations with in vivo responses.
- Specific elements that were highly correlated ($P < 0.001$) with in vitro ROS production in vascular endothelial cells were Cu, Sb, K, Sr, V, Fe, Co, Be, Ti, Ca, Sc, Mg, Ni, and P.
- Specific elements that were highly correlated ($P < 0.001$) with in vitro ROS production in airway epithelial cells were Cu, Sb, V, Co, Be, and Ni.
- Specific elements that were strongly correlated ($P < 0.01$) with mRNA levels in airway epithelial cells in vitro were Cu, K, Sr, V, and Ni for CSF-2; Cr, As, Cu, Sb, Sn, Sr, Ti, Tl, and Mn for HO-1; Cu, Sb, Sr, Fe, V, Ti, Ca, Sc, Ni, Mn, S, and Cr for IL-6; Cu, V, and Mn for IL-8; and Cu, Sr, Fe, K, Ti, Mn, Cr, and As for VEGF-A.
- Specific elements that were strongly correlated ($P < 0.01$) with mRNA levels in vascular endothelial cells in vitro were Cu, Sb, Fe, Co, Ti, Be, Ni, Mn, and Cr for HO-1; Sr, Co, Be, Mn, and Tl for ICAM-1; Co, Be, Ni, Pb, and Tl for IL-8; Co, Be, Ni, Pb, and Tl for TXNRD1; and K, Co, Be, Ni, Tl, and Pb for VEGF-A.
- A selection of PM samples that were tested in mice in vivo were also tested in vitro in cardiomyocytes. We found that some samples induced significant effects on spontaneous in vitro beat frequency: These included winter samples from Manhattan in the fine

and ultrafine particle size ranges that had unusually high concentrations of S and Zn.

EPIDEMIOLOGIC STUDIES (ITO STUDY 3 AND THURSTON STUDY 4)

Time-Series Studies of Daily Hospital Admissions and Mortality (Ito Study 3)

Multicity Daily Mortality

- More ambient air pollution components were associated with all-cause daily mortality in the warm season (NO₂, SO₂, EC, OC, Pb, Si, and V) than in the cold season (SO₂, Cu, K, OC, and Si), with some showing associations in both seasons (SO₂, OC, and Si).
- Of the six source categories examined, the Traffic, Soil, and Coal Combustion categories showed significant associations with all-cause mortality in an all-year analysis.
- In the second-stage analysis — which examined the heterogeneity of PM_{2.5} risk estimates across the cities as a function of city-level variables — SO₄²⁻, weekday excess PM_{2.5}, V, Pb, the berth volume of seaports within 60 miles of a city, and the sum of road lengths were important predictors in explaining the between-city variation of all-cause daily mortality risk estimates associated with PM_{2.5} exposure.

Multicity Daily CVD Hospitalizations

- In contrast to all-cause daily mortality, the associations of pollutants with CVD hospitalizations occurred mostly at 0-day lag in the cold season. Those pollutants were PM_{2.5}, NO₂, SO₂, CO, Cu, EC, Fe, OC, SO₄²⁻, Se, Si, and Zn. V showed a nearly significant association at 0-day lag and associations at 1- and 3-day lags. Several pollutants with 0-day lag associations (NO₂, SO₂, CO, EC, and OC) also showed associations at 3-day lag. In the warm season, NO₃⁻ (0-day lag) and Na (2-day lag) showed associations.
- Among the source categories examined, the Traffic and Salt categories showed significant associations with CVD hospitalization in the all-year analysis.
- In the second-stage analysis, Cu, NO₂, V, Ni, Fe, and the extent of land development were important positive predictors of the variation in risk estimates for CVD hospitalizations associated with PM_{2.5} across cities.

Multicity Daily Respiratory Hospitalizations

- In contrast to CVD hospitalizations, respiratory hospitalizations were associated with pollutants in both the

warm season (PM_{2.5}, CO, As, K, OC, and SO₄²⁻) and the cold season (PM_{2.5}, CO, Cu, EC, K, and Si).

- Of the six source categories examined, only the Traffic category showed significant associations with respiratory hospitalizations.
- In the second-stage analysis, Cu, Ni, V, SO₂, and the extent of land development ranked high as positive predictors of between-city variability in CVD risk estimates associated with PM_{2.5} concentrations.

Analysis of Daily Associations Between PM_{2.5} Components and Mortality and Hospital Admissions in Seattle and Detroit

These results are based on analyses of daily concentrations of PM_{2.5} components that were made possible by HEI supplemental funding (see Appendix F and Zhou et al. 2011). We used the city-specific daily data to examine the effects of distributed lags on subcategories of CVD and respiratory hospitalizations.

- In contrast to the combined effect estimates from the multicity mortality analysis, the estimates for the Seattle mortality analysis showed associations between a number of PM_{2.5} components (Al, K, Si, Zn, EC, CO, and NO₂) and mortality in the cold season. Consistent with the multicity analysis, the Detroit mortality analysis showed associations with PM_{2.5} mass and S in the warm season.
- Generally consistent with the multicity results, the associations between CVD hospitalizations and PM_{2.5} components or gaseous pollutants were limited in the cold season in both Seattle (Fe, Ni, and V) and Detroit (K, S, EC, and NO₂), and they were mainly driven by IHD hospitalizations.
- The multiday risk estimates computed using distributed-lag models in these two cities were generally larger than the individual-day effect estimates, which suggests that risk estimates for individual lag days may underestimate the effects of the pollutants.

Mortality Risk from Long-Term Exposure in the CPS-II Cohort (Thurston Study 4)

- A factor analysis of the 2000–2005 data from the 212 nationwide CSN monitoring sites for PM_{2.5} and its components identified major groupings of components, interpretable as being associated with specific PM_{2.5} source categories. The major source categories identified and their key tracer elements were as follows: Soil (Ca, Si); Metals (Pb, Zn); Traffic (OC, EC, Cu, NO₂); Salt (Na, Cl); Residual Oil Combustion (V, Ni); Steel Industry (Fe, Mn); Coal Combustion (As, Se); and Biomass Combustion (K).

- Inspection of spatial plots of the factor analysis scores (i.e., contributions of $PM_{2.5}$ mass from each source category) confirmed the anticipated interpretations: Contributions from Traffic were highest in Southern California; those from Soil were highest in the Southwest; those from Steel Industry were highest in cities with steelworks (e.g., Detroit, Michigan, and Birmingham, Alabama); those from Coal Combustion were highest in the Ohio River Valley region (e.g., Pittsburgh, Pennsylvania); and those from Residual Oil Combustion were highest in cities with wintertime residual fuel oil burning (e.g., New York City) or in cities with deepwater ports (e.g., Los Angeles and Long Beach, California; Savannah, Georgia; Newark, New Jersey–New York City), which is consistent with impacts by emissions from oceangoing ships burning highly polluting bunker fuel. The analysis revealed the same major source categories and spatial distribution of their contributions as those previously reported for the 1979–1983 Inhalable Particle Network data (Özkaynak and Thurston 1987) — although the levels of $PM_{2.5}$ have been lower in recent years than in the earlier period — which indicates a qualitative consistency in the spatial representativeness of these source categories over the past thirty years.
- Data on individual risk factors for approximately 446,000 adults (collected by the ACS for the CPS-II cohort) from 100 MSAs were linked with data on exposure to $PM_{2.5}$ mass, elements, and emissions from different source categories. The strongest associations for $PM_{2.5}$, its components, and source categories were with deaths from IHD and lung cancer, which is consistent with past analyses of the CPS-II data (Pope et al. 1995, 2002).
- Analyses of all-cause mortality data indicated that $PM_{2.5}$ and the elements associated with Coal Combustion (i.e., Se and As) were most significantly associated with increased risk of death.
- Although $PM_{2.5}$ mass from most industrial (Steel Industry, Metals) and fossil fuel combustion (Coal Combustion and Residual Oil Combustion) categories had relative risk estimates above 1.0 for IHD deaths, $PM_{2.5}$, S, and the elemental tracers for Coal Combustion (i.e., As and Se) were most strongly and consistently associated with IHD mortality across all of the various model specifications. $PM_{2.5}$ associated with Traffic and its key elemental tracer (EC) were also associated with IHD in some models. $PM_{2.5}$ mass originating from the Soil source category (wind-blown soil) or from Biomass Combustion was generally not

associated with increased risk of IHD mortality in this cohort.

- Respiratory mortality was most significantly associated with long-term exposure to secondary OC, but not with any specific source category.
- Only S, the Coal Combustion source category, and one of its key tracer elements (Se) were significantly associated with increased risk of death from lung cancer.
- The associations between $PM_{2.5}$ mass and health outcomes were more easily interpretable when mass was allocated to specific source categories than when individual components were analyzed alone, as components may have been emitted from a variety of sources.

Overall, modeling results from the main analyses indicated that long-term exposures to $PM_{2.5}$ and the tracers from a few key source categories were most explanatory of the associations between $PM_{2.5}$ and mortality found in past studies of the CPS-II cohort that were limited to studying $PM_{2.5}$ mass and SO_4^{2-} concentrations. In particular, the Coal Combustion source category was most consistently associated with increased risk of IHD mortality across the models (i.e., standard Cox or random effects Cox models with and without ecologic covariates). Traffic and Salt, and especially their respective tracers (EC and Cl), also showed significant associations with IHD mortality in some models. Associations between $PM_{2.5}$ and lung cancer mortality were also found for the $PM_{2.5}$ mass apportioned to SO_4^{2-} , the Coal Combustion source category, and one of its key tracer, Se, but not with other components of $PM_{2.5}$. This finding is particularly important in light of the reductions in annual mortality that have been attributed to reductions in long-term $PM_{2.5}$ exposures resulting from the implementation of the Clean Air Act. Soil and Biomass Combustion source categories were consistently not associated with any causes of death across all models.

The supplemental TRI analysis, which uses multipollutant models, found that the TRIs based on the source categories (especially when secondary aerosols were included in the analysis) were generally larger than the TRIs based on $PM_{2.5}$ mass alone. This suggests that the information related to specific source categories allows for a more accurate exposure and risk estimate, and that past estimates using nonspecific $PM_{2.5}$ mass have underestimated the total effect of $PM_{2.5}$ exposure on mortality. The TRIs from models that included the secondary aerosols (SO_4^{2-} , NO_3^- , OC) tended to be somewhat larger than the directly comparable TRIs from models without secondary aerosols. This analysis provided evidence that secondary aerosols contributed to the associations between $PM_{2.5}$ and mortality. Furthermore, the evidence from the TRI analysis of an association

between mortality and any of the three gaseous pollutants (analyzed separately from PM_{2.5} mass) was generally weak, which supports the hypothesis that the chronic health effects associated with ambient air pollution are primarily due to the components of PM_{2.5}. However, such inferences are somewhat constrained at this time because of the limited number of MSAs that have sufficient data on the concentrations of both PM_{2.5} components and criteria pollutant gases.

INTEGRATION OF RESULTS AMONG STUDIES

Our opportunities for integrating responses to PM_{2.5} and its components in humans, animals, and cells in vitro were limited to cardiovascular effects, because we generated no data on respiratory system responses or lung cancer in our toxicologic studies with mice that could be compared with respiratory mortality and hospital admissions data in humans. In our subchronic inhalation studies with mice — in collaboration with colleagues at other research laboratories — we generated data on the effects of CAPs on other organs (the brain and liver) and on fat metabolism and metabolic syndrome. However, we have no comparable data for such effects in human populations.

CVD Responses in the Time-Series Study of Humans (Ito Study 3)

One important limitation to be considered when integrating the results from observational time-series studies with those from toxicologic experiments is that the associations between PM_{2.5} components and the outcomes in time-series studies can be influenced by differential exposure errors. Unlike the toxicologic experiments, in which the reported concentrations of PM_{2.5} components are relatively accurate representations of the exposures to the cells or animals, the concentrations of PM_{2.5} components used in time-series analyses have varying levels of misclassification error related to population exposure. In a time-series analysis, the larger the exposure misclassification error, the more the association between the pollutant and the health effect will be biased toward the null. As shown in Figure 16 of Study 3, the expected exposure misclassification errors, as indicated by monitor-to-monitor correlations at sites with multiple monitors, varied widely across PM_{2.5} components. Generally, concentrations of secondary pollutants exhibited much less error than did primary components. In fact, two key elements of interest, As (a tracer element for Coal Combustion) and Ni (a tracer element for Residual Oil Combustion) exhibited the largest exposure errors among the components.

The following summarizes the associations of PM_{2.5} components with CVD hospitalizations (Study 3, Table 4):

- Associations with CVD hospitalizations occurred mostly in the cold season at 0-day lag.
- CVD hospitalizations were associated with primary gaseous pollutants (NO₂, SO₂, and CO), primary PM_{2.5} components (Cu, EC, Fe, Se, Zn, and V), and secondary PM_{2.5} components (SO₄²⁻, NO₃⁻, and OC).
- CVD hospitalizations were not associated with As or Ni, which was consistent with their expected large exposure misclassification errors. However, both Ni and V were associated with CVD hospitalizations in the cold season in Seattle (see Appendix F).
- The Traffic source category was associated with CVD hospitalizations in the cold season.

CVD Responses in Mice and Cells (Chen Study 1 and Gordon Study 2)

- Regression analysis of mean PM component concentrations across the five sites with three measures of cardiac function in ApoE^{-/-} mice (HR, SDNN, and RSSMD) indicated that the highest number of significant associations were observed for Ni ($r^2 = 0.96$), S ($r^2 = 0.65$), and V ($r^2 = 0.35$) (Chen Study 1). These elements were also among those most closely associated with ROS production and with mRNA levels in vascular endothelial and airway epithelial cells in vitro (Gordon Study 2).
- The winter samples from Manhattan in the fine and ultrafine PM size ranges, which were first tested by aspiration into the lungs of mice, induced significant effects on spontaneous in vitro beat frequency when applied to cardiomyocytes (Gordon Study 2). The samples were enriched with PM components associated with Residual Oil Combustion. These results were consistent with the finding in our 6-month CAPs inhalation study (Chen Study 1) that CAPs affected cardiac function to a much greater extent at Manhattan than at other sites with much lower Ni concentrations.

CVD Responses in the Time-Series Studies of Humans and Mice (Chen Study 1 and Ito Study 3)

- Daily hospitalizations for CVD causes, including IHD, were associated with PM_{2.5}. This is consistent with the observations of the following: (1) acute cardiac function changes in mice exposed 5 days each week by inhalation to CAPs (Chen Study 1); (2) increased HR in mice exposed in vivo by aspiration to fine and ultrafine PM collected in Manhattan; and (3) increased ROS production and mRNA levels in human endothelial and epithelial cells exposed to fine and ultrafine PM in vitro.

- Transition metals such as Cu, Fe, and V were among the PM_{2.5} components associated with CVD hospitalizations (Ito Study 3). This is consistent with our studies of mice exposed to PM_{2.5} in vitro and human cells exposed to PM_{2.5} in vitro.

Long-Term CVD Responses of Humans and Mice (Chen Study 1 and Thurston Study 4)

- Mortality for IHD in the CPS-II cohort analysis was related to the total PM_{2.5} mass concentration, S, and the elements associated with Coal Combustion (Se and As) (Thurston Study 4). Plaque progression in the BA of mice was seen after 6 months of CAPs exposure at Manhattan, Tuxedo, and East Lansing (which are all downwind of many coal-burning power plants), but not at Seattle or Irvine (where no coal-burning power plants are upwind). This suggests that the Coal Combustion source category accounted for the different responses in the different locations.

Summary of CVD Responses in Humans, Mice, and Cells (All Studies)

- We found both similarities and differences in the PM_{2.5} components associated with CVD effects in human populations and mice and in cells in vitro.
- An important similarity among results observed in humans and in laboratory studies with mice and cells was that specific components and source categories were more strongly associated with specific CVD health-related responses than PM_{2.5} mass was.
- One difference was that different PM components and PM source categories were most influential in short- and long-term effects. In Seattle — for which a direct comparison could be made between CVD outcomes in Study 1 (cardiac function changes in ApoE^{-/-} mice) and Study 3 (CVD hospitalizations [Appendix F, HEI Web site]) — Ni and V (both associated with Residual Oil Combustion) were important predictors of these CVD outcomes. Also, the second-stage analysis of the multicity results indicated that PM_{2.5} risks associated with CVD hospitalizations were larger in cities with high Ni and V (and Cu) levels.
- For responses to long-term exposure, the Coal Combustion source category was most closely associated with both increased mortality in the CPS-II cohort and with BA plaque progression in ApoE^{-/-} mice after 6 months of CAPs exposure.

PM Components and Sources Most Likely to Be Causal for Short-Term CVD Effects (Chen Study 1 and Ito Study 3)

Ito (Study 3) showed that many gaseous criteria pollutants and PM_{2.5} components were significantly associated nationwide with daily all-cause mortality (SO₂, NO₂, EC, OC, Si, K, Pb, and V; Study 3, Table 4). However, in the second-stage analysis, which attempted to explain the heterogeneity of all-cause mortality risk estimates associated with PM_{2.5} across cities, the most prominent predictors were SO₄²⁻, V, berth volume of seaports within 60 miles of a specific city, and the sum of road lengths; these predictors suggest that Residual Oil Combustion and Traffic were the source categories with the greatest impacts (Figure 14 in Study 3). These analyses lacked enough statistical power to differentiate by disease category, however. The second-stage analysis also indicated that Cu, NO₂, V, Ni, Fe, and the extent of land development were important positive predictors of the variation in risk estimates for daily CVD hospitalizations associated with PM_{2.5} across cities (Figure 14 in Study 3). These components are associated with the Residual Oil Combustion and Traffic source categories.

For Seattle, where air quality data were available daily and were sufficient to analyze mortality by cause, significant excess distributed lag risks were found in the cold season for IHD hospital admissions, but not for other CVD categories (see Appendix F on the HEI Web site). Only Fe, Ni, and V were associated with significant excess risks for total CVD hospitalizations. For the warm season in Seattle and Detroit, no significant excess distributed lag risks for total CVD hospital admissions or for any of the subcategories were found. The presence of a substantial Residual Oil Combustion source category in Seattle, and its absence in Detroit, suggest that the excess IHD hospitalizations in Seattle could be attributed to that source category.

In Chen (Study 1), the results of the time-series study of cardiac function in ApoE^{-/-} mice exposed to CAPs at five sites clearly showed that although variations in PM_{2.5} mass were not significantly associated with variations in cardiac function, the variations in cardiac function correlated highly with some of the PM_{2.5} components (Ni [$r^2 = 0.96$], Al [$r^2 = 0.81$], EC [$r^2 = 0.79$], P [$r^2 = 0.77$], S [$r^2 = 0.65$], and V [$r^2 = 0.35$]). With the exception of Al, these components are all known to be associated with Residual Oil Combustion. Other components had negative, less consistent exposure–response relationships with measures of cardiac function (Zn [$r^2 = 0.27$], Se [$r^2 = 0.19$], and K [$r^2 = 0.13$]), whereas concentrations of some elements (Cu, Si, Fe, Mg, Mn, and Pb) showed fewer exposure–response relationships, and these were inconsistent (i.e., both positive and negative associations for different lag days and times of day).

Among the source categories, Residual Oil Combustion was more closely associated with cardiac function changes than was Traffic or Coal Combustion; and two source categories that have generally been considered as not likely to be causal were among those associated with cardiac function changes at specific sites: Soil in Seattle and Tuxedo, and Salt in Seattle.

PM Components and Sources Most Likely to Be Causal for Short-Term Respiratory Effects (Ito Study 3)

In contrast to CVD hospitalizations, nationwide daily respiratory hospitalizations were associated with PM components in both the warm season (PM_{2.5}, CO, As, K, OC, Se, and SO₄²⁻) and the cold season (PM_{2.5}, CO, Cu, EC, K, Se, and Si). In the second-stage analysis, Cu, Ni, V, SO₂, and the extent of land development ranked high as positive predictors of city-to-city variation in excess risk estimates for daily respiratory hospitalizations. Among the six source categories examined, only Traffic showed significant associations with respiratory hospitalizations. These results did not clearly identify the most influential components or source categories associated with short-term respiratory effects.

PM Components and Sources Most Likely to Be Causal for Long-Term CVD Effects (Chen Study 1, Thurston Study 4)

For long-term CVD health effects, we lacked the statistical power to separate out the influences of individual components on BA plaque progression in ApoE^{-/-} mice exposed to CAPs. However, we did show that mortality resulting from IHD and lung cancer in humans and from plaque progression in mice was much more closely associated with exposure to the tracers for Coal Combustion than to any other mixture related to a source category.

PM Components Not Likely to Be Causal for Long-Term CVD Effects (Thurston Study 4)

With the exception of the Traffic source category, which had equivocal associations with IHD mortality associated with long-term exposure, the other source categories exhibited few, if any, such associations in the main analyses of the CPS-II cohort. The TRI analyses in Thurston Study 4 showed the following: (1) analyses using PM_{2.5} mass specific to a source category produced larger effect estimates than analyses that used component mass concentrations not specific to a source category; (2) inclusion of secondary aerosols (SO₄²⁻, NO₃⁻, and OC) increased the risk coefficient; and (3) inclusion of the gaseous criteria pollutants had a generally weak impact.

The Role of Traffic-Related Air Pollutants in Health Effects (All Studies)

In time-series analyses, the Traffic source category showed the most consistent associations with all-cause mortality, CVD hospitalizations, and respiratory hospitalizations, though the significance of the associations was less than that for some of the individual components of PM_{2.5} and the gaseous pollutants associated with traffic. Also, Traffic was considerably less closely associated with cardiac function in CAPs-exposed mice than were the metals associated with Residual Oil Combustion (Chen Study 1).

For effects of long-term exposure, the Traffic source category was less clearly associated with excess mortality in humans (Thurston Study 4) and aortic plaque progression in mice (Chen Study 1) than was the Coal Combustion category, but the Traffic source category (and its associated tracer, EC) was more closely associated with mortality in humans than any of the remaining source categories. Furthermore, no significant association of the Traffic source category with plaque volume progression was found in the mice exposed to CAPs at Irvine or Seattle, where there was no Coal Combustion source category to obscure the possible effect of the Traffic source category.

To the extent that the Traffic source category did affect either short- or long-term health effects, it was not at all clear whether the effects could be attributed to PM_{2.5} components or gas phase pollutants; however, the results of the TRI analysis of the CPS-II cohort indicated that the effects were less likely to be attributable to gaseous criteria pollutants. Although some of the associations of PM_{2.5} components with health outcomes may have been causal, our analyses lacked the power to distinguish between tailpipe emissions of transition metals and resuspended road dust containing mineral oxides.

Our studies also lacked the power to determine what role, if any, was played by OC and ultrafine PM. Our chemical analyses of PM_{2.5} were limited to determining total OC, which is generally dominated by aged secondary OC. Thus, we could not determine whether the freshly generated primary OC, a focus of the studies by Delfino and colleagues (2009, 2010a,b), was causally associated with the health endpoints that we studied. Furthermore, we also lacked particle number counts for ultrafine PM, which contains the highest number concentration of airborne particles; we had data on ultrafine mass concentrations only for the in vitro cellular toxicity analyses and the in vivo analyses of toxicity using lung aspiration of PM size classes (Gordon Study 2). In these analyses, the ultrafine samples were usually more toxic, per unit mass, than the other PM_{2.5} size classes that generally dominate the PM_{2.5}

mass. Given the small contribution of ultrafine PM to total PM mass, however, it is unlikely that ultrafine particles could have accounted for all of the short- and long-term health effects that were closely associated with the mass concentrations of PM_{2.5} components in Studies 1, 3, and 4.

DISCUSSION AND CONCLUSIONS

OVERALL ACCOMPLISHMENTS

The NPACT study at NYU, consisting of two toxicologic research projects, two epidemiologic research projects, and this integrative summary and discussion of the various research findings, has met nearly all of its original goals. In doing so, we have gained a much more thorough understanding of the particle size ranges and chemical characteristics of ambient PM that have the greatest influences on both short- and long-term health-related responses to PM exposure.

RESEARCH METHODS

Advantages of the NYU Study Design

One key to the success of this study is that we had access to chemical speciation data for short-term average concentrations of elemental and ionic components, as well as for EC and OC, within PM_{2.5}. For our subchronic mouse inhalation studies (Chen Study 1), we used our own speciation analyses of the elemental concentrations within the exposure chambers during the daily 6-hour exposures at five different sites having very different PM_{2.5} composition. For our time-series analyses of associations of ambient PM_{2.5} with daily mortality and hospital admissions (Ito Study 3), we had access to the 24-hour mean concentrations of PM_{2.5} ions and elemental components that were measured every third or sixth day in 150 MSAs. We used PM_{2.5} speciation data from EPA's CSN for 150 MSAs, as well as daily data for all the gaseous criteria pollutants in 64 of those cities.

We also had daily PM_{2.5} speciation data for Seattle and Detroit as a result of supplemental HEI support that enabled us to examine the lag structure and distributed lags in those two cities. Because we had these speciation data on short-term concentrations and their daily variations, we were able to identify source categories and specific elements that were most closely associated with the short-term health effects.

These PM_{2.5} concentration data also enabled us to examine how the differences in long-term average concentrations among many cities with different PM_{2.5} mixtures influenced mortality in humans (Thurston Study 4) and aortic plaque progression in mice (Chen Study 1).

Another key to our success is that we applied unique techniques that we developed or refined to measure health-related responses of mice to daily PM exposures. In particular, these included the systems we developed for (1) concentrating ambient air PM_{2.5}; (2) automating in vivo measurements of cardiac function indices in mice; (3) using statistical methods to efficiently analyze enormous volumes of cardiac function data; and (4) periodically performing in vivo measurements of plaque density in the mouse aorta.

Having standardized speciation data for both our mouse and cell-culture studies and for our epidemiologic studies was critical in terms of our ability to identify PM components that were particularly influential in eliciting health-related responses. It was especially critical for our studies of the effects of long-term exposure because the ranges of long-term mean concentrations were much less extreme than those observed in the studies of acute effects, and because the differences in mean concentrations for some components and source-category mixtures were relatively small among the cities with CPS-II cohort members.

We would also like to acknowledge that the success of our study depended in large part on effective collaboration with colleagues at other research institutions.

Limitations of the NYU Study Design

Although we made substantial progress in demonstrating that some PM components were considerably more closely associated with health-related effects than were others, many unresolved issues will need to be addressed in future studies. This was inevitable, considering the complexity of the overall goals of the NPACT Initiative, the limited knowledge base at the time we submitted our application for funding, and overall limitations in funding.

To meet the requirement for an integrated program of toxicology and epidemiology addressing effects of both short- and long-term exposures to ambient air pollution, we opted to rely on concentration data for PM components that were generated by XRF analyses of samples collected on Teflon filters, by thermal ramp assays of EC and total OC collected on quartz filters, and by optical reflectance of BC on Teflon filters. To do so within the budgetary limitations, we recognized that we would be unable to (1) obtain, for 24- or 6-hour samples, adequate numbers of measurements above the lower limit of detection for all of the elements of potential interest; (2) know the identity and solubility of the ions and crystalline forms associated with the elements; and (3) know the composition of the organic compounds within the total OC. In addition, the number of collocated sites where both PM and gaseous pollutant data

were collected (and made publicly available) limited our ability to discriminate more definitively between health outcomes associated with gaseous pollutants or with PM_{2.5}.

We also recognized that we were limited to the following health endpoints: (1) those for which data on human mortality and hospital admissions by cause were routinely collected by the NCHS and the Center for Medicare and Medicaid Services; (2) short-term health-related functional responses and longer-term pathological responses in mice *in vivo*; and (3) short-term health-related cellular responses *in vitro*. Finally, we recognized that — within the time frame and funding limits of this study — we would be unable to focus on elucidating the underlying biological mechanisms for the responses we hoped to characterize, especially in view of our limited

knowledge of the nature and duration of the effects we might observe. By focusing our characterization on both expected and unanticipated effects, we hoped this study would provide a firmer basis for future studies of the mechanisms of responses at realistic levels of pollutant exposures.

KEY RESULTS

Comparing Human CVD Hospitalizations and Cardiac Function Changes in ApoE^{-/-} Mice (Chen Study 1, Ito Study 3)

Any direct comparison of CVD effects in humans and mice can be challenged on the basis of species differences and the severity of the response — that is, a modest functional

Specific Results That Contribute to Identifying Specific PM_{2.5} Components or Source Categories That Are Most Closely Associated with Health Effects

TIME-SERIES CVD HOSPITALIZATION — 64 MSAS (ITO STUDY 3)

- When significant risk estimates were combined across the cities, CVD hospitalizations were mostly associated with the following PM_{2.5} components in the cold season: NO₂, SO₂, CO, Cu, EC, Fe, OC, SO₄²⁻, Se, Si, V, and Zn (all components at 0-day lag, except V, which was associated at 1- and 3-day lags).
- The second-stage regression of risk estimates for CVD hospitalizations at 0-day lag PM_{2.5} indicated a significant positive influence of local combustion-derived pollutants: Cu, Ni, V, NO₂, Fe, and Na. Among the source categories derived from factor analysis, the Traffic category had the strongest association with CVD hospitalizations at 0-day lag in the cold season.

DAILY IHD HOSPITALIZATION BASED ON CSN COMPONENT ANALYSES OF DAILY DATA FOR DETROIT AND SEATTLE (ITO STUDY 3)

- Seattle, cold season: significant associations with PM_{2.5}, transition metals (Fe, Ni, V, and Zn), soil-related elements (Al and Si), traffic-related pollutants (EC and CO), and S. No associations in the warm season. No associations for dysrhythmia, heart failure, and cerebrovascular diseases in either season.
- Detroit, cold season: significant associations with PM_{2.5}, Al, K, Se, Si, and NO₂. As in Seattle, the excess risk was

much smaller for total CVD, primarily due to the lack of excess risks reported for dysrhythmia, heart failure, and cerebrovascular diseases. In the warm season: excess risks for IHD associated with Al, Si, and Se.

TIME-SERIES ALL-CAUSE MORTALITY — MULTICITY RESULTS (ITO STUDY 3)

- In the second-stage regression analysis, to explain the city-to-city variation in PM_{2.5} mortality risk estimates in 148 cities, only SO₄²⁻ showed statistically significant positive influence, but weekday excess PM_{2.5} (an indicator of local PM_{2.5} sources), Pb, and V were nearly significant positive predictors.
- In the 64-cities analysis, in which PM_{2.5}, NO₂, SO₂, and CO were analyzed using all the available days (i.e., not restricted to the days for which speciation data were available), the combined estimates across the cities for all of these pollutants were significant for all-year data. Associations for PM_{2.5} were seen for both 0- and 1-day lags. The corresponding results for CVD mortality showed similar patterns of associations, but fewer significant results.
- Among the source categories derived by factor analysis, the Traffic and Soil source categories showed significantly positive associations at 1-day lag in all-year data.

(Continued next page)

response in mice versus a clearly adverse health effect in humans. However, because we were able to detect functional responses in an animal model of atherosclerosis, we maintain that an integrated consideration of these two very different short-term responses to PM_{2.5} exposure is justified in identifying PM_{2.5} components responsible for short-term cardiovascular responses. Still, for other short-term human responses that we saw in our time-series analyses, such as hospital admissions for respiratory diseases and all-cause mortality, and because of sample-size limitations, we lacked any comparable and measurable clinical responses in the mice (see sidebar Specific Results).

The comparison we did make was between changes in cardiac function in mice and CVD hospitalizations in humans; however, we were limited to comparisons of

PM_{2.5} components that could possibly play a causal role. In this regard, we have shown that the PM_{2.5} components most closely associated with cardiac function changes in the mice at five different sites were Ni, EC, S, and V, and that multiple PM_{2.5} components were associated with CVD hospital admissions in 64 MSAs: Cu, EC, Fe, OC, SO₄²⁻, Se, Si, V, and Zn. In addition, for the cold season in Seattle, significant excess distributed lag risks for IHD hospital admissions were associated with Al, Fe, Ni, S, Si, V, Zn, and EC. The correspondence of components associated with effects in humans and mice was close, but not perfect (common to all three analyses were EC, S or SO₄²⁻, and V). The influences of the other components that showed up in these three analyses were likely a result of the different PM_{2.5} mixtures in these locations, the number of which

VARIATIONS IN DAILY MORTALITY RISK BASED ON DAILY COMPONENT CONCENTRATION ANALYSES OF PM_{2.5} EXPOSURES IN SEATTLE AND DETROIT (ITO STUDY 3)

- The pattern of associations between mortality and PM_{2.5} components in Seattle and Detroit varied by season; more responses were observed in the warm season in Detroit (for secondary aerosols) and in the cold season in Seattle (for primary PM_{2.5} components). In contrast, few, if any, associations were found in the cold season in Detroit or in the warm season in Seattle.
- Seattle, cold season: significant excess distributed lag risks for IHD deaths associated with PM_{2.5}, K, S, Si, Zn, EC, CO, and NO₂; and for all causes of death associated with PM_{2.5}, Al, K, Si, Zn, EC, CO, and NO₂. No association for respiratory mortality (likely due to small daily counts).
- Detroit, warm season: only PM_{2.5} and S showed significant associations for both all-cause and CVD mortality; significant negative association found between Si and all-cause mortality. No association for respiratory mortality (likely due to small daily counts).

SHORT-TERM CHANGES IN CARDIAC FUNCTION INDICES (HR, SDNN, AND RMSSD) IN APOE^{-/-} MICE EXPOSED TO CAPS (CHEN STUDY 1)

- Many more differences in cardiac function indices were observed between CAPS-exposed and control mice at Manhattan and Tuxedo, where the mean PM_{2.5} concentrations were 123 and 136 µg/m³, respectively, than at East Lansing, Seattle, and Irvine.

- Fewer differences were observed at Seattle and East Lansing, where the mean PM_{2.5} concentrations were much lower — 61 and 68 µg/m³, respectively. However, we also observed few differences at Irvine, where the mean PM_{2.5} concentration was 138 µg/m³, which was similar to the means at Manhattan and Tuxedo.
- Several elements had positive associations that were as strong as, or stronger than, those for PM_{2.5} ($r = 0.52$). For data from the five sites, the components with positive r values were Ni ($r = 0.98$); P ($r = 0.88$); Al ($r = 0.88$); EC ($r = 0.86$); S ($r = 0.80$); and V ($r = 0.60$). Some had positive r values that were less strong than that of PM_{2.5} mass at predicting adverse exposure-related effects: Mg ($r = 0.52$); Ca ($r = 0.42$); Ti ($r = 0.21$); Si ($r = 0.11$); and Cr ($r = 0.01$).
- Other PM_{2.5} components had negative associations, implying beneficial or virtually no effects: Zn ($r = -0.51$); K ($r = -0.40$); Se ($r = -0.40$); Mn ($r = -0.33$); OC ($r = -0.29$); Na ($r = -0.22$); Fe ($r = -0.17$); and Cu ($r = -0.09$).
- Several individual PM_{2.5} components were associated with many more functional changes at Manhattan than at Tuxedo: BC, Al, Mg, Na, Ni, P, and V; whereas OC, Cr, Cu, K, Mn, and Zn were associated with many more functional changes at Tuxedo than at Manhattan. The correlations among the concentrations of these various elements may account for their associations with effects more so than their own individual influence on the effects.

varied from 150 or 64 MSAs in the national time-series analysis, to five sites in the mouse inhalation studies, and to one season in one city in the daily time-series study in Seattle. Ni was not associated with CVD hospitalizations in the multicity analysis (Study 3, Table 4), but this may be explained by the expected large exposure error associated with Ni (Figure 16 in Study 3). In an analysis of CVD hospitalizations in New York City by Ito and colleagues (2011), Ni (but not V) was associated with CVD hospitalizations.

It seems reasonable to conclude that, for short-term CVD effects, (1) the components associated with the Residual Oil Combustion source category (Ni, V, S, and EC) were particularly influential; (2) other combustion source categories that generate EC and S, such as diesel-related Traffic, were also important; and (3) the presence of other PM_{2.5} components may have exacerbated the effects of the most influential components. In a previous study relating heart rate in COPD patients to coarse and fine PM components in New York City and Seattle, the only component that had a significant association with health effects was Ni, but only in the PM_{2.5} fraction in New York City, which has notably high concentrations of Ni, V, S, and EC (Hsu et al. 2011).

Associations Between Mortality and Long-Term Exposures to PM_{2.5}, Its Components, and Source Categories (Thurston Study 4)

- The major source categories and their key elements that we evaluated for their associations with excess long-term IHD mortality were as follows: Metals (Pb, Zn); Soil (Ca, Si); Traffic (OC, EC, NO₂); Steel Industry (Fe, Mn); Coal Combustion (As, Se); Residual Oil Combustion (V, Ni); and Salt (Na, Cl).
- Of the above, our mortality analyses of the CPS-II cohort data identified Coal Combustion, and to a lesser extent Traffic, as the source categories that were most closely associated with excess IHD mortality.
- Factor analysis and source apportionment confirmed that the source categories that contributed most to the PM mixture, by location, were Traffic in Southern California; Soil in the Southwest; Steel Industry in steelworks cities (e.g., Detroit, Michigan, and Birmingham, Alabama); Coal Combustion in the Ohio Valley region (e.g., Pittsburgh, Pennsylvania); and Residual Oil Combustion in New York City (where residual fuel oil is heavily used for heat in the winter) and in coastal cities with deep ports that accommodate oceangoing ships.
- The strongest associations for PM_{2.5}, elemental components, and source categories were found with IHD and lung cancer deaths, which is consistent with past analyses of the CPS-II cohort (e.g., Pope et al. 2002).
- Although PM_{2.5} from most combustion source categories (Traffic, Coal Combustion, and Residual Oil Combustion) was associated with hazard ratio estimates above 1.0 for IHD deaths, PM_{2.5} from the Coal Combustion category, its correlated tracers (As and Se), and S yielded larger and more statistically significant IHD risks per unit mass than did total PM_{2.5} mass.
- PM_{2.5} from the Traffic category and (especially) one of its associated tracers (EC) were also somewhat associated with IHD. However, it should be noted that the Traffic category's mean nationwide contribution to the PM_{2.5} mass concentration was much higher than that of the Coal Combustion category.
- The data also suggested that the association between the Traffic category and IHD mortality was most related to EC (and therefore diesel combustion emissions), whereas the association between Traffic and respiratory mortality may have been related more to OC (and therefore to gasoline combustion emissions).
- PM_{2.5} mass originating from the Soil source category (wind-blown soil) or from Biomass Combustion (e.g., wood burning) was generally not associated with increased risk of IHD mortality in this cohort, and other source categories were more equivocal in their associations across our nationwide models.
- Although long-term exposure to the Residual Oil Combustion source category was generally not associated with an increased mortality risk in our ACS CPS-II cohort analyses, this negative finding may have been due to the current paucity of Residual Oil Combustion sources in most of the United States. Residual oil is no longer of significant use as a fuel for electric power and process heat production, and the major sources in the United States are largely oceangoing ships in seaports and adjacent coastal waters. Note that when crude oil was burned to produce process steam for heavy-oil recovery at an oil refinery in Bakersfield, California (before 1990), annual IHD mortality was markedly increased in the local area (Cahill et al. 2011a).
- For lung cancer mortality, only the Coal Combustion source category, its associated tracer (Se), and S were significantly associated with increased risk of death.
- Associations between PM_{2.5} mass from specific source categories and health impacts were consistent with, but more easily interpreted than, the associations of health impacts with individual components, many of which came from a variety of source categories.
- The TRI analyses based on the source categories resulted in risk impacts that were generally higher

than the risk impacts based on PM_{2.5} mass alone. The TRIs that included the secondary aerosols (SO₄²⁻, NO₃⁻, and OC) tended to be somewhat higher than the directly comparable TRIs that excluded them. This provides evidence that secondary aerosols contribute to the associations between PM_{2.5} and mortality.

- The evidence was generally weak for any additional contribution to the PM_{2.5} mass association between any cause of death and any of the three gaseous pollutants examined (SO₂, NO₂, and O₃) in the TRI analyses.

Atherosclerotic Plaque Progression in ApoE^{-/-} Mice Exposed to CAPs (Chen Study 1)

Long-term changes in plaque volume in the BA of CAPs-exposed ApoE^{-/-} mice varied by site location. When compared with the filtered air-exposed control animals, significantly increased plaque progression was found after 6 months in mice at Manhattan (40% occlusion versus 32%), Tuxedo (34% occlusion versus 23%), and to a lesser extent East Lansing (28% occlusion versus 23%, where Ni concentrations were the lowest of the five sites). No difference in progression between CAPs- and filtered air-exposed mice was found at Seattle (where the mean SO₄²⁻ and PM_{2.5} mass concentrations were the lowest) or at Irvine (where SO₄²⁻ was next to lowest, but PM_{2.5} mass was highest).

Since the PM_{2.5} mass concentration in East Lansing was only about half of that in Irvine, and the contribution of the Traffic source category to local PM_{2.5} was much lower in East Lansing than in Irvine, it appears that the PM_{2.5} attributable to Coal Combustion was more influential on plaque volume progression in the mice than that from all other source categories, including Traffic and Residual Oil Combustion. However, our additional analysis of plaque surface area coverage by quantitative image analysis in mice after 6 months of exposure at Irvine found evidence of plaque area progression, but the extent of progression at 6 months was less than that for mice exposed at East Lansing.

Comparing Long-Term Human IHD Mortality with Aortic Plaque Progression in ApoE^{-/-} Mice (Thurston Study 4 and Chen Study 1)

For long-term exposures, any direct comparison of IHD mortality in human populations and plaque progression in mice can be challenged on the basis of species differences and the severity of response — that is, a structural blood vessel response in mice versus a clearly adverse health effect in humans. However, because the structural response was seen in an animal model of atherosclerosis and the long-term exposure risk of IHD mortality in humans was largely confined to humans with IHD, we think an integrated consideration of these two very different long-term

responses to PM_{2.5} exposure has some justification. The comparison that we draw between BA plaque volume progression in mice and IHD mortality in humans is confined to the comparison of possibly causal PM_{2.5} source categories associated with the responses.

The sites at which mice showed significantly more BA plaque volume progression after 6 months of CAPs exposure were the two New York sites (Manhattan and Tuxedo) and East Lansing, which are the locations most affected by the Coal Combustion source category. We showed that S was present at both Irvine and East Lansing (6.5 and 7.6 µg/m³, respectively); however, S was largely from Coal Combustion at East Lansing (where plaque progression was observed), but at Irvine (where no plaque progression was observed) it was attributable, to a much greater extent, to liquid fuel combustion in motor vehicles and marine transport. This suggests that S may serve as a surrogate marker for, or enhance the effects of, other correlated components in the Coal Combustion source category.

IMPLICATIONS OF FINDINGS

Implications for Further Research

Our findings support our hypothesis that specific PM_{2.5} components and source categories are more closely associated with the health effects that have been attributed to PM_{2.5} mass concentrations than are other components and source categories, and consideration of these components may provide evidence of larger collective risk effects than the use of nonspecific PM_{2.5} mass alone. This is particularly the case for IHD-related mortality and morbidity, which accounts for a major part of the overall health impact and the tabulated benefits of ambient air pollution control.

Our findings also support the hypothesis that results obtained in toxicologic studies of subchronic CAPs inhalation exposure of mice in diverse airsheds with a range of PM_{2.5} compositions can provide biological plausibility for the effects observed in humans exposed at current ambient concentrations in epidemiologic studies of multiple cities. Not only can such toxicologic and epidemiologic study results be integrated, but the results of a range of subchronic CAPs inhalation studies of rodent responses related to the nervous system (Veronesi et al. 2005; Sama et al. 2007), liver (Tan et al. 2009), hypertension (Sun et al. 2008), metabolic syndrome (Sun et al. 2009), cardiac remodeling (Ying et al. 2009), obesity and diabetes (Xu et al. 2010), and so on, can help guide the design of future epidemiologic studies, especially those exploring the health benefits of specific interventions to control sources.

Future epidemiologic studies will need a more complete PM speciation and gaseous pollutant database than has been available until now in order to identify the following: (1) the PM components that are most likely causally related to health outcomes, and (2) the mortality and morbidity incidence of illnesses other than CVD and respiratory disease. Ideally, future studies should investigate communities where PM has a broad range of components and where multiple years of PM speciation and gaseous pollutant data from collocated monitors are available.

Findings from our NPACT study, and from additional such studies, can also aid in the design of future rodent inhalation studies that are focused on (1) evaluating additional health endpoints, (2) defining better the roles of specific PM components and their interactions with gaseous air pollutants, and (3) evaluating exposure atmospheres that include specific PM components that are added to or subtracted from controlled PM mixtures. When more such information becomes available, it may become possible to build realistic multipollutant models that can account for the observed associations of exposures and effects in humans and animals. We currently lack adequate knowledge and understanding of the effects of complex mixtures and of the interactions among the components in the mixtures. Based on currently available knowledge on exposures and exposure–response relationships, we doubt that anyone can overcome the inherent limitations of a study involving multiple concurrent exposures, multiple effects endpoints, and observations in multiple airsheds with different components and temporal patterns of exposure, especially when both short-term variations in exposure and acute responses and long-term exposures and chronic effects are being evaluated.

Implications for NAAQS for Coarse and Ultrafine PM

Although our epidemiologic studies and our subchronic mouse inhalation study were limited to the effects of PM_{2.5} exposures, some of our short-term *in vitro* and *in vivo* results from Gordon Study 2 suggest that a NAAQS for coarse PM combined with a monitoring program devoted to acquiring coarse PM speciation data could provide a more robust knowledge base for future considerations of the PM NAAQS. Although we did not compare the toxicity of urban and rural coarse PM, an issue that has complicated previous consideration of a coarse PM NAAQS, our findings did demonstrate that coarse PM from different source categories, as would be found at urban sites, produced different degrees of toxicity. Some of our *in vitro* and *in vivo* results also suggest that a separate nationwide monitoring program for ultrafine PM might also be warranted, at least for initial research purposes.

Implications for Setting PM_{2.5} NAAQS and Control Strategies

The associations between PM_{2.5} components and source-category-related PM_{2.5} mixtures and health-related effects were shown to be very much dependent on specific components and on the chemical composition of the mixtures; there were also differences in which components and sources were related to short-term or long-term effects.

Short-Term Effects The regional summary estimates of PM_{2.5} effects on all-cause mortality in our multicity time-series analysis showed the strongest associations in the northeastern United States (Appendix Figure B.3, Study 3), which is consistent with earlier findings on regional variations in daily mortality attributable to PM₁₀ (Peng et al. 2005) and PM_{2.5} (Dominici et al. 2007b) in the NMMAPS studies. This regional pattern is also partly consistent with the results of our second-stage analysis, which showed mortality risk estimates associated with PM_{2.5} to be larger in the cities with high SO₄²⁻ and V (Figure 14, Study 3).

The regional summary estimates of PM_{2.5} effects on CVD hospitalizations were more consistent, both in terms of lag days and season, across regions, except for the Southeast and Southwest (Appendix Figure B.3, Study 3). This is also consistent with the finding that Traffic, the most prevalent source category across cities, was the only source category significantly associated with CVD hospitalizations when the risk estimates were combined across the cities (Study 3, Table 4). The second-stage analysis of PM_{2.5} risk estimates for CVD hospitalizations also found that Cu (which is correlated with the Traffic source category; see the nationwide factor analysis [Figure 2, Study 3]) as well as Ni and V were important predictors of this health outcome.

In our time-series analysis of cardiac function in ApoE^{-/-} mice at five sites (two in the Northeast, one in the Midwest, and two on the West Coast) (Chen Study 1), the geographic differences were most noticeable in the measures of cardiac function in the ApoE^{-/-} mice: The responses in the mice to local CAPs were much greater at both Manhattan and at Tuxedo, which is generally upwind of New York City, than they were in East Lansing, Seattle, and Irvine. Some common PM_{2.5} components were observed at both New York sites because both had relatively high concentrations of regional secondary aerosols transported over long ranges. That PM resulted from chemical reactions of gaseous precursors whose origins were fossil fuel combustion in upwind coal-fired power plants and traffic exhaust throughout the eastern megalopolis. Also, both Manhattan and Tuxedo had much higher concentrations of Ni and S than did any of the other locations, and Manhattan had high concentrations of EC and V as well. In a previous study relating heart rate in COPD

patients to coarse and fine PM components at Manhattan and Seattle, the only component significantly associated with heart rate was Ni, but this was only observed for the Ni fraction of fine PM at Manhattan (Hsu et al. 2011).

Our time-series study of human populations in 64 cities (Ito Study 3) showed that adverse acute health effects related to PM_{2.5} exposure were occurring in cities that did not exceed, or barely exceeded, the current 24-hour PM_{2.5} NAAQS of 35 µg/m³. This emphasizes the need for a significantly more stringent 24-hour NAAQS if it is to be based on PM_{2.5} mass concentration. Furthermore, our additional time-series study based on daily measurements in Seattle and Detroit found that adverse short-term effects were occurring in Seattle, a city that would meet even the most stringent concentration that was considered for the 2012 revision of the 24-hour PM_{2.5} NAAQS — 25 µg/m³.

S, which is present in ambient air as sulfate ion, is unlikely to have any inherent toxicity in isolation, but it originates from the S content within coal, residual oil, and motor vehicle fuels and may interact with, and may enhance the effects of, other PM_{2.5} components, and thereby may show associations with health outcomes (Committee on the Medical Effects of Air Pollution 2009). OC, which is inconsistently associated with effects, is a very broad category of organic compounds. As demonstrated by Delfino and colleagues (2008, 2009), some primary OC species are likely to be toxic, whereas many secondary OC species have little or no acute toxicity. Furthermore, Delfino and colleagues found that total OC, as measured in the CSN, has little predictive power with respect to short-term health effects.

Although this NPACT study could not determine the extent to which individual PM_{2.5} components contributed causally to adverse short-term health effects, we did show that some were much more strongly associated than were others. Among those most strongly associated with short-term CVD effects in both humans and mice were Ni, V, Cu, EC, and S. Total OC, as well as Al, As, Ca, Fe, K, P, Se, Si, Ti, and Zn, however, were less consistently associated with such effects.

Our findings differed somewhat from those reported by Suh and colleagues (2011) for patients in Atlanta, Georgia, where hospital admissions for IHD, congestive heart failure, and atrial fibrillation were significantly associated with a group of transition metals (Cu, Mn, Zn, Ti, and Fe). By contrast, their microcrystalline oxide group (As, Br, Se, Pb, and Si) was associated with decreased CVD-related hospital admissions in their study. The differences between our NPACT study findings and those of Suh and associates (2011) may be evidence that — for at least some components — associations were likely due to the close correlation between the measured component's airborne concentrations and the concentrations of other components that may

be more causally related to health outcomes and come from the same pollutant sources. Both of these studies showed associations between Cu and CVD admissions for Medicare patients. However, Suh and colleagues didn't show associations with Ni and V (as did our study), which were not present at elevated concentrations in Atlanta. For both the NPACT and the Suh studies, CVD effects did not increase consistently with increases in As, Se, Pb, OC, and Si, which have often served as markers of the Coal Combustion and Traffic source categories.

Long-Term Effects Some IHD mortality outcomes were significantly associated with the long-term mean concentrations of PM_{2.5} mass in the 100 MSAs in which participants in the CPS-II cohort reside and in which CSN data were available (Thurston Study 4). Most of those cities did not exceed the current annual PM_{2.5} NAAQS concentration limit of 15 µg/m³. Our present analyses showed that the risks were primarily attributable to the PM_{2.5} related to the Coal Combustion source category and to a component related to the Traffic source category (EC). The exposure to PM_{2.5} derived from Coal Combustion was largely confined to the eastern half of the United States, whereas exposure to high concentrations of Traffic-derived PM_{2.5} is largely limited to residents of the largest cities. Since little of the mortality associated with long-term exposure was attributable to other nationwide PM_{2.5} source categories, the benefits of the more stringent annual PM_{2.5} NAAQS that was adopted in 2012 (12 µg/m³) may be optimized if the relative toxicity of PM_{2.5} mass sources can be considered in its implementation. Air pollution controls focused on emissions from coal-combustion and traffic-related sources and — in coastal regions affected by emissions from marine transport — on residual oil may be the most effective in reducing adverse chronic health effects.

Although our research could not define the extent to which individual PM_{2.5} components each contributed causally to adverse long-term health effects, it did show that the Coal Combustion source category was much more strongly associated with long-term effects (mortality in humans and aortic plaque progression in mice) than any of the other source categories, and it appeared to have a higher toxicity per unit mass than did other components for these chronic endpoints.

Need for a More Comprehensive Air Quality Monitoring Program

Our NPACT research has clearly demonstrated that (1) concentrations of PM mass in ambient air provide relatively crude indices of health risks, and that the risks associated with them vary considerably with particle size range, chemical composition, and season; (2) the PM components that are most closely associated with acute effects

differ from those that are most closely associated with chronic effects; and (3) the current CSN monitoring program has been of value for establishing these conclusions, but is inadequate for determining which specific PM components are causally related to either acute or chronic effects.

The limitations of the CSN monitoring network include the following: (1) too few monitoring sites for adequate characterization of the spatial distribution of PM components; (2) data collected with too little temporal resolution (measurements are currently limited to 24-hour concentrations every third or sixth day); (3) no detailed speciation of OC components; (4) no measurements for biogenic components; and (5) insufficient numbers of speciation sites with collocated monitors for gaseous air pollutants.

A more consistently comprehensive multipollutant monitoring network would make it possible to determine whether some of the associations of effects with PM components are causal, or are likely to be the result of associations with components whose concentrations are closely correlated with the monitored species. The data generated by such an enhanced CSN monitoring network would enable the EPA to establish better-targeted PM NAAQS and control strategies that are more clearly targeted at reducing the burden of adverse health effects.

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APPENDIX A. HEI Quality Assurance Statement

The conduct of this research project was subject to independent quality assurance (QA) oversight by Abt Associates. The audit team consisted of Dr. Sue Greco, who has more than 10 years of experience in both human health risk assessment and fine PM exposure assessment, and Mr. Jose Vallarino, who has overseen QA programs for the last 15 years. The QA oversight consisted of four on-site audits, a data regeneration exercise, and a review of the Investigators' Final Report. The dates of the QA oversight activities are listed below with the phase of the study examined.

November 3–5, 2008. Initial audit conducted on-site at the NYU Institute of Environmental Medicine. This technical systems audit was intended to review the standard operating procedures and data management practices used in the research to ensure that these procedures were followed. We were unable to observe the particle concentration mechanism in operation for Study 1.

April 8, 2009. Interim Audit conducted on-site in Seattle, WA. The auditors conducted an audit in Seattle, Washington to observe the particle concentrator in operation. This was one of the five sites where concentrated air pollutants

were being collected and animal exposures were carried out.

September 19–20, 2011. Data regeneration exercise and audit of Draft Final Report on-site at the NYU Institute of Environmental Medicine. To audit the August 23, 2011, Investigators' Draft Final Report that the researchers submitted to HEI, we selected tables and figures from the report and asked the investigators to present how they were generated starting from raw data. We also asked about data quality and management. As part of the Final Report Audit, for a portion of the concentration data we requested the raw study data, written instructions on how these data were processed, and the final calculated data. We were able to regenerate this portion of the final data, starting from the raw data and using the researchers' instructions. No issues impacting data quality were identified in the final datasets. At this time, we were not able to review the standard Cox and random effects survival models because they were processed at the University of Ottawa.

November 12, 2012. Audit of survival model data on-site at the University of Ottawa. This audit was conducted to corroborate all steps by which data were modified for Study 4 led by Professor George D. Thurston of NYU.

November 2012. Review of Revised Final Report. We reviewed the Investigators' Revised Final Report dated July 24, 2012, to determine if our recommendations on the Draft Final Report had been followed.

Overall, we found the researchers to be well-organized and cooperative during the audits. The study procedures, analysis steps, and data storage were systematic, consistent, and well-designed to manage the various and complex data and analytical streams necessary to complete the study.



Sue Greco, Sc.D.



Jose Vallarino, M.Sc.

APPENDIX B. Ito Study 3. Additional Results and Supporting Information

Table B.1. All 150 MSAs and the Subset of 64 MSAs with City Characteristics

City Number	MSA Name ^a	MSA Code (Region) ^b	Population (2000)	Housing (units)	Area (sq. miles)	Population Density (people per sq. mi.)
1	Akron, OH	80 (IM)	542,899	230,880	413	1,315
2	Albany–Schenectady–Troy, NY	160 (NE)	875,583	386,262	3,222	272
3	Albuquerque, NM	200 (SW)	712,738	298,583	5,943	120
4	Allentown–Bethlehem–Easton, PA	240 (NE)	579,156	235,620	720	804
5	Anchorage, AK	380 (OT)	260,283	100,368	1,697	153
6	Ann Arbor, MI	440 (IM)	479,846	189,988	1,278	375
7	Asheville, NC	480 (SE)	225,965	103,695	1,105	204
8	Athens, GA	500 (SE)	153,444	62,174	590	260
9	Atlanta, GA	520 (SE)	4,066,054	1,572,264	5,962	682
10	Augusta–Aiken, GA–SC	600 (SE)	388,153	162,438	2,158	180
11	Bakersfield, CA	680 (SC)	661,645	231,564	8,141	81
12	Baltimore, MD	720 (NE)	2,552,994	1,048,046	2,609	979
13	Baton Rouge, LA	760 (SE)	526,267	213,655	1,295	407
14	Beaumont–Port Arthur, TX	840 (SE)	337,017	136,861	1,260	268
15	Biloxi–Gulfport–Pascagoula, MS	920 (SE)	321,021	131,314	1,308	246
16	Birmingham, AL	1000 (SE)	870,082	374,767	2,541	342
17	Bismarck, ND	1010 (UM)	94,719	39,590	3,559	27
18	Boise City, ID	1080 (NW)	432,345	166,481	1,645	263
19	Boston, MA–NH	1120 (NE)	4,306,655	1,717,061	4,150	1,038
20	Bridgeport, CT	1160 (NE)	1,706,575	680,198	1,231	1,386
21	Buffalo–Niagara Falls, NY	1280 (IM)	1,170,111	511,583	1,567	747
22	Burlington, VT	1305 (NE)	198,889	82,718	1,259	158
23	Canton–Massillon, OH	1320 (IM)	378,098	157,024	576	656
24	Cedar Rapids, IA	1360 (UM)	191,701	80,551	717	267
25	Charleston–North Charleston, SC	1440 (SE)	406,382	178,268	1,493	272
26	Charleston, WV	1480 (IM)	251,662	115,409	1,249	201
27	Charlotte–Gastonia–Rock Hill, NC–SC	1520 (SE)	1,368,230	563,075	3,012	454
28	Chattanooga, TN–GA	1560 (SE)	411,879	178,633	1,661	248
29	Chicago, IL	1600 (IM)	8,272,768	3,132,638	5,062	1,634
30	Chico–Paradise, CA	1620 (NW)	203,171	85,523	1,639	124
31	Cincinnati, OH–KY–IN	1640 (IM)	1,600,286	673,172	3,037	527
32	Clarksville–Hopkinsville, TN–KY	1660 (SE)	134,768	52,167	539	250
33	Cleveland–Lorain–Elyria, OH	1680 (IM)	2,148,143	911,356	2,004	1,072
34	Colorado Springs, CO	1720 (NW)	516,929	202,428	2,126	243
35	Columbia, SC	1760 (SE)	320,677	129,793	756	424
36	Columbus, GA–AL	1800 (SE)	274,624	112,617	1,570	175

Table continues next page^a Cities in **bold** are part of the 64-city subset.^b Regions: IM: Industrial Midwest; NE: Northeast; NW: Northwest; SW: Southwest; OT: Others; SE: Southeast; SC: Southern California; UM: Upper Midwest.

Table B.1 (Continued). All 150 MSAs and the Subset of 64 MSAs with City Characteristics

City Number	MSA Name ^a	MSA Code (Region) ^b	Population (2000)	Housing (units)	Area (sq. miles)	Population Density (people per sq. mi.)
37	Columbus, OH	1840 (IM)	1,430,168	610,693	2,699	530
38	Corpus Christi, TX	1880 (SW)	380,783	147,905	1,527	249
39	Dallas, TX	1920 (SE)	3,027,501	1,171,168	5,338	567
40	Davenport–Moline–Rock Island, IA–IL	1960 (IM)	308,042	130,138	885	348
41	Dayton–Springfield, OH	2000 (IM)	805,816	347,221	1,284	628
42	Decatur, AL	2030 (SE)	111,064	47,388	582	191
43	Decatur, IL	2040 (IM)	114,706	50,241	581	198
44	Denver, CO	2080 (NW)	2,109,282	856,685	3,761	561
45	Des Moines, IA	2120 (UM)	415,272	171,736	1,141	364
46	Detroit, MI	2160 (IM)	4,353,647	1,762,005	3,243	1,343
47	Dover, DE	2190 (NE)	126,697	50,481	590	215
48	El Paso, TX	2320 (SW)	679,622	224,447	1,013	671
49	Elkhart–Goshen, IN	2330 (IM)	182,791	69,791	464	394
50	Erie, PA	2360 (IM)	280,843	114,322	802	350
51	Eugene–Springfield, OR	2400 (NW)	322,959	138,946	4,554	71
52	Evansville–Henderson, IN–KY	2440 (IM)	269,134	116,312	1,059	254
53	Fargo–Moorhead, ND–MN	2520 (UM)	123,138	53,790	1,765	70
54	Fayetteville, NC	2560 (SE)	302,963	118,425	653	464
55	Fort Lauderdale, FL	2680 (SE)	1,623,018	741,043	1,205	1,347
56	Fort Wayne, IN	2760 (IM)	468,516	193,833	2,108	222
57	Fresno, CA	2840 (SC)	922,516	311,154	8,099	114
58	Galveston–Texas City, TX	2920 (SE)	250,158	111,733	398	628
59	Gary, IN	2960 (IM)	631,362	252,608	915	690
60	Grand Junction, CO	2995 (NW)	116,255	48,427	3,328	35
61	Grand Rapids–Muskegon–Holland, MI	3000 (IM)	1,088,514	422,704	2,758	395
62	Greeley, CO	3060 (NW)	180,936	66,194	3,992	45
63	Greensboro–Winston-Salem–High Point, NC	3120 (SE)	1,120,709	480,374	3,451	325
64	Greenville–Spartanburg–Anderson, SC	3160 (SE)	796,701	338,189	2,491	320
65	Hamilton–Middletown, OH	3200 (IM)	332,807	129,793	467	712
66	Harrisburg–Lebanon–Carlisle, PA	3240 (NE)	415,727	179,394	1,441	289
67	Hattiesburg, MS	3285 (SE)	111,674	45,346	964	116
68	Hickory–Morganton–Lenoir, NC	3290 (SE)	308,248	130,776	1,378	224
69	Honolulu, HI	3320 (OT)	876,156	315,988	600	1,461
70	Houston, TX	3360 (SE)	4,151,615	1,565,205	5,321	780
71	Huntington–Ashland, WV–KY–OH	3400 (IM)	315,538	141,398	2,159	146
72	Huntsville, AL	3440 (SE)	276,700	120,288	805	344

Table continues next page

^a Cities in **bold** are part of the 64-city subset.

^b Regions: IM: Industrial Midwest; NE: Northeast; NW: Northwest; SW: Southwest; OT: Others; SE: Southeast; SC: Southern California; UM: Upper Midwest.

Table B.1 (Continued). All 150 MSAs and the Subset of 64 MSAs with City Characteristics

City Number	MSA Name ^a	MSA Code (Region) ^b	Population (2000)	Housing (units)	Area (sq. miles)	Population Density (people per sq. mi.)
73	Indianapolis, IN	3480 (IM)	1,561,379	663,215	3,100	504
74	Jackson, MS	3560 (SE)	440,801	174,138	2,361	187
75	Johnson City–Kingsport–Bristol, TN–VA	3660 (SE)	423,349	192,270	2,524	168
76	Kalamazoo–Battle Creek, MI	3720 (IM)	314,866	133,225	1,173	269
77	Kansas City, MO–KS	3760 (UM)	1,324,976	559,272	4,929	269
78	Kenosha, WI	3800 (IM)	149,577	59,989	273	548
79	Knoxville, TN	3840 (SE)	615,919	280,943	2,112	292
80	Lancaster, PA	4000 (NE)	470,658	179,990	949	496
81	Las Vegas, NV–AZ	4120 (SW)	1,408,250	575,733	26,057	54
82	Lexington, KY	4280 (IM)	459,838	196,508	1,628	282
83	Little Rock–North Little Rock, AR	4400 (SE)	497,831	215,709	2,260	220
84	Longview–Marshall, TX	4420 (SE)	97,401	41,201	1,486	66
85	Los Angeles–Long Beach, CA	4480 (SC)	9,519,338	3,270,909	4,061	2,344
86	Louisville, KY–IN	4520 (IM)	929,126	397,059	1,697	548
87	Lowell, MA–NH	4560 (NE)	380,841	149,961	876	435
88	Lubbock, TX	4600 (SW)	242,628	100,595	899	270
89	Macon, GA	4680 (SE)	322,549	134,359	1,532	211
90	Medford–Ashland, OR	4890 (NW)	181,269	75,737	2,785	65
91	Memphis, TN–AR–MS	4920 (SE)	1,084,748	434,027	2,396	453
92	Miami, FL	5000 (SE)	2,253,362	852,278	1,946	1,158
93	Middlesex–Somerset–Hunterdon, NJ	5015 (NE)	1,047,652	385,660	614	1,705
94	Milwaukee–Waukesha, WI	5080 (IM)	1,500,741	618,244	1,460	1,028
95	Minneapolis–St. Paul, MN–WI	5120 (UM)	2,670,722	1,061,684	5,639	474
96	Missoula, MT	5140 (NW)	95,802	41,319	2,598	37
97	Mobile, AL	5160 (SE)	399,843	165,101	1,233	324
98	Modesto, CA	5170 (NW)	446,997	150,807	1,494	299
99	Montgomery, AL	5240 (SE)	289,384	121,170	1,411	205
100	Nashville, TN	5360 (SE)	1,195,399	495,785	3,770	317
101	New York, NY	5600 (NE)	9,314,235	3,680,360	1,142	8,159
102	Newark, NJ	5640 (NE)	1,239,356	465,009	1,451	854
103	Oklahoma City, OK	5880 (SW)	995,649	432,261	3,347	298
104	Omaha, NE–IA	5920 (UM)	629,294	255,240	1,521	414
105	Owensboro, KY	5990 (IM)	91,545	38,432	462	198
106	Pensacola, FL	6080 (SE)	412,153	173,766	1,679	245
107	Philadelphia, PA–NJ	6160 (NE)	4,677,537	1,886,532	3,051	1,533
108	Phoenix–Mesa, AZ	6200 (SW)	3,251,876	1,331,385	14,573	223

Table continues next page^a Cities in **bold** are part of the 64-city subset.^b Regions: IM: Industrial Midwest; NE: Northeast; NW: Northwest; SW: Southwest; OT: Others; SE: Southeast; SC: Southern California; UM: Upper Midwest.

Table B.1 (Continued). All 150 MSAs and the Subset of 64 MSAs with City Characteristics

City Number	MSA Name ^a	MSA Code (Region) ^b	Population (2000)	Housing (units)	Area (sq. miles)	Population Density (people per sq. mi.)
109	Pittsburgh, PA	6280 (IM)	2,358,695	1,046,094	4,626	510
110	Portland–Vancouver, OR–WA	6440 (NW)	1,579,618	649,346	3,159	500
111	Providence–Fall River–Warwick, RI–MA	6480 (NE)	924,773	383,021	712	1,299
112	Provo–Orem, UT	6520 (NW)	368,536	104,315	1,998	184
113	Raleigh–Durham–Chapel Hill, NC	6640 (SE)	1,138,612	474,254	2,806	406
114	Reading, PA	6680 (NE)	373,638	150,222	859	435
115	Reno, NV	6720 (NW)	339,486	143,908	6,342	54
116	Richmond–Petersburg, VA	6760 (NE)	989,586	407,499	2,762	358
117	Riverside–San Bernardino, CA	6780 (SC)	3,254,821	1,186,043	27,260	119
118	Roanoke, VA	6800 (IM)	205,436	91,781	308	666
119	Rochester, MN	6820 (UM)	124,277	49,422	653	190
120	Rochester, NY	6840 (NE)	1,037,831	427,172	2,931	354
121	Sacramento, CA	6920 (NW)	1,471,898	582,116	2,370	621
123	Salt Lake City–Ogden, UT	7160 (NW)	2,590,876	1,089,960	6,660	389
124	San Diego, CA	7320 (SC)	1,333,914	455,556	1,617	825
125	San Jose, CA	7400 (NW)	2,813,833	1,040,149	4,200	670
126	Savannah, GA	7520 (SE)	1,682,585	579,329	1,291	1,304
127	Scranton–Wilkes-Barre–Hazleton, PA	7560 (NE)	269,583	113,852	918	294
128	Seattle–Bellevue–Everett, WA	7600 (NW)	560,625	252,761	1,747	321
129	Shreveport–Bossier City, LA	7680 (SE)	2,343,058	978,442	4,215	556
130	Sioux Falls, SD	7760 (UM)	392,302	167,573	2,316	169
131	South Bend, IN	7800 (IM)	148,281	60,237	810	183
132	Spokane, WA	7840 (NW)	265,559	107,013	457	581
133	Springfield, MA	8000 (NE)	417,939	175,005	1,764	237
122	St. Louis, MO–IL	7040 (IM)	608,479	244,520	1,147	530
134	State College, PA	8050 (NE)	135,758	53,161	1,108	123
135	Steubenville–Weirton, OH–WV	8080 (IM)	132,008	59,169	581	227
136	Tacoma, WA	8200 (NW)	700,820	277,060	1,679	417
137	Tallahassee, FL	8240 (SE)	239,452	103,974	667	359
138	Tampa–St. Petersburg–Clearwater, FL	8280 (SE)	2,265,195	1,081,252	2,076	1,091
139	Toledo, OH	8400 (IM)	576,119	243,727	958	602
140	Tucson, AZ	8520 (SW)	843,746	366,737	9,186	92
141	Tulsa, OK	8560 (SE)	735,868	313,429	4,059	181
142	Ventura, CA	8735 (SC)	753,197	251,712	1,845	408
143	Visalia–Tulare–Porterville, CA	8780 (SC)	368,021	119,639	4,824	76
144	Washington, DC–MD–VA–WV	8840 (NE)	4,923,153	1,942,641	6,509	756
145	Wheeling, WV–OH	9000 (IM)	82,946	37,980	413	201
146	Wichita, KS	9040 (UM)	485,738	204,511	1,539	316
147	Wilmington–Newark, DE–MD	9160 (NE)	586,216	233,982	774	757
148	Yakima, WA	9260 (NW)	222,581	79,174	4,296	52
149	York, PA	9280 (NE)	381,751	156,720	904	422
150	Youngstown–Warren, OH	9320 (IM)	482,671	206,879	1,032	468

^a Cities in **bold** are part of the 64-city subset.

^b Regions: IM: Industrial Midwest; NE: Northeast; NW: Northwest; SW: Southwest; OT: Others; SE: Southeast; SC: Southern California; UM: Upper Midwest.

Table B.2. Mean Daily Death Counts by City and Cause, 2001–2006

City	All Causes (\pm SD)	Cardiovascular (\pm SD)	Respiratory (\pm SD)
Akron, OH	12.4 \pm 3.7	4.6 \pm 2.2	1.5 \pm 1.3
Albany, NY	20.2 \pm 4.9	8.4 \pm 3.0	2.1 \pm 1.6
Albuquerque, NM	13.5 \pm 3.9	4.5 \pm 2.1	1.5 \pm 1.3
Allentown, PA	14.1 \pm 4.0	5.4 \pm 2.4	1.3 \pm 1.2
Anchorage, AK	2.7 \pm 1.6	0.9 \pm 0.9	0.2 \pm 0.5
Ann Arbor, MI	6.5 \pm 2.7	2.4 \pm 1.5	0.6 \pm 0.8
Asheville, NC	5.8 \pm 2.4	2.1 \pm 1.5	0.7 \pm 0.8
Athens, GA	2.3 \pm 1.6	0.9 \pm 0.9	0.2 \pm 0.5
Atlanta, GA	63.0 \pm 9.5	22.3 \pm 5.2	6.1 \pm 2.8
Augusta, GA	9.2 \pm 3.1	3.2 \pm 1.8	1.0 \pm 1.0
Bakersfield, CA	11.9 \pm 3.6	5.0 \pm 2.3	1.5 \pm 1.3
Baltimore, MD	56.7 \pm 8.9	21.0 \pm 5.1	5.4 \pm 2.5
Baton Rouge, LA	10.3 \pm 3.4	3.8 \pm 2.0	0.9 \pm 1.0
Beaumont, TX	7.8 \pm 3.0	3.3 \pm 1.9	0.8 \pm 0.9
Biloxi, MS	6.8 \pm 2.8	2.8 \pm 1.7	0.7 \pm 0.8
Birmingham, AL	21.9 \pm 5.0	8.0 \pm 3.0	2.2 \pm 1.5
Bismarck, ND	1.8 \pm 1.4	0.7 \pm 0.8	0.2 \pm 0.4
Boston, MA	86.3 \pm 12.5	29.8 \pm 6.7	9.9 \pm 3.8
Bridgeport, CT	35.7 \pm 6.9	13.5 \pm 4.1	3.8 \pm 2.1
Buffalo, NY	31.0 \pm 6.3	12.6 \pm 3.9	3.0 \pm 1.9
Burlington, VT	3.3 \pm 1.9	1.2 \pm 1.1	0.3 \pm 0.6
Canton, OH	9.2 \pm 3.3	3.6 \pm 2.0	1.1 \pm 1.1
Cedar Rapids, IA	3.6 \pm 2.0	1.2 \pm 1.1	0.5 \pm 0.7
Charleston, SC	8.5 \pm 3.0	3.1 \pm 1.8	0.8 \pm 0.9
Charleston, WV	6.9 \pm 2.6	2.5 \pm 1.6	0.8 \pm 0.8
Charlotte, NC	25.6 \pm 5.5	9.0 \pm 3.1	2.7 \pm 1.7
Chattanooga, TN	10.0 \pm 3.2	3.8 \pm 1.9	1.1 \pm 1.0
Chicago, IL	153.5 \pm 17.1	59.4 \pm 9.8	14.0 \pm 4.4
Chico, CA	5.3 \pm 2.4	2.2 \pm 1.5	0.7 \pm 0.8
Cincinnati, OH	34.2 \pm 6.5	12.1 \pm 3.6	3.6 \pm 2.0
Clarksville, TN	1.8 \pm 1.3	0.7 \pm 0.8	0.2 \pm 0.5
Cleveland, OH	53.3 \pm 8.4	22.0 \pm 5.4	4.5 \pm 2.2
Colorado Springs, CO	7.8 \pm 2.8	2.6 \pm 1.6	0.9 \pm 0.9
Columbia, SC	6.0 \pm 2.5	2.0 \pm 1.4	0.5 \pm 0.7
Columbus, GA	6.2 \pm 2.6	2.3 \pm 1.5	0.6 \pm 0.8
Columbus, OH	28.2 \pm 5.9	9.9 \pm 3.4	3.0 \pm 1.8
Corpus Christi, TX	7.1 \pm 2.7	2.4 \pm 1.6	0.7 \pm 0.8
Dallas, TX	44.9 \pm 7.7	17.1 \pm 4.5	4.3 \pm 2.3
Davenport, IA	6.8 \pm 2.7	2.7 \pm 1.7	0.8 \pm 0.9
Dayton, OH	18.2 \pm 4.5	6.8 \pm 2.8	1.8 \pm 1.4
Decatur, AL	2.2 \pm 1.5	0.8 \pm 0.9	0.3 \pm 0.5
Decatur, IL	2.8 \pm 1.7	1.2 \pm 1.1	0.3 \pm 0.5
Denver, CO	32.3 \pm 6.0	11.0 \pm 3.5	3.8 \pm 2.1
Des Moines, IA	7.8 \pm 2.8	2.9 \pm 1.7	1.0 \pm 1.0

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Table B.2 (Continued). Mean Daily Death Counts by City and Cause, 2001–2006

City	All Causes (± SD)	Cardiovascular (± SD)	Respiratory (± SD)
Detroit, MI	92.7 ± 11.7	39.5 ± 7.3	8.0 ± 3.1
Dover, DE	2.3 ± 1.5	0.9 ± 1.0	0.2 ± 0.5
El Paso, TX	10.6 ± 3.5	3.7 ± 1.9	0.9 ± 1.0
Elkhart, IN	3.3 ± 1.8	1.3 ± 1.2	0.4 ± 0.6
Erie, PA	6.7 ± 2.7	2.6 ± 1.6	0.6 ± 0.8
Eugene, OR	7.2 ± 2.7	2.4 ± 1.5	0.8 ± 0.9
Evansville, IN	6.8 ± 2.7	2.6 ± 1.6	0.8 ± 0.9
Fargo, ND	1.9 ± 1.4	0.7 ± 0.9	0.2 ± 0.5
Fayetteville, NC	4.6 ± 2.2	1.7 ± 1.3	0.5 ± 0.7
Fort Lauderdale, FL	35.7 ± 6.4	15.3 ± 4.2	2.9 ± 1.7
Fort Wayne, IN	9.5 ± 3.2	3.6 ± 1.9	1.0 ± 1.0
Fresno, CA	16.1 ± 4.2	6.5 ± 2.7	1.8 ± 1.4
Galveston, TX	4.4 ± 2.1	1.6 ± 1.2	0.4 ± 0.6
Gary, IN	14.1 ± 4.0	5.5 ± 2.4	1.2 ± 1.1
Grand Junction, CO	3.0 ± 1.8	1.1 ± 1.0	0.4 ± 0.7
Grand Rapids, MI	19.4 ± 4.6	7.3 ± 2.7	2.1 ± 1.5
Greeley, CO	2.5 ± 1.6	0.9 ± 1.0	0.3 ± 0.6
Greensboro, NC	24.4 ± 5.4	8.5 ± 3.0	2.7 ± 1.7
Greenville, SC	17.6 ± 4.6	6.3 ± 2.7	2.0 ± 1.4
Hamilton, OH	5.8 ± 2.5	2.0 ± 1.4	0.8 ± 0.9
Harrisburg, PA	9.4 ± 3.2	3.7 ± 1.9	0.9 ± 1.0
Hattiesburg, MS	2.4 ± 1.6	1.0 ± 1.0	0.3 ± 0.5
Hickory, NC	6.7 ± 2.6	2.4 ± 1.6	0.8 ± 0.9
Houston, TX	61.1 ± 8.7	23.0 ± 5.3	5.0 ± 2.4
Huntington, WV	8.6 ± 3.0	3.2 ± 1.9	1.0 ± 1.0
Huntsville, AL	5.5 ± 2.4	2.0 ± 1.4	0.6 ± 0.8
Indianapolis, IN	32.6 ± 6.4	12.0 ± 3.7	3.8 ± 2.1
Jackson, MS	9.5 ± 3.1	3.9 ± 2.0	0.9 ± 0.9
Johnson City, TN	12.0 ± 3.7	4.5 ± 2.1	1.4 ± 1.2
Kalamazoo, MI	6.0 ± 2.5	2.2 ± 1.5	0.6 ± 0.8
Kansas City, MO	28.1 ± 5.8	10.4 ± 3.4	3.0 ± 1.9
Kenosha, WI	2.7 ± 1.6	1.1 ± 1.0	0.3 ± 0.6
Knoxville, TN	14.5 ± 3.9	5.4 ± 2.4	1.6 ± 1.4
Lancaster, PA	10.5 ± 3.4	4.1 ± 2.1	1.1 ± 1.1
Las Vegas, NV	30.1 ± 6.0	11.1 ± 3.6	3.4 ± 1.9
Lexington, KY	8.7 ± 3.1	3.2 ± 1.8	0.9 ± 1.0
Little Rock, AR	11.0 ± 3.5	4.0 ± 2.1	1.1 ± 1.0
Longview, TX	1.4 ± 1.2	0.6 ± 0.8	0.1 ± 0.4
Los Angeles, CA	146.4 ± 17.6	63.6 ± 10.6	14.9 ± 4.8
Louisville, KY	21.8 ± 5.2	8.1 ± 3.0	2.5 ± 1.6
Lowell, MA	6.4 ± 2.6	2.4 ± 1.6	0.7 ± 0.9
Lubbock, TX	4.8 ± 2.3	1.7 ± 1.3	0.6 ± 0.8
Macon, GA	7.0 ± 2.7	2.7 ± 1.7	0.7 ± 0.9
Medford, OR	4.7 ± 2.2	1.6 ± 1.3	0.5 ± 0.7

Table continues next page

Table B.2 (Continued). Mean Daily Death Counts by City and Cause, 2001–2006

City	All Causes (± SD)	Cardiovascular (± SD)	Respiratory (± SD)
Memphis, TN	23.2 ± 5.3	9.4 ± 3.3	2.0 ± 1.5
Miami, FL	45.4 ± 7.2	19.4 ± 4.8	3.7 ± 1.9
Middlesex, NJ	15.6 ± 4.2	5.9 ± 2.6	1.4 ± 1.2
Milwaukee, WI	32.0 ± 6.2	12.0 ± 3.8	3.1 ± 1.8
Minneapolis, MN	42.3 ± 7.6	12.8 ± 3.9	4.0 ± 2.2
Missoula, MT	1.6 ± 1.3	0.5 ± 0.7	0.2 ± 0.5
Mobile, AL	9.4 ± 3.1	3.8 ± 2.0	0.8 ± 0.9
Modesto, CA	8.4 ± 3.0	3.7 ± 2.0	1.0 ± 1.0
Montgomery, AL	6.3 ± 2.6	2.3 ± 1.5	0.6 ± 0.8
Nashville, TN	24.1 ± 5.3	9.3 ± 3.2	2.7 ± 1.7
New York, NY	166.7 ± 19.6	80.1 ± 12.5	14.5 ± 4.7
Newark, NJ	21.0 ± 5.0	8.2 ± 3.1	2.0 ± 1.5
Oklahoma City, OK	22.3 ± 5.1	9.2 ± 3.2	2.4 ± 1.7
Omaha, NE	11.3 ± 3.5	3.9 ± 2.0	1.3 ± 1.2
Owensboro, KY	2.1 ± 1.5	0.8 ± 0.9	0.2 ± 0.4
Pensacola, FL	9.3 ± 3.1	3.5 ± 1.9	0.9 ± 1.0
Philadelphia, PA	111.2 ± 15.0	41.6 ± 8.0	10.8 ± 4.0
Phoenix, AZ	61.9 ± 9.9	22.4 ± 5.1	7.1 ± 3.2
Pittsburgh, PA	69.1 ± 10.5	27.4 ± 6.2	6.8 ± 2.9
Portland, OR	28.7 ± 5.8	10.1 ± 3.3	2.8 ± 1.8
Providence, RI	22.2 ± 5.4	8.8 ± 3.2	2.3 ± 1.6
Provo, UT	3.9 ± 2.0	1.4 ± 1.2	0.3 ± 0.6
Raleigh, NC	17.6 ± 4.5	6.3 ± 2.6	1.7 ± 1.4
Reading, PA	8.2 ± 3.0	3.3 ± 1.9	0.8 ± 1.0
Reno, NV	7.0 ± 2.7	2.8 ± 1.7	0.9 ± 1.0
Richmond, VA	21.2 ± 5.0	7.5 ± 2.9	2.3 ± 1.7
Riverside, CA	60.2 ± 9.6	25.0 ± 5.7	6.8 ± 3.0
Roanoke, VA	6.0 ± 2.5	2.3 ± 1.6	0.7 ± 0.8
Rochester, MN	1.9 ± 1.4	0.6 ± 0.8	0.2 ± 0.5
Rochester, NY	22.5 ± 5.0	8.2 ± 3.0	2.4 ± 1.6
Sacramento, CA	29.1 ± 6.0	11.6 ± 3.6	3.4 ± 2.0
Salt Lake City, UT	60.5 ± 9.4	24.3 ± 5.6	6.1 ± 2.8
San Diego, CA	19.0 ± 4.5	6.1 ± 2.4	2.0 ± 1.5
San Jose, CA	49.2 ± 8.2	19.2 ± 4.8	5.1 ± 2.5
Savannah, GA	21.2 ± 4.9	8.1 ± 3.0	2.3 ± 1.6
Scranton, PA	6.2 ± 2.5	2.4 ± 1.6	0.6 ± 0.8
Seattle, WA	18.2 ± 4.6	8.0 ± 3.0	1.6 ± 1.3
Shreveport, LA	38.0 ± 6.8	13.7 ± 4.0	4.0 ± 2.1
Sioux Falls, SD	9.9 ± 3.2	3.5 ± 1.9	1.0 ± 1.0
South Bend, IN	2.7 ± 1.6	0.9 ± 1.0	0.3 ± 0.6
Spokane, WA	5.9 ± 2.5	2.4 ± 1.6	0.6 ± 0.8
Springfield, MA	9.0 ± 3.0	3.3 ± 1.9	1.1 ± 1.1
St. Louis, MO	14.2 ± 4.1	5.0 ± 2.4	1.7 ± 1.4
State College, PA	2.0 ± 1.4	0.8 ± 0.9	0.2 ± 0.5

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Table B.2 (Continued). Mean Daily Death Counts by City and Cause, 2001–2006

City	All Causes (± SD)	Cardiovascular (± SD)	Respiratory (± SD)
Steubenville, OH	3.4 ± 1.9	1.4 ± 1.2	0.4 ± 0.6
Tacoma, WA	12.6 ± 3.7	4.8 ± 2.3	1.3 ± 1.2
Tallahassee, FL	3.6 ± 1.9	1.2 ± 1.1	0.4 ± 0.6
Tampa, FL	65.0 ± 9.6	24.1 ± 5.7	6.0 ± 2.6
Toledo, OH	13.1 ± 3.7	5.5 ± 2.4	1.3 ± 1.2
Tucson, AZ	18.8 ± 4.7	6.7 ± 2.7	2.3 ± 1.6
Tulsa, OK	16.1 ± 4.3	6.5 ± 2.6	1.7 ± 1.4
Ventura, CA	10.9 ± 3.4	4.3 ± 2.2	1.2 ± 1.1
Visalia, CA	5.9 ± 2.4	2.4 ± 1.6	0.6 ± 0.8
Washington, DC	74.9 ± 10.3	27.3 ± 5.9	6.5 ± 2.8
Wheeling, WV	2.2 ± 1.5	0.9 ± 0.9	0.2 ± 0.5
Wichita, KS	10.0 ± 3.3	3.6 ± 1.9	1.1 ± 1.1
Wilmington, DE	11.5 ± 3.5	4.3 ± 2.1	1.1 ± 1.1
Yakima, WA	4.2 ± 2.1	1.7 ± 1.3	0.4 ± 0.7
York, PA	7.6 ± 2.8	2.9 ± 1.7	0.7 ± 0.8
Youngstown, OH	12.9 ± 3.9	5.3 ± 2.4	1.2 ± 1.1

Table B.3. Mean Daily Emergency Hospitalizations for Cardiovascular Causes by City, 2000–2008

City	All CVD Causes (± SD)	Acute Myocardial Infarction (± SD)	Ischemic Heart Disease (± SD)	Dysrhythmias (± SD)	Heart Failure (± SD)	Cerebrovascular Disease (± SD)
Akron, OH	10.5 ± 3.5	1.5 ± 1.3	1.2 ± 1.1	1.8 ± 1.4	3.2 ± 1.9	2.6 ± 1.6
Albany, NY	15.3 ± 4.4	2.5 ± 1.7	1.3 ± 1.2	2.8 ± 1.7	5.1 ± 2.4	3.5 ± 1.8
Albuquerque, NM	2.5 ± 1.7	0.5 ± 0.8	0.3 ± 0.6	0.4 ± 0.6	0.6 ± 0.8	0.6 ± 0.8
Allentown, PA	14.9 ± 4.6	2.4 ± 1.6	1.7 ± 1.4	2.5 ± 1.6	4.8 ± 2.4	3.2 ± 1.8
Anchorage, AK	1.5 ± 1.2	0.2 ± 0.5	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.6	0.4 ± 0.6
Ann Arbor, MI	5.6 ± 2.5	0.9 ± 1.0	0.6 ± 0.8	1.1 ± 1.1	1.6 ± 1.3	1.4 ± 1.2
Asheville, NC	3.4 ± 2.0	0.7 ± 0.9	0.3 ± 0.6	0.7 ± 0.8	0.8 ± 0.9	0.9 ± 0.9
Athens, GA	2.0 ± 1.4	0.3 ± 0.5	0.2 ± 0.5	0.3 ± 0.6	0.6 ± 0.8	0.5 ± 0.8
Atlanta, GA	36.5 ± 7.2	5.5 ± 2.4	4.2 ± 2.2	5.4 ± 2.4	11.6 ± 4.1	9.1 ± 3.2
Augusta, GA	5.3 ± 2.6	0.7 ± 0.8	1.1 ± 1.2	0.8 ± 0.9	1.3 ± 1.2	1.2 ± 1.2
Bakersfield, CA	3.9 ± 2.0	0.6 ± 0.8	0.5 ± 0.7	0.5 ± 0.7	1.2 ± 1.1	1.0 ± 1.0
Baltimore, MD	56.7 ± 11.4	8.2 ± 3.4	8.2 ± 3.9	9.0 ± 3.3	16.8 ± 5.0	13.5 ± 3.8
Baton Rouge, LA	5.8 ± 2.5	0.8 ± 1.0	0.6 ± 0.8	0.8 ± 0.9	1.8 ± 1.3	1.5 ± 1.3
Beaumont, TX	6.4 ± 2.7	0.8 ± 0.9	0.9 ± 1.0	0.9 ± 0.9	2.0 ± 1.5	1.7 ± 1.3
Biloxi, MS	4.9 ± 2.4	0.7 ± 0.8	0.7 ± 0.8	0.6 ± 0.8	1.7 ± 1.4	1.1 ± 1.1
Birmingham, AL	12.8 ± 4.4	1.9 ± 1.5	2.3 ± 1.7	1.9 ± 1.4	3.1 ± 1.9	3.4 ± 2.0
Bismarck, ND	0.2 ± 0.4	0.1 ± 0.3	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2
Boise, ID	2.4 ± 1.6	0.4 ± 0.6	0.3 ± 0.5	0.4 ± 0.7	0.6 ± 0.8	0.6 ± 0.8
Boston, MA	61.5 ± 11.6	10.0 ± 3.5	7.4 ± 3.4	10.9 ± 4.0	20.5 ± 5.9	12.2 ± 3.6
Bridgeport, CT	30.4 ± 6.9	4.7 ± 2.3	3.3 ± 2.0	5.5 ± 2.6	9.8 ± 3.6	6.6 ± 2.6
Buffalo, NY	20.4 ± 6.0	3.6 ± 2.1	2.1 ± 1.5	3.2 ± 2.0	6.5 ± 2.9	4.7 ± 2.3
Burlington, VT	2.3 ± 1.6	0.4 ± 0.6	0.3 ± 0.6	0.4 ± 0.6	0.6 ± 0.8	0.5 ± 0.7
Canton, OH	7.2 ± 2.9	1.1 ± 1.1	0.8 ± 0.9	1.3 ± 1.2	2.1 ± 1.5	1.7 ± 1.3
Cedar Rapids, IA	1.6 ± 1.3	0.3 ± 0.6	0.2 ± 0.4	0.2 ± 0.5	0.4 ± 0.6	0.4 ± 0.6
Charleston, SC	6.0 ± 2.6	0.7 ± 0.9	0.7 ± 0.9	1.1 ± 1.0	1.8 ± 1.4	1.6 ± 1.2
Charleston, WV	6.5 ± 2.8	1.2 ± 1.1	0.9 ± 1.1	1.1 ± 1.1	1.7 ± 1.4	1.5 ± 1.3
Charlotte, NC	18.1 ± 4.6	2.6 ± 1.6	2.2 ± 1.6	3.0 ± 1.8	5.2 ± 2.4	4.8 ± 2.2
Chattanooga, TN	8.3 ± 3.1	1.1 ± 1.0	1.1 ± 1.1	1.4 ± 1.2	2.3 ± 1.6	2.3 ± 1.5
Chicago, IL	126.7 ± 18.6	18.3 ± 4.8	14.4 ± 4.7	20.0 ± 5.4	40.4 ± 9.2	31.0 ± 5.9
Chico, CA	1.2 ± 1.3	0.3 ± 0.5	0.1 ± 0.4	0.2 ± 0.5	0.3 ± 0.6	0.3 ± 0.6
Cincinnati, OH	24.6 ± 5.9	3.5 ± 1.9	3.2 ± 1.9	3.8 ± 2.0	8.2 ± 3.2	5.6 ± 2.4
Clarksville, TN	1.3 ± 1.1	0.2 ± 0.5	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 0.6	0.3 ± 0.5
Cleveland, OH	43.9 ± 8.8	6.5 ± 2.7	5.9 ± 2.8	6.9 ± 2.9	13.6 ± 4.4	10.1 ± 3.3
Colorado Springs, CO	3.4 ± 2.0	0.6 ± 0.8	0.4 ± 0.6	0.6 ± 0.8	0.9 ± 1.0	1.0 ± 1.0
Columbia, SC	3.6 ± 2.0	0.5 ± 0.8	0.6 ± 0.8	0.5 ± 0.7	1.0 ± 1.0	0.9 ± 0.9
Columbus, GA	4.5 ± 2.3	0.7 ± 0.9	0.7 ± 0.9	0.7 ± 0.8	1.2 ± 1.1	1.1 ± 1.1
Columbus, OH	19.3 ± 5.1	2.8 ± 1.8	2.0 ± 1.5	3.6 ± 2.0	6.1 ± 2.7	4.4 ± 2.2
Corpus Christi, TX	5.0 ± 2.4	0.7 ± 0.8	0.6 ± 0.8	0.6 ± 0.8	1.9 ± 1.4	1.2 ± 1.1
Dallas, TX	27.3 ± 6.4	4.0 ± 2.1	3.0 ± 1.9	4.1 ± 2.1	8.4 ± 3.2	7.2 ± 2.8
Davenport, IA	4.4 ± 2.2	0.9 ± 1.0	0.6 ± 0.8	0.6 ± 0.8	1.3 ± 1.2	1.1 ± 1.1

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Table B.3 (Continued). Mean Daily Emergency Hospitalizations for Cardiovascular Causes by City, 2000–2008

City	All CVD Causes (± SD)	Acute Myocardial Infarction (± SD)	Ischemic Heart Disease (± SD)	Dysrhythmias (± SD)	Heart Failure (± SD)	Cerebrovascular Disease (± SD)
Dayton, OH	13.0 ± 3.9	1.9 ± 1.4	1.4 ± 1.2	2.0 ± 1.4	4.1 ± 2.1	3.2 ± 1.8
Decatur, AL	2.9 ± 1.8	0.4 ± 0.6	0.4 ± 0.6	0.4 ± 0.7	1.0 ± 1.0	0.7 ± 0.8
Decatur, IL	2.4 ± 1.6	0.4 ± 0.6	0.3 ± 0.6	0.4 ± 0.6	0.8 ± 0.9	0.6 ± 0.8
Denver, CO	10.8 ± 3.8	2.0 ± 1.5	1.2 ± 1.2	1.9 ± 1.5	2.8 ± 1.8	2.7 ± 1.7
Des Moines, IA	5.1 ± 2.4	0.8 ± 0.9	0.7 ± 0.9	0.9 ± 1.0	1.5 ± 1.2	1.2 ± 1.1
Detroit, MI	96.1 ± 15.1	14.2 ± 4.2	11.9 ± 4.5	15.2 ± 4.7	29.6 ± 7.4	22.2 ± 5.1
Dover, DE	2.4 ± 1.6	0.5 ± 0.7	0.3 ± 0.6	0.4 ± 0.6	0.7 ± 0.8	0.5 ± 0.7
El Paso, TX	6.9 ± 2.9	1.0 ± 1.0	0.9 ± 1.0	0.9 ± 1.0	2.1 ± 1.6	1.8 ± 1.4
Elkhart, IN	2.1 ± 1.6	0.4 ± 0.6	0.3 ± 0.6	0.3 ± 0.6	0.6 ± 0.8	0.5 ± 0.7
Erie, PA	4.4 ± 2.3	0.7 ± 0.8	0.7 ± 0.8	0.7 ± 0.9	1.3 ± 1.2	1.0 ± 1.0
Eugene, OR	1.8 ± 1.4	0.4 ± 0.6	0.1 ± 0.4	0.3 ± 0.6	0.4 ± 0.7	0.5 ± 0.7
Evansville, IN	4.7 ± 2.3	0.8 ± 0.9	0.5 ± 0.7	0.8 ± 0.9	1.4 ± 1.2	1.2 ± 1.1
Fargo, ND	0.6 ± 0.8	0.2 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3
Fayetteville, NC	3.4 ± 1.9	0.5 ± 0.7	0.4 ± 0.7	0.5 ± 0.7	1.0 ± 1.0	0.9 ± 0.9
Fort Lauderdale, FL	20.4 ± 5.6	3.3 ± 1.9	2.2 ± 1.5	3.5 ± 2.1	6.1 ± 2.8	4.9 ± 2.3
Fort Wayne, IN	6.4 ± 2.8	1.0 ± 1.0	0.8 ± 0.9	1.1 ± 1.0	1.9 ± 1.4	1.5 ± 1.2
Fresno, CA	8.8 ± 3.2	1.6 ± 1.3	1.3 ± 1.2	1.2 ± 1.1	2.5 ± 1.7	2.1 ± 1.4
Galveston, TX	5.0 ± 2.4	0.8 ± 0.9	0.6 ± 0.8	0.8 ± 0.9	1.5 ± 1.3	1.2 ± 1.1
Gary, IN	13.5 ± 3.9	1.5 ± 1.3	1.7 ± 1.3	1.9 ± 1.4	4.6 ± 2.2	3.4 ± 1.9
Grand Junction, CO	0.7 ± 0.8	0.2 ± 0.4	0.1 ± 0.3	0.1 ± 0.4	0.1 ± 0.4	0.2 ± 0.4
Grand Rapids, MI	14.3 ± 4.3	2.2 ± 1.5	1.7 ± 1.4	2.1 ± 1.6	4.3 ± 2.2	3.8 ± 2.0
Greeley, CO	0.4 ± 0.7	0.1 ± 0.3	0.0 ± 0.2	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3
Greensboro, NC	16.0 ± 4.7	2.6 ± 1.6	2.0 ± 1.5	2.7 ± 1.7	4.4 ± 2.3	4.2 ± 2.1
Greenville, SC	12.7 ± 4.2	2.1 ± 1.5	1.8 ± 1.5	1.9 ± 1.4	3.6 ± 2.0	3.2 ± 1.9
Hamilton, OH	4.7 ± 2.3	0.7 ± 0.9	0.8 ± 0.9	0.7 ± 0.9	1.2 ± 1.1	1.1 ± 1.1
Harrisburg, PA	4.8 ± 2.4	1.0 ± 1.0	0.7 ± 0.9	0.7 ± 0.9	1.4 ± 1.2	0.9 ± 1.0
Hattiesburg, MS	2.2 ± 1.5	0.3 ± 0.6	0.3 ± 0.6	0.3 ± 0.6	0.6 ± 0.8	0.5 ± 0.7
Hickory, NC	4.4 ± 2.2	0.8 ± 0.9	0.6 ± 0.8	0.8 ± 0.9	1.1 ± 1.1	1.1 ± 1.0
Honolulu, HI	6.9 ± 2.7	1.2 ± 1.1	0.6 ± 0.8	1.1 ± 1.0	1.8 ± 1.4	2.1 ± 1.5
Houston, TX	36.4 ± 7.9	5.3 ± 2.4	4.4 ± 2.3	5.3 ± 2.5	11.3 ± 4.0	9.5 ± 3.2
Huntington, WV	8.6 ± 3.2	1.6 ± 1.3	1.1 ± 1.1	1.2 ± 1.1	2.9 ± 1.8	1.7 ± 1.4
Huntsville, AL	4.5 ± 2.2	0.7 ± 0.9	0.6 ± 0.8	0.7 ± 0.8	1.4 ± 1.2	1.1 ± 1.1
Indianapolis, IN	22.8 ± 5.6	3.7 ± 2.0	2.7 ± 1.8	3.5 ± 2.0	6.7 ± 2.8	5.5 ± 2.4
Jackson, MS	6.3 ± 2.7	0.9 ± 1.0	0.6 ± 0.8	0.8 ± 0.9	2.1 ± 1.5	1.8 ± 1.3
Johnson City, TN	7.9 ± 3.1	1.4 ± 1.2	1.0 ± 1.0	1.3 ± 1.2	2.3 ± 1.6	1.8 ± 1.4
Kalamazoo, MI	3.3 ± 1.9	0.5 ± 0.7	0.5 ± 0.7	0.5 ± 0.7	1.0 ± 1.0	0.8 ± 0.9
Kansas City, MO	12.0 ± 3.9	1.9 ± 1.4	1.4 ± 1.2	2.0 ± 1.5	3.4 ± 1.9	3.1 ± 1.8
Kenosha, WI	1.6 ± 1.3	0.2 ± 0.5	0.1 ± 0.4	0.3 ± 0.5	0.5 ± 0.7	0.4 ± 0.6
Knoxville, TN	8.2 ± 3.0	1.5 ± 1.2	0.9 ± 1.0	1.3 ± 1.2	2.3 ± 1.5	2.0 ± 1.4
Lancaster, PA	8.4 ± 3.0	1.4 ± 1.2	1.0 ± 1.0	1.6 ± 1.3	2.1 ± 1.5	2.0 ± 1.4

Table continues next page

Table B.3 (Continued). Mean Daily Emergency Hospitalizations for Cardiovascular Causes by City, 2000–2008

City	All CVD Causes (± SD)	Acute Myocardial Infarction (± SD)	Ischemic Heart Disease (± SD)	Dysrhythmias (± SD)	Heart Failure (± SD)	Cerebrovascular Disease (± SD)
Las Vegas, NV	12.0 ± 4.3	1.6 ± 1.3	1.7 ± 1.3	2.1 ± 1.6	3.5 ± 2.1	3.1 ± 1.9
Lexington, KY	4.7 ± 2.4	0.9 ± 1.0	0.7 ± 0.9	0.8 ± 0.9	1.2 ± 1.2	1.1 ± 1.1
Little Rock, AR	5.8 ± 2.6	0.8 ± 0.9	0.8 ± 0.9	0.9 ± 1.0	1.9 ± 1.4	1.3 ± 1.1
Longview, TX	1.9 ± 1.4	0.3 ± 0.6	0.2 ± 0.5	0.3 ± 0.5	0.6 ± 0.8	0.5 ± 0.7
Los Angeles, CA	48.7 ± 10.8	7.6 ± 3.1	5.5 ± 2.5	7.5 ± 3.4	14.1 ± 5.0	12.6 ± 4.0
Louisville, KY	15.8 ± 4.4	2.6 ± 1.6	2.0 ± 1.5	2.5 ± 1.6	4.9 ± 2.4	3.6 ± 1.9
Lowell, MA	3.9 ± 2.1	0.7 ± 0.9	0.4 ± 0.7	0.7 ± 0.8	1.2 ± 1.1	0.9 ± 1.0
Lubbock, TX	4.7 ± 2.3	0.6 ± 0.8	1.0 ± 1.1	0.7 ± 0.8	1.5 ± 1.3	0.9 ± 0.9
Macon, GA	5.6 ± 2.5	0.6 ± 0.8	1.0 ± 1.0	0.8 ± 0.9	1.8 ± 1.4	1.3 ± 1.2
Medford, OR	2.8 ± 1.8	0.5 ± 0.7	0.4 ± 0.6	0.5 ± 0.7	0.6 ± 0.8	0.7 ± 0.9
Memphis, TN	18.0 ± 4.9	2.1 ± 1.5	2.7 ± 1.9	2.5 ± 1.6	5.5 ± 2.5	4.7 ± 2.2
Miami, FL	28.6 ± 7.7	4.6 ± 2.3	3.0 ± 2.0	4.0 ± 2.4	9.3 ± 3.5	7.1 ± 3.0
Middlesex, NJ	15.4 ± 4.5	2.3 ± 1.5	2.0 ± 1.5	2.6 ± 1.7	4.7 ± 2.4	3.3 ± 1.8
Milwaukee, WI	22.4 ± 5.4	3.5 ± 1.9	3.0 ± 1.9	3.6 ± 1.9	6.5 ± 2.8	5.6 ± 2.4
Minneapolis, MN	19.9 ± 5.1	3.6 ± 2.0	2.2 ± 1.6	3.8 ± 2.0	4.9 ± 2.4	5.3 ± 2.4
Missoula, MT	0.5 ± 0.7	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.4
Mobile, AL	8.4 ± 3.4	0.7 ± 0.9	1.9 ± 1.6	1.4 ± 1.3	2.5 ± 1.7	1.7 ± 1.3
Modesto, CA	1.5 ± 1.5	0.3 ± 0.5	0.1 ± 0.4	0.2 ± 0.5	0.5 ± 0.8	0.3 ± 0.6
Montgomery, AL	4.2 ± 2.2	0.5 ± 0.7	0.5 ± 0.7	0.6 ± 0.8	1.2 ± 1.1	1.2 ± 1.1
Nashville, TN	13.2 ± 3.9	1.8 ± 1.4	1.6 ± 1.3	2.1 ± 1.5	4.0 ± 2.0	3.4 ± 1.9
New York, NY	134.6 ± 26.5	18.9 ± 5.2	19.4 ± 7.2	22.2 ± 7.0	42.9 ± 10.6	28.9 ± 6.5
Newark, NJ	21.3 ± 5.4	2.9 ± 1.8	2.2 ± 1.6	3.8 ± 2.1	6.7 ± 2.8	4.9 ± 2.3
Oklahoma City, OK	14.0 ± 4.0	2.1 ± 1.5	2.3 ± 1.6	2.2 ± 1.5	3.6 ± 2.0	3.6 ± 1.9
Omaha, NE	6.0 ± 2.6	1.0 ± 1.0	0.7 ± 0.8	1.0 ± 1.0	1.6 ± 1.3	1.6 ± 1.2
Owensboro, KY	2.1 ± 1.5	0.4 ± 0.6	0.3 ± 0.6	0.3 ± 0.6	0.6 ± 0.8	0.5 ± 0.7
Pensacola, FL	7.5 ± 2.9	1.1 ± 1.0	1.1 ± 1.0	1.2 ± 1.1	2.3 ± 1.6	1.7 ± 1.3
Philadelphia, PA	79.5 ± 17.2	10.1 ± 3.7	8.5 ± 3.7	14.4 ± 5.2	26.2 ± 7.5	18.8 ± 5.2
Phoenix, AZ	28.2 ± 8.1	4.3 ± 2.2	3.8 ± 2.1	5.4 ± 3.1	7.0 ± 3.2	7.3 ± 2.9
Pittsburgh, PA	48.6 ± 12.3	7.1 ± 3.2	5.4 ± 3.1	7.8 ± 3.3	16.9 ± 5.3	10.6 ± 3.8
Portland, OR	9.9 ± 4.1	1.9 ± 1.5	1.1 ± 1.1	1.7 ± 1.4	2.7 ± 1.9	2.5 ± 1.7
Providence, RI	13.6 ± 4.3	2.2 ± 1.5	1.4 ± 1.2	2.2 ± 1.6	4.9 ± 2.4	2.8 ± 1.7
Provo, UT	0.7 ± 0.8	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.4	0.1 ± 0.4	0.2 ± 0.4
Raleigh, NC	12.9 ± 3.9	2.2 ± 1.5	1.6 ± 1.3	2.1 ± 1.5	3.4 ± 1.9	3.4 ± 1.9
Reading, PA	4.2 ± 2.2	0.9 ± 1.0	0.5 ± 0.8	0.6 ± 0.8	1.1 ± 1.1	0.9 ± 1.0
Reno, NV	2.2 ± 1.6	0.4 ± 0.7	0.2 ± 0.4	0.4 ± 0.6	0.5 ± 0.8	0.6 ± 0.8
Richmond, VA	16.5 ± 4.6	2.3 ± 1.5	1.9 ± 1.4	2.2 ± 1.5	4.7 ± 2.3	4.7 ± 2.3
Riverside, CA	15.4 ± 5.0	2.6 ± 1.7	2.0 ± 1.5	2.6 ± 1.8	4.0 ± 2.2	3.8 ± 2.1
Roanoke, VA	4.8 ± 2.3	0.8 ± 0.9	0.6 ± 0.8	0.7 ± 0.8	1.4 ± 1.2	1.3 ± 1.1
Rochester, MN	1.7 ± 1.4	0.3 ± 0.5	0.3 ± 0.5	0.4 ± 0.7	0.4 ± 0.6	0.3 ± 0.6
Rochester, NY	13.6 ± 4.9	2.4 ± 1.7	1.4 ± 1.3	2.2 ± 1.5	4.4 ± 2.4	2.9 ± 1.8

Table continues next page

Table B.3 (Continued). Mean Daily Emergency Hospitalizations for Cardiovascular Causes by City, 2000–2008

City	All CVD Causes (± SD)	Acute Myocardial Infarction (± SD)	Ischemic Heart Disease (± SD)	Dysrhythmias (± SD)	Heart Failure (± SD)	Cerebrovascular Disease (± SD)
Sacramento, CA	6.8 ± 3.8	1.3 ± 1.2	0.6 ± 0.8	1.1 ± 1.2	2.0 ± 1.7	1.8 ± 1.5
Salt Lake City, UT	35.5 ± 6.8	6.0 ± 2.5	3.9 ± 2.1	5.2 ± 2.4	11.3 ± 3.8	8.4 ± 3.1
San Diego, CA	4.3 ± 2.2	0.8 ± 0.9	0.6 ± 0.8	0.7 ± 0.8	1.0 ± 1.0	1.2 ± 1.1
San Jose, CA	12.8 ± 4.2	2.3 ± 1.6	1.4 ± 1.2	1.9 ± 1.5	3.5 ± 2.0	3.4 ± 2.0
Savannah, GA	5.7 ± 2.8	1.0 ± 1.0	0.6 ± 0.8	0.9 ± 1.0	1.6 ± 1.4	1.5 ± 1.3
Scranton, PA	3.8 ± 2.0	0.4 ± 0.7	0.3 ± 0.6	0.5 ± 0.7	1.2 ± 1.1	1.1 ± 1.0
Seattle, WA	11.0 ± 3.9	1.8 ± 1.4	1.5 ± 1.3	1.8 ± 1.4	3.1 ± 1.9	2.6 ± 1.7
Shreveport, LA	15.9 ± 4.5	2.8 ± 1.7	1.7 ± 1.4	2.6 ± 1.7	4.1 ± 2.2	4.4 ± 2.2
Sioux Falls, SD	6.2 ± 2.7	0.7 ± 0.8	0.9 ± 1.0	1.0 ± 1.0	1.9 ± 1.4	1.7 ± 1.3
South Bend, IN	1.9 ± 1.4	0.4 ± 0.6	0.2 ± 0.5	0.3 ± 0.6	0.5 ± 0.7	0.5 ± 0.7
Spokane, WA	4.2 ± 2.1	0.7 ± 0.8	0.4 ± 0.6	0.7 ± 0.8	1.3 ± 1.2	1.1 ± 1.0
Springfield, MA	1.9 ± 1.6	0.4 ± 0.6	0.2 ± 0.5	0.3 ± 0.6	0.4 ± 0.7	0.6 ± 0.8
St. Louis, MO	10.3 ± 3.4	2.1 ± 1.5	1.0 ± 1.0	1.9 ± 1.4	2.9 ± 1.8	2.3 ± 1.5
State College, PA	1.7 ± 1.3	0.3 ± 0.5	0.2 ± 0.4	0.3 ± 0.5	0.6 ± 0.8	0.3 ± 0.6
Steubenville, OH	4.9 ± 2.5	0.7 ± 0.8	0.7 ± 0.9	0.6 ± 0.8	1.9 ± 1.4	0.9 ± 1.0
Tacoma, WA	6.6 ± 2.7	1.1 ± 1.0	0.7 ± 0.8	1.1 ± 1.1	1.9 ± 1.4	1.7 ± 1.3
Tallahassee, FL	1.6 ± 1.3	0.3 ± 0.5	0.2 ± 0.5	0.2 ± 0.5	0.4 ± 0.6	0.4 ± 0.7
Tampa, FL	40.3 ± 8.0	6.7 ± 2.8	5.2 ± 2.6	6.6 ± 2.8	10.5 ± 3.7	10.3 ± 3.3
Toledo, OH	8.9 ± 3.2	1.5 ± 1.3	1.0 ± 1.0	1.4 ± 1.2	2.8 ± 1.8	2.0 ± 1.4
Tucson, AZ	8.6 ± 3.2	1.4 ± 1.2	1.2 ± 1.1	1.8 ± 1.4	2.1 ± 1.6	2.0 ± 1.5
Tulsa, OK	9.3 ± 3.3	1.7 ± 1.3	1.1 ± 1.0	1.5 ± 1.3	2.5 ± 1.7	2.5 ± 1.6
Ventura, CA	5.8 ± 2.6	1.0 ± 1.0	0.8 ± 0.9	0.9 ± 1.0	1.4 ± 1.2	1.6 ± 1.3
Visalia, CA	1.5 ± 1.3	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.5	0.5 ± 0.7	0.5 ± 0.7
Washington, DC	55.4 ± 9.6	7.7 ± 3.1	5.8 ± 3.1	9.0 ± 3.3	16.3 ± 4.7	14.8 ± 4.0
Wheeling, WV	2.2 ± 1.6	0.4 ± 0.7	0.3 ± 0.5	0.3 ± 0.6	0.7 ± 0.9	0.5 ± 0.7
Wichita, KS	6.1 ± 2.5	1.1 ± 1.1	0.9 ± 0.9	1.0 ± 1.0	1.5 ± 1.2	1.7 ± 1.3
Wilmington, DE	11.8 ± 4.0	1.8 ± 1.4	1.5 ± 1.3	1.9 ± 1.4	3.5 ± 2.0	2.9 ± 1.8
Yakima, WA	2.8 ± 1.7	0.5 ± 0.7	0.3 ± 0.6	0.5 ± 0.7	0.8 ± 0.9	0.6 ± 0.8
York, PA	6.8 ± 2.7	1.3 ± 1.2	1.0 ± 1.0	1.0 ± 1.0	1.9 ± 1.4	1.5 ± 1.2
Youngstown, OH	12.1 ± 3.8	1.7 ± 1.3	1.4 ± 1.3	2.0 ± 1.5	4.0 ± 2.1	2.7 ± 1.7

Table B.4. Mean Daily Emergency Hospitalizations for Respiratory Causes by City, 2000–2008

City	All Respiratory Causes (\pm SD)	Pneumonia (\pm SD)	COPD (\pm SD)
Akron, OH	6.7 \pm 3.3	3.1 \pm 2.1	1.7 \pm 1.4
Albany, NY	10.2 \pm 4.4	4.7 \pm 2.7	3.2 \pm 2.1
Albuquerque, NM	1.5 \pm 1.4	0.9 \pm 1.1	0.4 \pm 0.6
Allentown, PA	7.5 \pm 3.6	3.1 \pm 2.2	2.6 \pm 1.8
Anchorage, AK	0.9 \pm 1.0	0.4 \pm 0.6	0.3 \pm 0.5
Ann Arbor, MI	3.3 \pm 2.1	1.6 \pm 1.4	1.0 \pm 1.0
Asheville, NC	2.3 \pm 1.8	0.9 \pm 1.0	0.8 \pm 0.9
Athens, GA	1.3 \pm 1.2	0.5 \pm 0.7	0.5 \pm 0.7
Atlanta, GA	24.4 \pm 9.0	11.0 \pm 5.3	7.6 \pm 3.5
Augusta, GA	2.9 \pm 2.0	1.6 \pm 1.4	0.8 \pm 0.9
Bakersfield, CA	2.8 \pm 1.9	1.4 \pm 1.3	0.7 \pm 0.9
Baltimore, MD	30.7 \pm 10.3	12.5 \pm 5.6	10.5 \pm 4.5
Baton Rouge, LA	3.2 \pm 2.1	1.4 \pm 1.3	0.8 \pm 1.0
Beaumont, TX	3.8 \pm 2.3	1.8 \pm 1.5	1.2 \pm 1.2
Biloxi, MS	2.6 \pm 1.9	1.2 \pm 1.2	1.0 \pm 1.1
Birmingham, AL	7.4 \pm 3.7	3.3 \pm 2.2	2.1 \pm 1.6
Bismarck, ND	0.1 \pm 0.3	0.0 \pm 0.2	0.0 \pm 0.1
Boise, ID	1.6 \pm 1.4	0.9 \pm 1.0	0.4 \pm 0.7
Boston, MA	40.0 \pm 13.6	18.8 \pm 7.8	12.4 \pm 5.2
Bridgeport, CT	19.5 \pm 7.2	9.9 \pm 4.7	4.7 \pm 2.6
Buffalo, NY	10.2 \pm 4.4	4.9 \pm 2.7	3.2 \pm 2.2
Burlington, VT	1.4 \pm 1.2	0.8 \pm 1.0	0.4 \pm 0.6
Canton, OH	4.7 \pm 2.9	2.3 \pm 1.8	1.5 \pm 1.3
Cedar Rapids, IA	1.2 \pm 1.2	0.5 \pm 0.8	0.2 \pm 0.5
Charleston, SC	3.2 \pm 2.0	1.5 \pm 1.4	1.0 \pm 1.1
Charleston, WV	4.0 \pm 2.3	2.0 \pm 1.6	1.4 \pm 1.2
Charlotte, NC	10.9 \pm 4.8	4.8 \pm 2.8	3.3 \pm 2.1
Chattanooga, TN	5.2 \pm 2.8	2.5 \pm 1.8	1.5 \pm 1.3
Chicago, IL	73.3 \pm 21.8	33.9 \pm 12.8	21.8 \pm 7.6
Chico, CA	0.9 \pm 1.2	0.4 \pm 0.7	0.2 \pm 0.5
Cincinnati, OH	16.4 \pm 6.3	8.0 \pm 4.0	4.4 \pm 2.5
Clarksville, TN	1.1 \pm 1.1	0.5 \pm 0.7	0.4 \pm 0.6
Cleveland, OH	25.9 \pm 9.3	11.4 \pm 5.2	8.8 \pm 4.1
Colorado Springs, CO	2.5 \pm 1.8	1.1 \pm 1.1	0.7 \pm 0.9
Columbia, SC	1.6 \pm 1.3	0.7 \pm 0.9	0.4 \pm 0.6
Columbus, GA	2.0 \pm 1.6	1.0 \pm 1.1	0.5 \pm 0.8
Columbus, OH	12.5 \pm 5.2	5.7 \pm 3.1	4.4 \pm 2.4
Corpus Christi, TX	2.9 \pm 2.0	1.5 \pm 1.4	0.8 \pm 1.0
Dallas, TX	17.4 \pm 7.3	8.1 \pm 4.4	5.4 \pm 3.0
Davenport, IA	2.7 \pm 2.0	1.4 \pm 1.3	0.8 \pm 1.0
Dayton, OH	8.8 \pm 4.0	3.6 \pm 2.3	2.9 \pm 1.9
Decatur, AL	2.2 \pm 1.7	0.9 \pm 1.0	0.8 \pm 0.9
Decatur, IL	1.5 \pm 1.4	0.7 \pm 0.9	0.5 \pm 0.8
Denver, CO	7.4 \pm 3.9	3.5 \pm 2.3	2.2 \pm 1.7

Table continues next page

Table B.4 (Continued). Mean Daily Emergency Hospitalizations for Respiratory Causes by City, 2000–2008

City	All Respiratory Causes (± SD)	Pneumonia (± SD)	COPD (± SD)
Des Moines, IA	3.4 ± 2.1	1.4 ± 1.3	0.9 ± 1.0
Detroit, MI	51.1 ± 15.3	21.3 ± 8.0	18.4 ± 6.9
Dover, DE	1.5 ± 1.3	0.6 ± 0.8	0.5 ± 0.7
El Paso, TX	4.8 ± 3.1	2.2 ± 1.9	1.4 ± 1.3
Elkhart, IN	1.1 ± 1.1	0.5 ± 0.7	0.4 ± 0.6
Erie, PA	2.3 ± 1.6	1.0 ± 1.1	0.7 ± 0.9
Eugene, OR	1.2 ± 1.2	0.6 ± 0.8	0.3 ± 0.6
Evansville, IN	3.1 ± 2.2	1.4 ± 1.3	1.0 ± 1.1
Fargo, ND	0.3 ± 0.5	0.1 ± 0.4	0.1 ± 0.3
Fayetteville, NC	1.9 ± 1.5	0.7 ± 0.9	0.7 ± 0.9
Fort Lauderdale, FL	11.0 ± 4.7	3.7 ± 2.2	4.2 ± 2.7
Fort Wayne, IN	3.7 ± 2.3	1.9 ± 1.6	1.2 ± 1.2
Fresno, CA	5.5 ± 2.9	3.1 ± 2.1	1.2 ± 1.1
Galveston, TX	2.8 ± 1.9	1.2 ± 1.2	1.1 ± 1.1
Gary, IN	6.8 ± 3.2	2.9 ± 2.0	2.3 ± 1.7
Grand Junction, CO	0.4 ± 0.7	0.2 ± 0.5	0.1 ± 0.3
Grand Rapids, MI	8.8 ± 4.1	4.4 ± 2.7	2.3 ± 1.7
Greeley, CO	0.2 ± 0.5	0.1 ± 0.3	0.1 ± 0.3
Greensboro, NC	9.9 ± 4.5	4.4 ± 2.7	2.8 ± 2.0
Greenville, SC	7.6 ± 4.0	3.5 ± 2.4	2.3 ± 1.8
Hamilton, OH	3.0 ± 2.0	1.4 ± 1.3	0.8 ± 1.0
Harrisburg, PA	1.9 ± 1.5	0.8 ± 1.0	0.5 ± 0.8
Hattiesburg, MS	1.4 ± 1.3	0.6 ± 0.8	0.5 ± 0.7
Hickory, NC	2.9 ± 2.0	1.5 ± 1.4	0.7 ± 0.9
Honolulu, HI	4.6 ± 2.4	2.2 ± 1.6	1.0 ± 1.0
Houston, TX	21.4 ± 7.8	9.4 ± 4.4	7.1 ± 3.5
Huntington, WV	6.3 ± 3.1	2.9 ± 2.1	2.1 ± 1.6
Huntsville, AL	2.5 ± 1.8	1.0 ± 1.1	0.8 ± 0.9
Indianapolis, IN	16.1 ± 6.5	7.4 ± 3.8	5.2 ± 2.8
Jackson, MS	3.9 ± 2.5	1.7 ± 1.5	1.4 ± 1.3
Johnson City, TN	5.7 ± 3.0	2.7 ± 2.0	1.6 ± 1.4
Kalamazoo, MI	1.8 ± 1.4	0.9 ± 1.0	0.5 ± 0.7
Kansas City, MO	8.0 ± 4.0	3.6 ± 2.4	2.5 ± 1.9
Kenosha, WI	1.0 ± 1.1	0.5 ± 0.7	0.3 ± 0.6
Knoxville, TN	5.8 ± 2.9	2.7 ± 1.9	1.6 ± 1.4
Lancaster, PA	4.0 ± 2.4	1.7 ± 1.5	1.3 ± 1.2
Las Vegas, NV	7.9 ± 3.9	3.3 ± 2.1	2.7 ± 2.0
Lexington, KY	3.0 ± 2.1	1.5 ± 1.4	0.9 ± 1.0
Little Rock, AR	3.2 ± 2.3	1.5 ± 1.5	0.9 ± 1.0
Longview, TX	1.3 ± 1.2	0.6 ± 0.8	0.4 ± 0.7
Los Angeles, CA	32.4 ± 11.9	14.5 ± 6.4	8.4 ± 4.2
Louisville, KY	11.2 ± 4.8	4.4 ± 2.7	3.1 ± 2.1
Lowell, MA	2.6 ± 1.8	1.2 ± 1.2	0.9 ± 1.0
Lubbock, TX	2.8 ± 2.0	1.4 ± 1.3	0.7 ± 0.9

Table continues next page

Table B.4 (Continued). Mean Daily Emergency Hospitalizations for Respiratory Causes by City, 2000–2008

City	All Respiratory Causes (± SD)	Pneumonia (± SD)	COPD (± SD)
Macon, GA	3.0 ± 2.0	1.3 ± 1.3	0.9 ± 1.0
Medford, OR	1.5 ± 1.3	0.8 ± 1.0	0.5 ± 0.7
Memphis, TN	8.6 ± 4.0	3.9 ± 2.5	3.0 ± 2.0
Miami, FL	19.6 ± 6.3	7.3 ± 3.1	6.8 ± 3.3
Middlesex, NJ	7.9 ± 3.6	3.5 ± 2.1	2.2 ± 1.7
Milwaukee, WI	12.6 ± 5.2	6.1 ± 3.3	3.8 ± 2.2
Minneapolis, MN	11.0 ± 4.6	5.2 ± 2.9	3.0 ± 2.0
Missoula, MT	0.3 ± 0.5	0.2 ± 0.4	0.1 ± 0.3
Mobile, AL	4.0 ± 2.5	1.6 ± 1.4	1.4 ± 1.3
Modesto, CA	1.1 ± 1.2	0.6 ± 0.8	0.3 ± 0.6
Montgomery, AL	2.3 ± 1.7	1.1 ± 1.1	0.8 ± 0.9
Nashville, TN	10.3 ± 4.5	5.0 ± 2.9	3.1 ± 2.1
New York, NY	79.0 ± 22.8	35.8 ± 12.5	25.1 ± 9.2
Newark, NJ	12.3 ± 4.9	5.8 ± 3.0	3.7 ± 2.2
Oklahoma City, OK	8.9 ± 4.3	4.1 ± 2.6	2.8 ± 1.9
Omaha, NE	3.9 ± 2.4	2.1 ± 1.7	1.0 ± 1.1
Owensboro, KY	1.3 ± 1.2	0.5 ± 0.8	0.4 ± 0.7
Pensacola, FL	4.2 ± 2.4	1.8 ± 1.5	1.5 ± 1.3
Philadelphia, PA	45.1 ± 15.6	17.9 ± 7.5	15.2 ± 6.7
Phoenix, AZ	16.3 ± 7.8	7.9 ± 4.4	4.5 ± 3.0
Pittsburgh, PA	28.9 ± 10.9	11.7 ± 5.5	9.6 ± 4.8
Portland, OR	6.2 ± 3.4	3.1 ± 2.1	1.5 ± 1.5
Providence, RI	9.5 ± 4.4	4.5 ± 2.7	2.9 ± 2.0
Provo, UT	0.4 ± 0.6	0.3 ± 0.6	0.0 ± 0.2
Raleigh, NC	7.6 ± 3.7	3.3 ± 2.3	2.1 ± 1.6
Reading, PA	2.0 ± 1.5	0.9 ± 1.0	0.6 ± 0.8
Reno, NV	1.5 ± 1.4	0.7 ± 0.9	0.4 ± 0.7
Richmond, VA	9.0 ± 3.9	3.2 ± 2.1	2.8 ± 1.9
Riverside, CA	9.7 ± 4.5	5.0 ± 2.8	2.5 ± 1.8
Roanoke, VA	2.8 ± 1.9	1.4 ± 1.3	0.8 ± 0.9
Rochester, MN	0.9 ± 1.0	0.4 ± 0.7	0.3 ± 0.5
Rochester, NY	8.6 ± 4.1	4.1 ± 2.5	2.5 ± 1.8
Sacramento, CA	4.7 ± 3.3	2.1 ± 1.8	1.2 ± 1.4
Salt Lake City, UT	22.0 ± 8.3	10.0 ± 5.0	5.3 ± 2.8
San Diego, CA	2.7 ± 1.9	1.5 ± 1.4	0.4 ± 0.7
San Jose, CA	7.6 ± 4.0	3.4 ± 2.4	2.1 ± 1.6
Savannah, GA	3.5 ± 2.3	1.6 ± 1.4	0.7 ± 0.9
Scranton, PA	2.3 ± 1.7	0.9 ± 1.0	0.6 ± 0.8
Seattle, WA	5.7 ± 3.0	2.6 ± 1.8	1.8 ± 1.5
Shreveport, LA	10.0 ± 4.2	4.3 ± 2.4	2.3 ± 1.7
Sioux Falls, SD	3.9 ± 2.4	1.7 ± 1.5	1.3 ± 1.2
South Bend, IN	1.2 ± 1.2	0.6 ± 0.8	0.4 ± 0.6
Spokane, WA	2.4 ± 1.8	1.1 ± 1.2	0.8 ± 1.0
Springfield, MA	1.3 ± 1.4	0.6 ± 0.9	0.3 ± 0.6

Table continues next page

Table B.4 (Continued). Mean Daily Emergency Hospitalizations for Respiratory Causes by City, 2000–2008

City	All Respiratory Causes (\pm SD)	Pneumonia (\pm SD)	COPD (\pm SD)
St. Louis, MO	5.8 \pm 3.0	2.8 \pm 1.9	1.8 \pm 1.5
State College, PA	0.9 \pm 1.0	0.4 \pm 0.7	0.3 \pm 0.5
Steubenville, OH	3.1 \pm 2.0	1.4 \pm 1.3	1.2 \pm 1.1
Tacoma, WA	4.0 \pm 2.4	1.8 \pm 1.5	1.1 \pm 1.1
Tallahassee, FL	0.7 \pm 0.9	0.2 \pm 0.5	0.2 \pm 0.4
Tampa, FL	22.1 \pm 7.9	8.4 \pm 3.9	7.5 \pm 3.7
Toledo, OH	5.3 \pm 2.9	2.3 \pm 1.7	2.0 \pm 1.6
Tucson, AZ	5.0 \pm 3.0	2.4 \pm 1.9	1.5 \pm 1.4
Tulsa, OK	5.7 \pm 3.2	2.9 \pm 2.1	1.7 \pm 1.5
Ventura, CA	3.4 \pm 2.1	1.7 \pm 1.4	0.8 \pm 0.9
Visalia, CA	1.2 \pm 1.3	0.7 \pm 0.9	0.3 \pm 0.6
Washington, DC	31.9 \pm 10.1	14.1 \pm 5.9	9.3 \pm 4.0
Wheeling, WV	1.4 \pm 1.3	0.7 \pm 0.9	0.4 \pm 0.7
Wichita, KS	3.2 \pm 2.2	1.6 \pm 1.5	0.8 \pm 1.0
Wilmington, DE	6.6 \pm 3.3	2.8 \pm 1.9	2.2 \pm 1.7
Yakima, WA	1.6 \pm 1.4	0.9 \pm 1.0	0.5 \pm 0.7
York, PA	3.1 \pm 2.0	1.4 \pm 1.3	1.0 \pm 1.1
Youngstown, OH	6.4 \pm 3.3	2.8 \pm 1.9	1.9 \pm 1.6

Table B.5. Percentage of Measurements Below the Detection Limit for PM_{2.5} and its Key Chemical Components

Pollutant	2000	2001	2002	2003	2004	2005	2006	2007
As	69.9	68.7	67.1	64.9	67.0	65.6	64.4	71.1
Al	40.1	46.8	57.6	64.1	60.5	60.3	47.0	40.9
Ba	45.4	55.2	57.6	74.0	77.2	79.7	88.3	97.8
Br	27.5	30.5	27.0	31.5	31.3	24.0	26.0	31.0
Cd	99.6	98.3	95.3	91.3	92.2	94.2	97.9	99.1
Ca	0.4	0.2	3.7	5.1	4.7	5.4	8.3	8.4
Cr	81.6	68.9	67.2	71.2	74.0	68.1	74.0	81.3
Cu	25.7	28.4	35.6	40.1	46.8	39.2	34.9	25.4
Fe	0.3	0.1	2.7	3.7	3.0	2.7	3.3	3.3
Pb	39.0	50.0	50.8	57.5	61.2	52.3	53.4	71.8
Mn	38.7	38.3	44.7	56.4	54.9	48.4	49.5	62.2
Ni	37.3	48.0	65.9	68.9	68.9	68.6	67.8	62.6
Mg	73.3	79.9	86.2	88.3	81.9	84.6	78.8	79.7
Hg	99.8	92.6	89.8	92.3	86.7	80.7	85.7	95.9
P	99.3	84.3	80.4	92.9	88.1	92.5	95.7	97.1
Se	81.5	69.9	69.5	80.0	85.3	76.3	70.6	84.9
V	58.3	54.8	67.8	68.4	67.4	64.6	65.7	73.4
Si	0.8	0.3	3.3	6.4	13.6	26.4	11.3	9.0
Zn	12.4	9.3	12.7	13.9	14.7	8.5	9.1	15.1
S	0.4	0.1	2.9	2.9	2.6	2.7	3.1	3.2
K	1.4	0.8	3.2	3.3	3.4	3.1	3.5	3.9
Na	32.3	43.6	64.9	76.7	66.7	65.8	61.4	60.0
NH ₄ ⁺	3.8	1.7	0.4	0.4	0.3	0.2	0.3	0.2
OC	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
NO ₃ ⁻	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
EC	6.7	7.6	9.7	11.4	15.8	14.4	15.6	9.5
SO ₄ ²⁻	0.0	0.0	0.0	0.2	0.0	0.1	0.1	0.1
PM _{2.5}	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table B.6. Percentage of Zeros for PM_{2.5} and its Key Chemical Components

Pollutant	2000	2001	2002	2003	2004	2005	2006	2007
As	26.6	28.0	29.2	27.7	26.9	32.6	31.9	40.3
Al	25.8	26.4	38.5	48.0	45.1	45.9	25.6	22.7
Ba	2.1	7.9	13.5	34.9	39.5	52.9	64.3	83.6
Br	5.8	5.5	4.4	7.2	6.7	3.3	7.6	7.6
Cd	53.4	51.2	49.1	54.3	60.8	63.4	72.8	85.5
Ca	0.1	0.0	0.2	0.9	0.3	0.3	1.7	1.4
Cr	38.2	8.1	11.0	22.5	31.1	30.4	38.0	45.3
Cu	4.0	7.9	10.5	15.4	11.3	8.7	10.0	4.6
Fe	0.0	0.0	0.0	0.8	0.5	0.0	0.3	0.1
Pb	2.5	7.9	10.4	17.8	23.1	15.5	19.3	35.5
Mn	5.4	5.4	14.1	21.7	17.0	13.3	13.4	23.9
Ni	8.5	10.2	18.2	24.5	21.1	23.0	18.8	16.6
Mg	49.1	57.3	69.3	72.0	64.5	65.0	60.3	62.1
Hg	47.7	41.9	40.3	52.2	47.6	50.6	58.4	78.3
P	96.8	72.7	66.6	80.2	71.5	86.3	88.1	92.0
Se	31.3	25.4	21.1	24.0	25.9	23.6	24.2	41.4
V	32.6	12.4	19.2	29.3	27.6	19.3	22.4	33.8
Si	0.3	0.1	0.1	1.1	5.6	17.7	2.4	1.1
Zn	5.4	3.1	3.5	3.7	3.4	0.8	0.7	3.9
S	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.1
K	0.9	0.5	0.1	0.1	0.1	0.0	0.1	0.2
Na	22.8	28.8	43.7	59.1	44.5	39.3	39.9	35.1
NH ₄ ⁺	3.6	1.2	0.3	0.3	0.2	0.1	0.2	0.1
OC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NO ₃ ⁻	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
EC	0.0	0.2	0.1	0.3	0.8	0.6	1.2	0.6
SO ₄ ²⁻	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
PM _{2.5}	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table B.7. Speciation Monitors Considered for Each City

City Number	City Name	MSA Code	FIPS Code ^a	AQS Site ID
1	Akron, OH	80	39153	391530023
2	Albany, NY	160	36001	360010005
3	Albuquerque, NM	200	35001	350010023
4	Allentown, PA	240	42095	420950025
5	Anchorage, AK	380	2020	20200018
6	Ann Arbor, MI	440	26161	261610008
7	Asheville, NC	480	37021	370210034
8	Athens, GA	500	13059	130590001
9	Atlanta, GA	520	13089	130890002
10	Augusta, GA	600	13245	132450091
11	Bakersfield, CA	680	6029	60290014
12	Baltimore, MD	720	24003	240030019
12	Baltimore, MD	720	24005	240053001
12	Baltimore, MD	720	24510	245100053
13	Baton Rouge, LA	760	22033	220330009
14	Beaumont, TX	840	48245	482450022
14	Beaumont, TX	840	48361	483611100
15	Biloxi, MS	920	28047	280470008
16	Birmingham, AL	1000	1073	10730023
16	Birmingham, AL	1000	1073	10731009
16	Birmingham, AL	1000	1073	10732003
17	Bismarck, ND	1010	38015	380150003
18	Boise, ID	1080	16001	160010010
18	Boise, ID	1080	16027	160270004
19	Boston, MA	1120	25025	250250042
19	Boston, MA	1120	33015	330150014
20	Bridgeport, CT	1160	9001	90019003
20	Bridgeport, CT	1160	9009	90090027
20	Bridgeport, CT	1160	9009	90091123
21	Buffalo, NY	1280	36029	360290005
22	Burlington, VT	1305	50007	500070012
23	Canton, OH	1320	39151	391510017
23	Canton, OH	1320	39151	391510020
24	Cedar Rapids, IA	1360	19113	191130037
25	Charleston, SC	1440	45019	450190049
26	Charleston, WV	1480	54039	540390011
26	Charleston, WV	1480	54039	540391005
27	Charlotte, NC	1520	37119	371190041
27	Charlotte, NC	1520	37159	371590021
28	Chattanooga, TN	1560	47065	470654002

Table continues next page^a The Federal Information Processing Standard Codes are uniform designations for geographic entities throughout all federal agencies.

Table B.7 (Continued). Speciation Monitors Considered for Each City

City Number	City Name	MSA Code	FIPS Code ^a	AQS Site ID
29	Chicago, IL	1600	17031	170310057
29	Chicago, IL	1600	17031	170310076
29	Chicago, IL	1600	17031	170314201
29	Chicago, IL	1600	17043	170434002
30	Chico, CA	1620	6007	60070002
31	Cincinnati, OH	1640	21117	211170007
31	Cincinnati, OH	1640	39061	390610040
31	Cincinnati, OH	1640	39061	390610042
31	Cincinnati, OH	1640	39061	390618001
32	Clarksville, TN	1660	47125	471251009
33	Cleveland, OH	1680	39035	390350038
33	Cleveland, OH	1680	39035	390350060
33	Cleveland, OH	1680	39093	390930016
33	Cleveland, OH	1680	39093	390933002
34	Colorado Springs, CO	1720	8041	80410011
35	Columbia, SC	1760	45079	450790019
36	Columbus, GA	1800	1113	11130001
36	Columbus, GA	1800	13215	132150011
37	Columbus, OH	1840	39049	390490081
38	Corpus Christi, TX	1880	48355	483550034
39	Dallas, TX	1920	48113	481130050
39	Dallas, TX	1920	48113	481130069
39	Dallas, TX	1920	48139	481390015
39	Dallas, TX	1920	48257	482570005
40	Davenport, IA	1960	19163	191630015
41	Dayton, OH	2000	39113	391130031
41	Dayton, OH	2000	39113	391130032
42	Decatur, AL	2030	1103	11030011
43	Decatur, IL	2040	17115	171150013
44	Denver, CO	2080	8001	80010006
45	Des Moines, IA	2120	19153	191530030
45	Des Moines, IA	2120	19153	191532520
46	Detroit, MI	2160	26115	261150005
46	Detroit, MI	2160	26125	261250010
46	Detroit, MI	2160	26163	261630001
46	Detroit, MI	2160	26163	261630019
46	Detroit, MI	2160	26163	261630033
47	Dover, DE	2190	10001	100010003
48	El Paso, TX	2320	48141	481410044
48	El Paso, TX	2320	48141	481410053

Table continues next page

^a The Federal Information Processing Standard Codes are uniform designations for geographic entities throughout all federal agencies.

Table B.7 (Continued). Speciation Monitors Considered for Each City

City Number	City Name	MSA Code	FIPS Code ^a	AQS Site ID
49	Elkhart, IN	2330	18039	180390003
49	Elkhart, IN	2330	18039	180390008
50	Erie, PA	2360	42049	420490003
51	Eugene, OR	2400	41039	410390060
52	Evansville, IN	2440	18163	181630012
53	Fargo, ND	2520	38017	380171004
54	Fayetteville, NC	2560	37051	370510009
55	Fort Lauderdale, FL	2680	12011	120111002
56	Fort Wayne, IN	2760	18003	180030004
57	Fresno, CA	2840	6019	60190008
58	Galveston, TX	2920	48167	481670014
59	Gary, IN	2960	18089	180890022
59	Gary, IN	2960	18089	180892004
60	Grand Junction, CO	2995	8077	80770003
60	Grand Junction, CO	2995	8077	80770017
61	Grand Rapids, MI	3000	26005	260050003
61	Grand Rapids, MI	3000	26081	260810020
62	Greeley, CO	3060	8123	81230008
63	Greensboro, NC	3120	37057	370570002
63	Greensboro, NC	3120	37067	370670022
63	Greensboro, NC	3120	37081	370810013
64	Greenville, SC	3160	45045	450450008
64	Greenville, SC	3160	45045	450450009
65	Hamilton, OH	3200	39017	390171004
66	Harrisburg, PA	3240	42043	420430401
66	Harrisburg, PA	3240	42099	420990301
67	Hattiesburg, MS	3285	28035	280350004
68	Hickory, NC	3290	37035	370350004
69	Honolulu, HI	3320	15003	150032004
70	Houston, TX	3360	48201	482010024
70	Houston, TX	3360	48201	482010026
70	Houston, TX	3360	48201	482010055
70	Houston, TX	3360	48201	482010803
70	Houston, TX	3360	48201	482011034
70	Houston, TX	3360	48201	482011039
70	Houston, TX	3360	48339	483390078
70	Houston, TX	3360	48339	483390089
71	Huntington, WV	3400	21019	210190017
71	Huntington, WV	3400	39087	390870010
71	Huntington, WV	3400	39087	390870012

Table continues next page^a The Federal Information Processing Standard Codes are uniform designations for geographic entities throughout all federal agencies.

Table B.7 (Continued). Speciation Monitors Considered for Each City

City Number	City Name	MSA Code	FIPS Code ^a	AQS Site ID
72	Huntsville, AL	3440	1089	10890014
73	Indianapolis, IN	3480	18097	180970078
74	Jackson, MS	3560	28049	280490018
75	Johnson City, TN	3660	47163	471631007
75	Johnson City, TN	3660	51520	515200006
76	Kalamazoo, MI	3720	26077	260770008
77	Kansas City, MO	3760	20209	202090021
77	Kansas City, MO	3760	29047	290470005
78	Kenosha, WI	3800	55059	550590019
79	Knoxville, TN	3840	47093	470931020
80	Lancaster, PA	4000	42071	420710007
81	Las Vegas, NV	4120	32003	320030020
81	Las Vegas, NV	4120	32003	320030560
81	Las Vegas, NV	4120	32003	320030561
82	Lexington, KY	4280	21067	210670012
83	Little Rock, AR	4400	5119	51190007
84	Longview, TX	4420	48203	482030002
85	Los Angeles, CA	4480	6037	60371103
86	Louisville, KY	4520	21111	211110043
86	Louisville, KY	4520	21111	211110048
87	Lowell, MA	4560	33011	330110020
88	Lubbock, TX	4600	48303	483030001
89	Macon, GA	4680	13021	130210007
90	Medford, OR	4890	41029	410290133
91	Memphis, TN	4920	47157	471570024
91	Memphis, TN	4920	47157	471570047
92	Miami, FL	5000	12086	120861016
93	Middlesex, NJ	5015	34023	340230006
94	Milwaukee, WI	5080	55079	550790026
94	Milwaukee, WI	5080	55133	551330027
95	Minneapolis, MN	5120	27053	270530963
95	Minneapolis, MN	5120	27123	271230871
96	Missoula, MT	5140	30063	300630024
96	Missoula, MT	5140	30063	300630031
97	Mobile, AL	5160	1097	10970003
98	Modesto, CA	5170	6099	60990005
99	Montgomery, AL	5240	1101	11011002
100	Nashville, TN	5360	47037	470370023
100	Nashville, TN	5360	47165	471650007
101	New York, NY	5600	36005	360050083

Table continues next page

^a The Federal Information Processing Standard Codes are uniform designations for geographic entities throughout all federal agencies.

Table B.7 (Continued). Speciation Monitors Considered for Each City

City Number	City Name	MSA Code	FIPS Code ^a	AQS Site ID
101	New York, NY	5600	36005	360050110
101	New York, NY	5600	36061	360610062
101	New York, NY	5600	36061	360610134
101	New York, NY	5600	36081	360810124
102	Newark, NJ	5640	34027	340273001
102	Newark, NJ	5640	34039	340390004
103	Oklahoma City, OK	5880	40109	401091037
104	Omaha, NE	5920	31055	310550019
105	Owensboro, KY	5990	21059	210590005
105	Owensboro, KY	5990	21059	210590014
106	Pensacola, FL	6080	12033	120330004
107	Philadelphia, PA	6160	34007	340070003
107	Philadelphia, PA	6160	42029	420290100
107	Philadelphia, PA	6160	42045	420450002
107	Philadelphia, PA	6160	42101	421010004
107	Philadelphia, PA	6160	42101	421010055
107	Philadelphia, PA	6160	42101	421010136
108	Phoenix, AZ	6200	4013	40130019
108	Phoenix, AZ	6200	4013	40134009
108	Phoenix, AZ	6200	4013	40137003
108	Phoenix, AZ	6200	4013	40137020
108	Phoenix, AZ	6200	4013	40138006
108	Phoenix, AZ	6200	4013	40139997
108	Phoenix, AZ	6200	4013	40139998
109	Pittsburgh, PA	6280	42003	420030008
109	Pittsburgh, PA	6280	42003	420030021
109	Pittsburgh, PA	6280	42003	420030064
109	Pittsburgh, PA	6280	42125	421255001
109	Pittsburgh, PA	6280	42129	421290008
110	Portland, OR	6440	41051	410510080
110	Portland, OR	6440	41051	410510246
111	Providence, RI	6480	44007	440070022
111	Providence, RI	6480	44007	440071010
112	Provo, UT	6520	49049	490494001
113	Raleigh, NC	6640	37183	371830014
114	Reading, PA	6680	42011	420110010
114	Reading, PA	6680	42011	420110011
115	Reno, NV	6720	32031	320310016
116	Richmond, VA	6760	51087	510870014
116	Richmond, VA	6760	51760	517600020

Table continues next page^a The Federal Information Processing Standard Codes are uniform designations for geographic entities throughout all federal agencies.

Table B.7 (Continued). Speciation Monitors Considered for Each City

City Number	City Name	MSA Code	FIPS Code ^a	AQS Site ID
117	Riverside, CA	6780	6065	60658001
118	Roanoke, VA	6800	51770	517700014
119	Rochester, MN	6820	27109	271095008
120	Rochester, NY	6840	36055	360551007
120	Rochester, NY	6840	36055	360556001
121	Sacramento, CA	6920	6067	60670006
121	Sacramento, CA	6920	6067	60670010
122	St. Louis, MO	7040	17119	171192009
122	St. Louis, MO	7040	17119	171190024
122	St. Louis, MO	7040	29510	295100089
122	St. Louis, MO	7040	29099	290990012
122	St. Louis, MO	7040	29099	290990019
122	St. Louis, MO	7040	29510	295100085
123	Salt Lake City, UT	7160	49035	490353006
123	Salt Lake City, UT	7160	49011	490110001
123	Salt Lake City, UT	7160	49011	490110004
124	San Diego, CA	7320	6073	60730003
124	San Diego, CA	7320	6073	60731002
125	San Jose, CA	7400	6085	60850005
125	San Jose, CA	7400	6085	60850004
126	Savannah, GA	7520	13051	130510017
127	Scranton, PA	7560	42069	420692006
128	Seattle, WA	7600	53033	530330048
128	Seattle, WA	7600	53033	530330024
128	Seattle, WA	7600	53033	530330032
128	Seattle, WA	7600	53033	530330038
128	Seattle, WA	7600	53033	530330057
128	Seattle, WA	7600	53033	530330080
129	Shreveport, LA	7680	22015	220150008
130	Sioux Falls, SD	7760	46099	460990006
131	South Bend, IN	7800	18141	181411008
132	Spokane, WA	7840	53063	530630016
133	Springfield, MA	8000	25013	250130008
134	State College, PA	8050	42027	420270100
135	Steubenville, OH	8080	39081	390810017
135	Steubenville, OH	8080	39081	390811001

Table continues next page

^a The Federal Information Processing Standard Codes are uniform designations for geographic entities throughout all federal agencies.

Table B.7 (Continued). Speciation Monitors Considered for Each City

City Number	City Name	MSA Code	FIPS Code ^a	AQS Site ID
136	Tacoma, WA	8200	53053	530530029
137	Tallahassee, FL	8240	12073	120730012
138	Tampa, FL	8280	12057	120571075
138	Tampa, FL	8280	12057	120573002
138	Tampa, FL	8280	12103	121030026
139	Toledo, OH	8400	39095	390950026
140	Tucson, AZ	8520	4019	40191028
141	Tulsa, OK	8560	40143	401431127
142	Ventura, CA	8735	6111	61112002
143	Visalia, CA	8780	6107	61072002
144	Washington, DC	8840	11001	110010042
144	Washington, DC	8840	11001	110010043
144	Washington, DC	8840	24033	240330030
145	Wheeling, WV	9000	54051	540511002
146	Wichita, KS	9040	20173	201730010
147	Wilmington, DE	9160	10003	100032004
148	Yakima, WA	9260	53077	530770009
149	York, PA	9280	42133	421330008
150	Youngstown, OH	9320	39099	390990014

^a The Federal Information Processing Standard Codes are uniform designations for geographic entities throughout all federal agencies.

Table B.8. Factor Loadings (Correlations Between Pollutants and Source Categories) for the Nationwide 64-Cities Factor Analysis

Pollutant	Traffic	Soil	Metals	Coal Combustion	Salt	Residual Oil Combustion
NO ₂	0.78	0.07	0.18	0.05	-0.06	0.14
SO ₂	0.35	0.06	0.12	0.36	-0.05	0.27
CO	0.80	0.07	0.10	-0.07	-0.01	0.01
Al	-0.04	0.88	0.01	0.01	0.01	0.03
As	0.24	0.03	0.09	0.51	-0.02	-0.07
Br	0.48	0.08	0.08	0.37	0.32	0.10
Ca	0.23	0.68	0.19	-0.05	0.06	0.02
Cl	0.05	0.02	0.11	0.03	0.80	-0.04
Cu	0.45	0.07	0.20	-0.06	-0.10	0.02
EC	0.80	0.12	0.20	0.08	0.03	0.06
Fe	0.31	0.58	0.59	0.03	0.02	0.08
Hg	-0.01	0.04	-0.09	0.52	-0.01	-0.07
K	0.35	0.42	0.05	0.19	0.16	-0.02
Mn	0.12	0.23	0.76	-0.01	0.03	0.05
Na	-0.03	0.06	-0.02	-0.01	0.80	0.09
Ni	0.02	0.02	0.09	-0.02	-0.01	0.80
OC	0.78	0.12	0.08	0.19	0.09	0.03
Pb	0.25	0.02	0.44	0.32	0.05	0.00
Se	0.09	0.02	0.13	0.69	0.03	0.08
Si	0.05	0.93	0.06	0.05	0.03	0.02
Ti	0.12	0.84	0.08	-0.01	0.02	0.03
V	0.23	0.14	0.03	0.08	0.10	0.67
Zn	0.25	0.02	0.75	0.12	0.07	0.06

Table B.9. Convergence Status of Regression Results, Based on the Default Generalized Linear Model Control^a, for Data Displayed in Figures 4 Through 12

Outcome	Excluded Cities (City No.) ^c	Convergence of Components or Source Categories ^b		
		All Year City No. (lag days)	Warm Season City No. (lag days)	Cold Season City No. (lag days)
Figure 4, Mortality, 150 Cities; PM_{2.5}				
All-cause	8	All converged	All converged	All converged
CVD	8	All converged	All converged	All converged
Respiratory	8	All converged	All converged	All converged
Figure 5, CVD Hospitalizations, 150 Cities; PM_{2.5}				
All-CVD		All converged	All converged	All converged
Acute myocardial infarction	17, 62	5 (3), 96 (0,2,3), 143 (2)	5 (3), 143 (2)	5 (3), 143 (2)
Ischemic heart disease	17, 60, 62	30 (0,1,3), 32 (3), 96 (2), 98 (2), 115 (1,2), 134 (2), 137 (0,3), 143 (0,1,3)	32 (3), 98 (2), 115 (1,2), 134 (2), 137 (0,3), 143 (0,1,3)	32 (3), 98 (2), 115 (1,2), 134 (2), 137 (0,3), 143 (0,1,3)
Dysrhythmias	17, 60, 62	43 (1), 134 (1), 143 (1,3)	43 (1), 134 (1), 143 (1,3)	43 (1), 134 (1), 143 (1,3)
Heart failure	17, 30, 53, 60, 62, 96	All converged	All converged	All converged
Cerebrovascular disease	17, 30, 53, 60, 62, 96	All converged	All converged	All converged
Figure 6, Respiratory Hospitalizations, 150 Cities; PM_{2.5}				
Respiratory		17 (1), 30 (1)	17 (1), 30 (1)	17 (1), 30 (1)
Pneumonia	17, 30, 53, 60, 62, 96	All converged	All converged	All converged
COPD	17, 30, 53, 60, 62, 96	119 (2), 148 (2)	119 (2), 143 (0,3), 143 (2)	119 (2), 143 (0,3), 143 (2)

Table continues next page^a Epsilon = 1e-08; maximum iterations = 25 in the GLM function of R Software. See Appendix Table B.1 for the list of cities and numbers.^b The pollutant or source category did not converge at the lag day given in parentheses after the city number. For example, in the rows for Figure 5 for acute myocardial infarction, the regression for city 5 (Anchorage, AK) did not converge at the 3-day lag.^c Cities listed failed to run because of small daily counts of hospitalizations or deaths (i.e., they were small cities).

Table B.9 (Continued). Convergence Status of Regression Results, Based on the Default Generalized Linear Model Control^a, for Data Displayed in Figures 4 Through 12

Outcome	Excluded Cities (City No.) ^c	Convergence of Components or Source Categories ^b		
		All Year City No. (lag days)	Warm Season City No. (lag days)	Cold Season City No. (lag days)
Figure 7, All-Cause Mortality, 64 Cities; PM_{2.5}, Its Components, and Gaseous Pollutants				
All-cause		PM _{2.5} and gases — 92 (2,3); All components except Cu, Na, OC, SO ₄ ²⁻ — 92 (0,1); Cu, Na, OC, SO ₄ ²⁻ — 92 (0,1,2)	PM _{2.5} and gases — 92 (2,3); All components except Cu, Ni, NO ₃ ⁻ , OC — 92 (0,1); Cu, Ni, NO ₃ ⁻ , OC — 92 (0,1,2)	PM _{2.5} and gases — 92 (2,3); All components except Cu, Ni, NO ₃ ⁻ , OC — 92 (0,1); Cu, Ni, NO ₃ ⁻ , OC — 92 (0,1,2)
Figure 8, All-Cause Mortality, 64 Cities; Source Categories (Factor Scores)				
All-cause		All source categories except Metals — 92 (0, 1); Metals — all converged	All source categories except Metals — 92 (0, 1); Metals — all converged	All source categories except Metals — 92 (0, 1); Metals — all converged
Figure 9, CVD Hospitalizations, 64 Cities; PM_{2.5}, Its Components, and Gaseous Pollutants				
CVD		PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2)	PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2)	PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2)

Table continues next page

^a Epsilon = 1e-08; maximum iterations = 25 in the GLM function of R Software. See Appendix Table B.1 for the list of cities and numbers.

^b The pollutant or source category did not converge at the lag day given in parentheses after the city number. For example, in the rows for Figure 5 for acute myocardial infarction, the regression for city 5 (Anchorage, AK) did not converge at the 3-day lag.

^c Cities listed failed to run because of small daily counts of hospitalizations or deaths (i.e., they were small cities).

Table B.9 (Continued). Convergence Status of Regression Results, Based on the Default Generalized Linear Model Control^a, for Data Displayed in Figures 4 Through 12

Outcome	Excluded Cities (City No.) ^c	Convergence of Components or Source Categories ^b		
		All Year City No. (lag days)	Warm Season City No. (lag days)	Cold Season City No. (lag days)
Figure 10, CVD Hospitalizations, 64 Cities; Source Categories (Factor Scores)				
CVD		Traffic, Salt, and Residual Oil Combustion — 85 (0,1,2); Soil and Metals — 85 (0,1,2, 3); Coal Combustion — all converged	Traffic, Soil, Salt, Residual Oil Combustion, and Metals — 85 (0,1,2); Coal Combustion — all converged	Traffic, Soil, Salt, Residual Oil Combustion, and Metals — 85 (0,1,2); Coal Combustion — all converged
Figure 11, Respiratory Hospitalizations, 64 Cities; PM_{2.5}, Its Components, and Gaseous Pollutants				
Respiratory		PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 81 (0,1), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2)	PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 8 (0,1), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2), 95 (0)	PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 81 (0,1), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2), 95 (0)
Figure 12, Respiratory Hospitalizations, 64 Cities; Source Categories (Factor Scores)				
Respiratory		Coal Combustion — all converged; Traffic, Soil, Residual Oil Combustion— 85 (0,1,2); Salt — 85 (0,1,2), 95 (0); Metals — 85 (0,1,2,3)	Coal Combustion — all converged; Traffic, Salt — 85 (0,1,2), 95 (0); Soil — 85 (0,1,2); Residual Oil Combustion — 85 (0,1,2,3), 95 (0); Metals — 85 (0,1,2,3)	Coal Combustion — all converged; Traffic, Salt — 85 (0,1,2), 95 (0); Soil — 85 (0,1,2); Residual Oil Combustion — 85 (0,1,2,3), 95 (0); Metals — 85 (0,1,2,3)

^a Epsilon = 1e-08; maximum iterations = 25 in the GLM function of R Software. See Appendix Table B.1 for the list of cities and numbers.

^b The pollutant or source category did not converge at the lag day given in parentheses after the city number. For example, in the rows for Figure 5 for acute myocardial infarction, the regression for city 5 (Anchorage, AK) did not converge at the 3-day lag.

^c Cities listed failed to run because of small daily counts of hospitalizations or deaths (i.e., they were small cities).

Table B.10. Convergence Status of Regression Results, Based on the Default Generalized Linear Model Control^a, for Data Displayed in Figures 4 through 12

Outcome	Excluded Cities (City No.) ^c	Convergence of Components or Source Categories ^b		
		All Year City No. (lag days)	Warm Season City No. (lag days)	Cold Season City No. (lag days)
Figure B.4, All-Cause Mortality, 150 Cities; PM_{2.5} and Its Components				
All-cause	2, 32, 84, 88, 105, 148	PM _{2.5} — 54 (3), 56 (3), 67 (2,3), 78 (2,3), 87 (2,3), 92 (2,3), 118 (3), 129 (0,1,2), 131 (3); All components except Cu, Ni, NO ₃ ⁻ , and OC — 54 (3), 92 (0,1); Cu, Ni, NO ₃ ⁻ , and OC — 54 (3), 92 (0,1,2)	PM _{2.5} — 54 (3), 56 (3), 67 (2,3), 78 (2,3), 87 (2,3), 92 (2,3), 118 (3), 129 (0,1,2), 131 (3); All components except Cu, Ni, NO ₃ ⁻ , and OC — 54 (3), 92 (0,1); Cu, Ni, NO ₃ ⁻ , and OC — 54 (3), 92 (0,1,2)	PM _{2.5} — 54 (3), 56 (3), 67 (2,3), 78 (2,3), 87 (2,3), 92 (2,3), 118 (3), 129 (0,1,2), 131 (3); All components except Cu, Ni, NO ₃ ⁻ , and OC — 54 (3), 92 (0,1); Cu, Ni, NO ₃ ⁻ , and OC — 54 (3), 92 (0,1,2)
Figure B.4, CVD Hospitalizations, 150 Cities; PM_{2.5} and Its Components				
All-CVD	2, 17, 32, 34, 84, 88, 105, 148	PM _{2.5} — 8 (0,1,2,3), 14 (0,1,2,3), 35 (1,2,3), 54 (3), 56 (3), 78 (2,3), 92 (2,3), 118 (3), 131 (3); All components except EC and OC — 60 (0), 126 (0,1,2,3); EC and OC — 18 (0,1,2,3), 26 (0,1,2,3), 36 (0,1,2,3), 60 (0), 85 (0,1,2)	PM _{2.5} — 8 (0,1,2,3), 14 (0,1,2,3), 35 (1,2,3), 54 (3), 56 (3), 78 (2,3), 92 (2,3), 118 (3), 131 (3); All components except EC and OC — 60 (0), 126 (0,1,2,3); EC and OC — 18 (0,1,2,3), 26 (0,1,2,3), 36 (0,1,2,3), 60 (0), 85 (0,1,2)	PM _{2.5} — 8 (0,1,2,3), 14 (0,1,2,3), 35 (1,2,3), 54 (3), 56 (3), 78 (2,3), 92 (2,3), 118 (3), 131 (3); All components except EC and OC — 60 (0), 126 (0,1,2,3); EC and OC — 18 (0,1,2,3), 26 (0,1,2,3), 36 (0,1,2,3), 60 (0), 85 (0,1,2)
Figure B.4, Respiratory Hospitalizations, 150 Cities; PM_{2.5} and Its Components				
Respiratory	2, 17, 32, 34, 84, 88, 105, 148	PM _{2.5} — 8 (0,1,2,3), 14 (0,1,2,3), 35 (1,2,3), 54 (3), 56 (3), 78 (2,3), 92 (2,3), 131 (3); All components except EC and OC — 126 (0,1,2,3); EC and OC — 18 (0,1,2,3), 26 (0,1,2,3), 36 (0,1,2,3), 85 (0,1,2), 112 (2), 135 (1)	PM _{2.5} — 8 (0,1,2,3), 14 (0,1,2,3), 35 (1,2,3), 54 (3), 56 (3), 78 (2,3), 92 (2,3), 131 (3); All components except EC and OC — 126 (0,1,2,3); EC and OC — 18 (0,1,2,3), 26 (0,1,2,3), 36 (0,1,2,3), 85 (0,1,2), 95 (0), 112 (2), 135 (1)	PM _{2.5} — 8 (0,1,2,3), 14 (0,1,2,3), 35 (1,2,3), 54 (3), 56 (3), 78 (2,3), 92 (2,3), 131 (3); All components except EC and OC — 126 (0,1,2,3); EC and OC — 18 (0,1,2,3), 26 (0,1,2,3), 36 (0,1,2,3), 85 (0,1,2), 95 (0), 112 (2), 135 (1)

Table continues next page

^a Epsilon = 1e-08; maximum iterations = 25 in the GLM function of R Software. See Appendix Table B.1 for the list of cities and numbers.

^b The pollutant or source category did not converge at the lag day given in parentheses after the city number. For example, in the rows for Figure B.4 All-Cause Mortality, the regression for city 54 (Fayetteville, NC) did not converge at the 3-day lag.

^c Cities listed failed to run because of small daily counts of hospitalizations or deaths (i.e., they were small cities).

Table B.10 (Continued). Convergence Status of Regression Results, Based on the Default Generalized Linear Model Control^a, for Data Displayed in Figures 4 through 12

Outcome	Excluded Cities (City No.) ^c	Convergence of Components or Source Categories ^b		
		All Year City No. (lag days)	Warm Season City No. (lag days)	Cold Season City No. (lag days)
Figure B.5, All-Cause Mortality, 64 Cities; PM_{2.5}, Its Components, and Gaseous Pollutants (Untransformed)				
All-cause		PM _{2.5} and gases — 92 (2,3); All components converged except As, Na, NO ₃ ⁻ , Pb, SO ₄ ²⁻ — 92 (0,1); As, Na, NO ₃ ⁻ , Pb, SO ₄ ²⁻ — 92 (0,1,2)	PM _{2.5} and gases — 92 (2,3) As, EC, K, Ni, NO ₃ ⁻ , OC, Pb, Si, V — 92 (0,1,2); Cu, Fe, Na, Se, Zn — 92 (0,1)	PM _{2.5} and gases — 92 (2,3); As, EC, K, Ni, NO ₃ ⁻ , OC, Pb, Si, V — 92 (0,1,2); Cu, Fe, Na, Se, Zn — 92 (0,1)
Figure B.5, CVD Hospitalizations, 64 Cities; PM_{2.5}, Its Components, and Gaseous Pollutants (Untransformed)				
CVD		PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 81 (0), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2)	PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2)	PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 44 (1), 75 (0,1,2,3), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2)

Table continues next page^a Epsilon = 1e-08; maximum iterations = 25 in the GLM function of R Software. See Appendix Table B.1 for the list of cities and numbers.^b The pollutant or source category did not converge at the lag day given in parentheses after the city number. For example, in the rows for Figure B.4 All-Cause Mortality, the regression for city 54 (Fayetteville, NC) did not converge at the 3-day lag.^c Cities listed failed to run because of small daily counts of hospitalizations or deaths (i.e., they were small cities).

Table B.10 (Continued). Convergence Status of Regression Results, Based on the Default Generalized Linear Model Control^a, for Data Displayed in Figures 4 through 12

Outcome	Excluded Cities (City No.) ^c	Convergence of Components or Source Categories ^b		
		All Year City No. (lag days)	Warm Season City No. (lag days)	Cold Season City No. (lag days)
Figure B.5, Respiratory Hospitalizations, 64 Cities; PM_{2.5}, Its Components, and Gaseous Pollutants (Untransformed)				
Respiratory		PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 75 (0,1,2,3), 81 (0), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC — 85 (0,1,2); OC — 85 (0,1,2,3), 95 (0)	PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 1 47 (0,1,2,3); SO ₂ — 75 (0,1,2,3), 81 (0,1), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 85 (0,1,2)	PM _{2.5} — 35 (1,2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); NO ₂ — 35 (1,2,3), 81 (2,3), 92 (2,3), 128 (0,1,2,3), 147 (0,1,2,3); SO ₂ — 75 (0,1,2,3), 81 (0,1), 95 (0), 128 (0,1,2,3); CO — 61 (2,3), 81 (2,3), 92 (2,3), 147 (0,1,2,3); All components converged except EC and OC — 5 (0,1,2)
Figure B.6, All-Cause Mortality, 64 Cities; Source Categories (Factor Scores) from Nationwide Factor Analysis				
All-cause		All source categories — 92 (0,1)	All source categories — 92 (0,1)	All source categories — 92 (0,1)
Figure B.6, CVD Hospitalizations, 64 Cities; Source Categories (Factor Scores) from Nationwide Factor Analysis				
CVD		All source categories — 85 (0,1,2)	All source categories — 85 (0,1,2)	All source categories — 85 (0,1,2)
Figure B.6, Respiratory Hospitalizations, 64 Cities; Source Categories (Factor Scores) from Nationwide Factor Analysis				
Respiratory		Traffic — 85 (0,1,2,3), 95 (0); Soil — 85 (0,1,2,3); Metals, Coal Combustion, Residual Oil Combustion— 85 (0,1,2), 95 (0); Salt — 85 (0,1,2)	Traffic, Soil, Metals — 85 (0,1,2), 95 (0); Coal Combustion — 85 (0,1,2); Salt — 81 (0), 85 (0,1,2,3), 95 (0); Residual Oil Combustion — 85 (0,1,2,3)	Traffic, Soil, Metals — 85 (0,1,2), 95 (0); Coal Combustion — 85 (0,1,2); Salt — 81 (0), 85 (0,1,2,3), 95 (0); Residual Oil Combustion — 85 (0,1,2,3)

^a Epsilon = 1e-08; maximum iterations = 25 in the GLM function of R Software. See Appendix Table B.1 for the list of cities and numbers.

^b The pollutant or source category did not converge at the lag day given in parentheses after the city number. For example, in the rows for Figure B.4 All-Cause Mortality, the regression for city 54 (Fayetteville, NC) did not converge at the 3-day lag.

^c Cities listed failed to run because of small daily counts of hospitalizations or deaths (i.e., they were small cities).

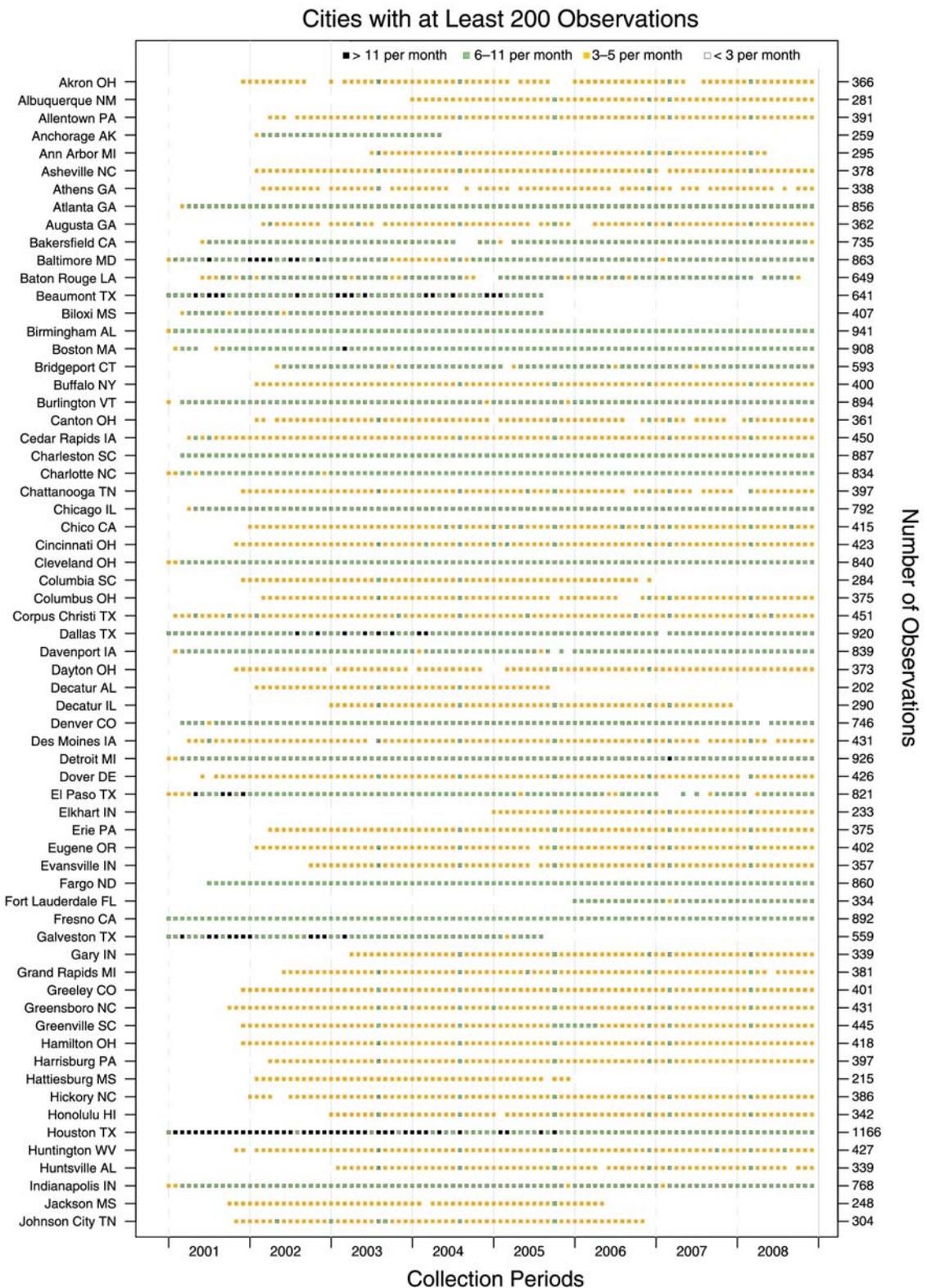


Figure B.1. Sampling frequency patterns in cities with at least 200 observations. Included are the number of observation days and collection periods for the CSN speciation data. (Figure continues next page.)

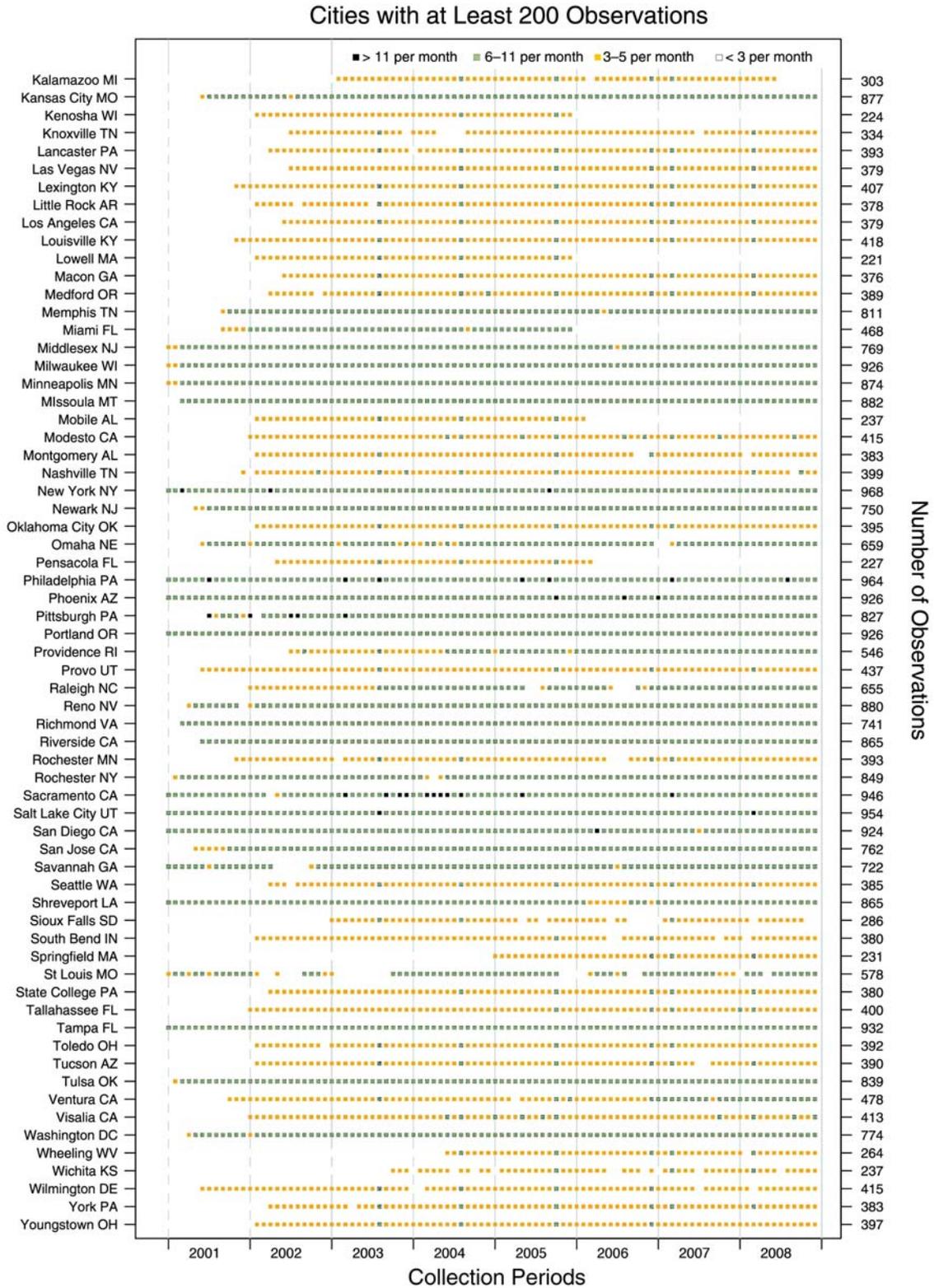


Figure B.1 (Continued).

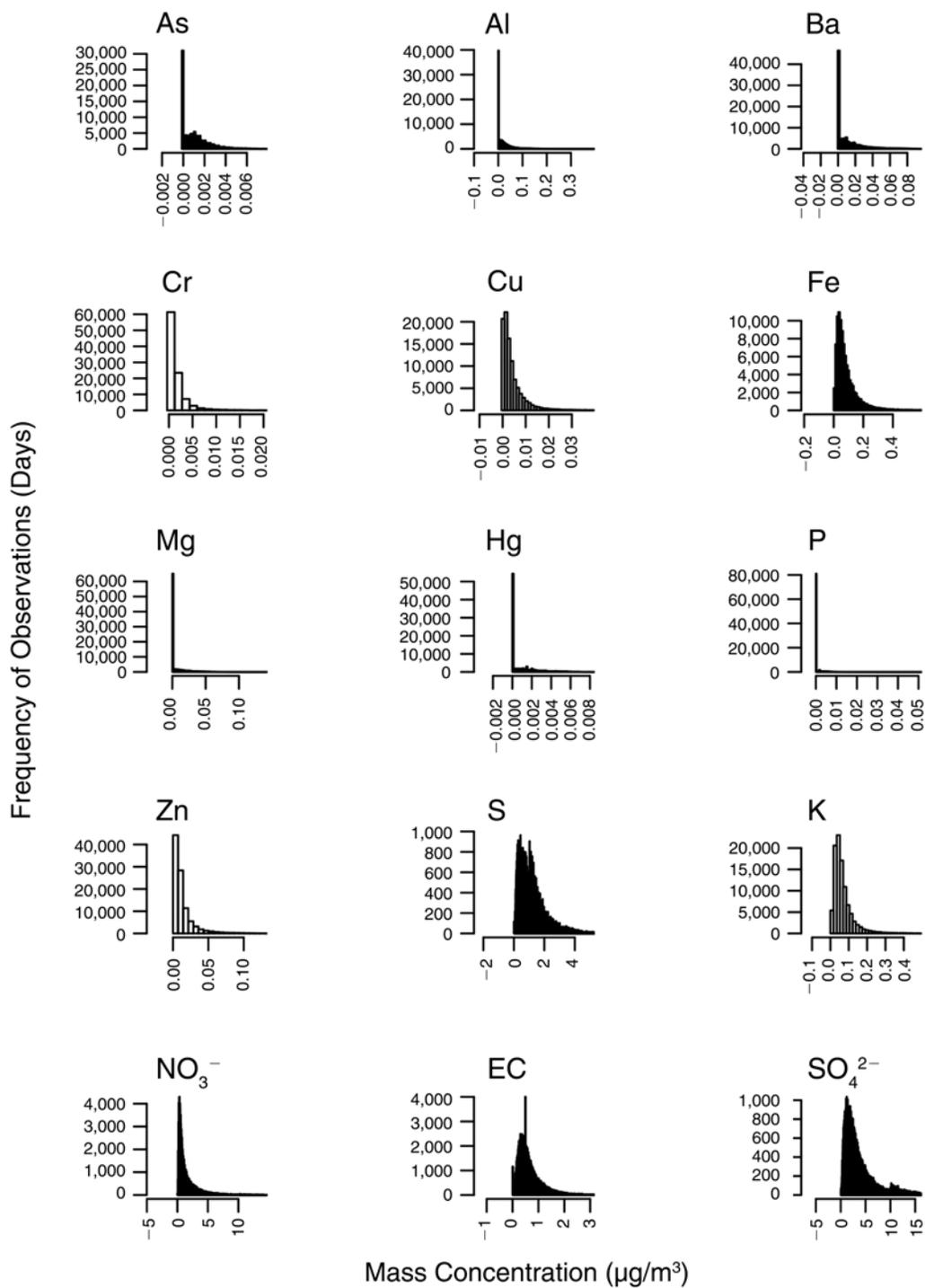


Figure B.2. Histograms of $\text{PM}_{2.5}$ and its 27 chemical components, measured by XRF, in the nationwide $\text{PM}_{2.5}$ chemical speciation data (2000–2007). (Figure continues next page.)

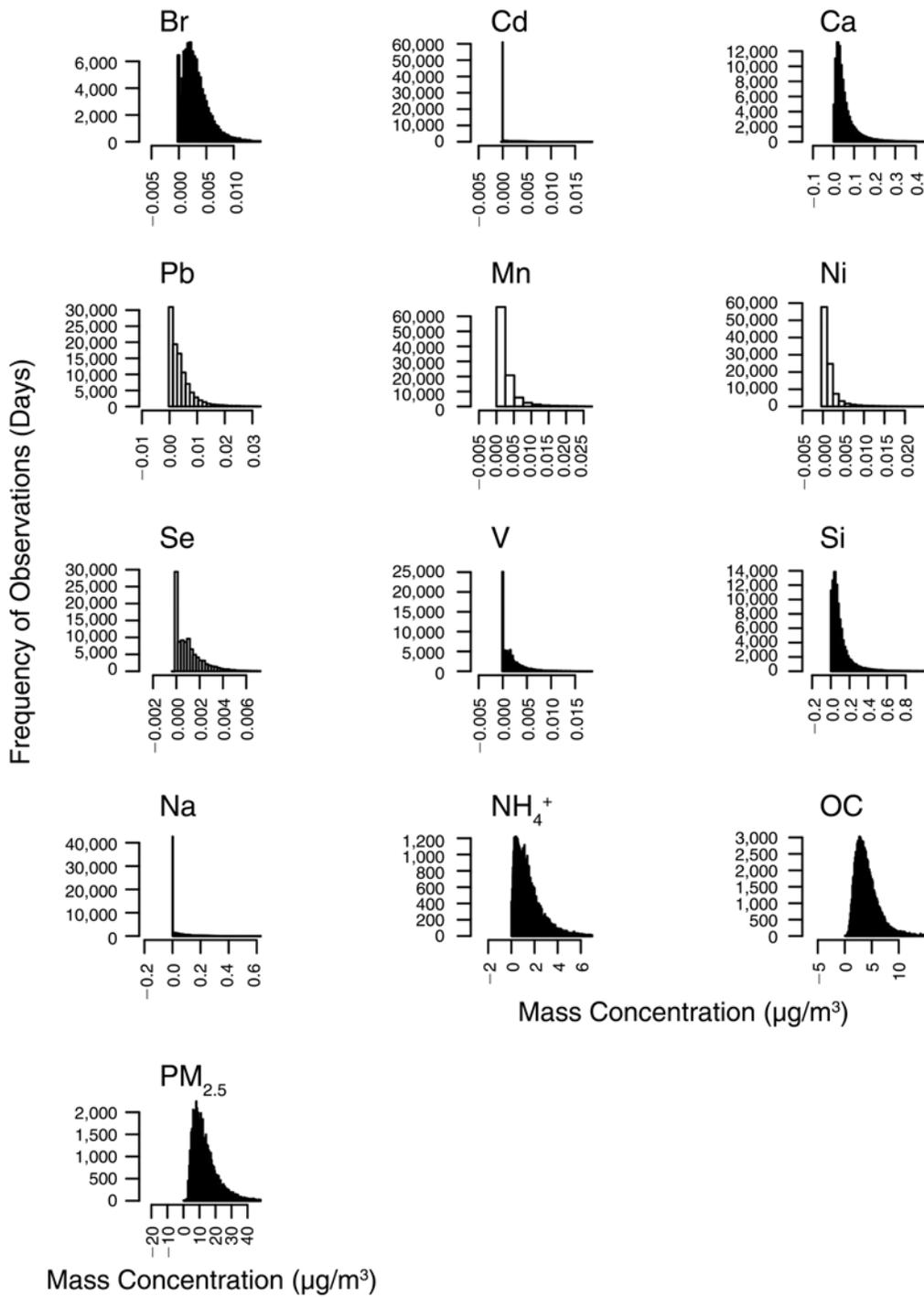


Figure B.2. (Continued).

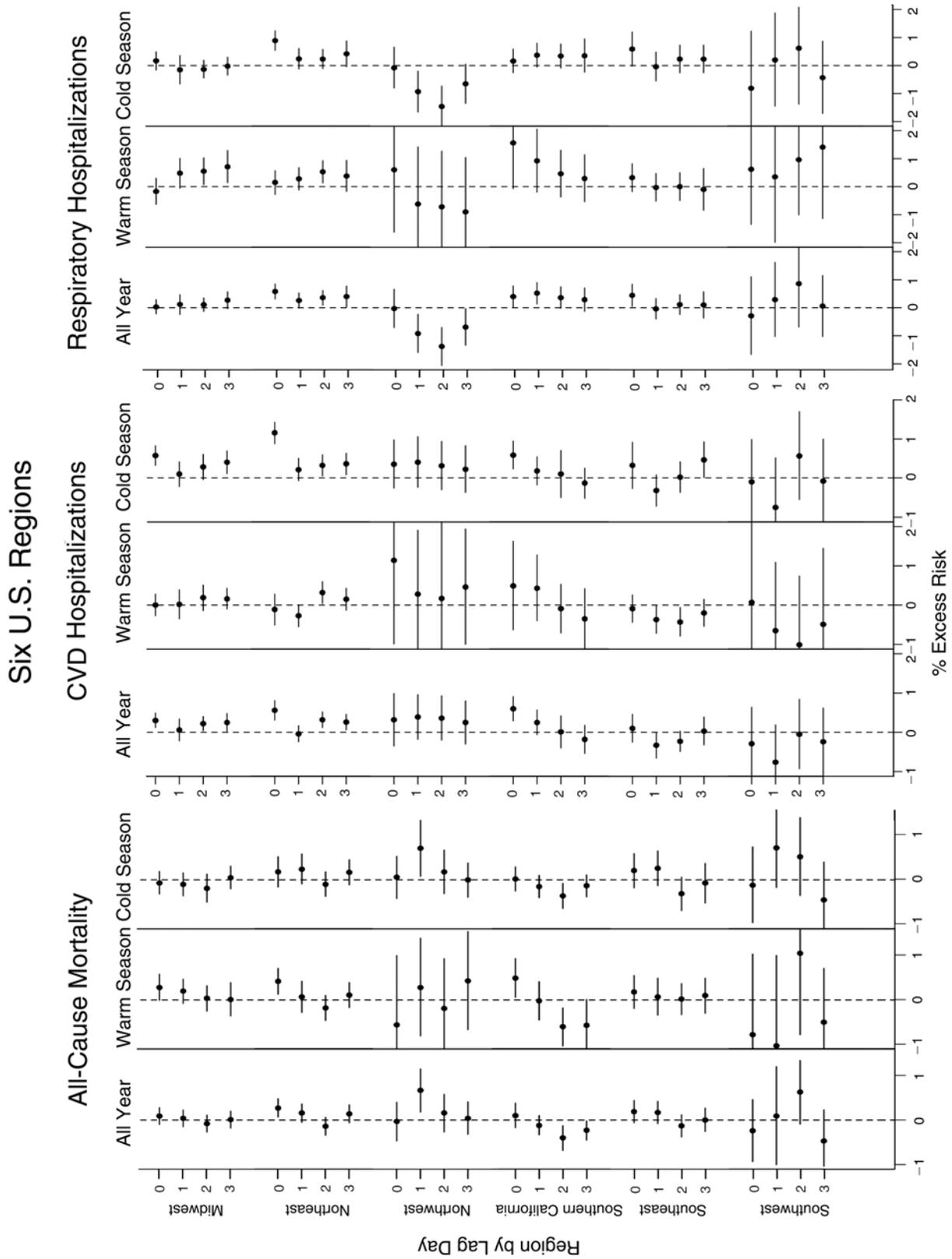


Figure B.3. Association of $PM_{2.5}$ with all-cause mortality and CVD and respiratory hospitalizations in 150 cities (148 for all-cause mortality) summarized for six U.S. regions. The estimated percentage of excess risk at 0- through 3-day lags for all-cause mortality (left panel), CVD hospitalizations (center panel), and respiratory hospitalizations (right panel) is per the median IQR for the 150 cities.

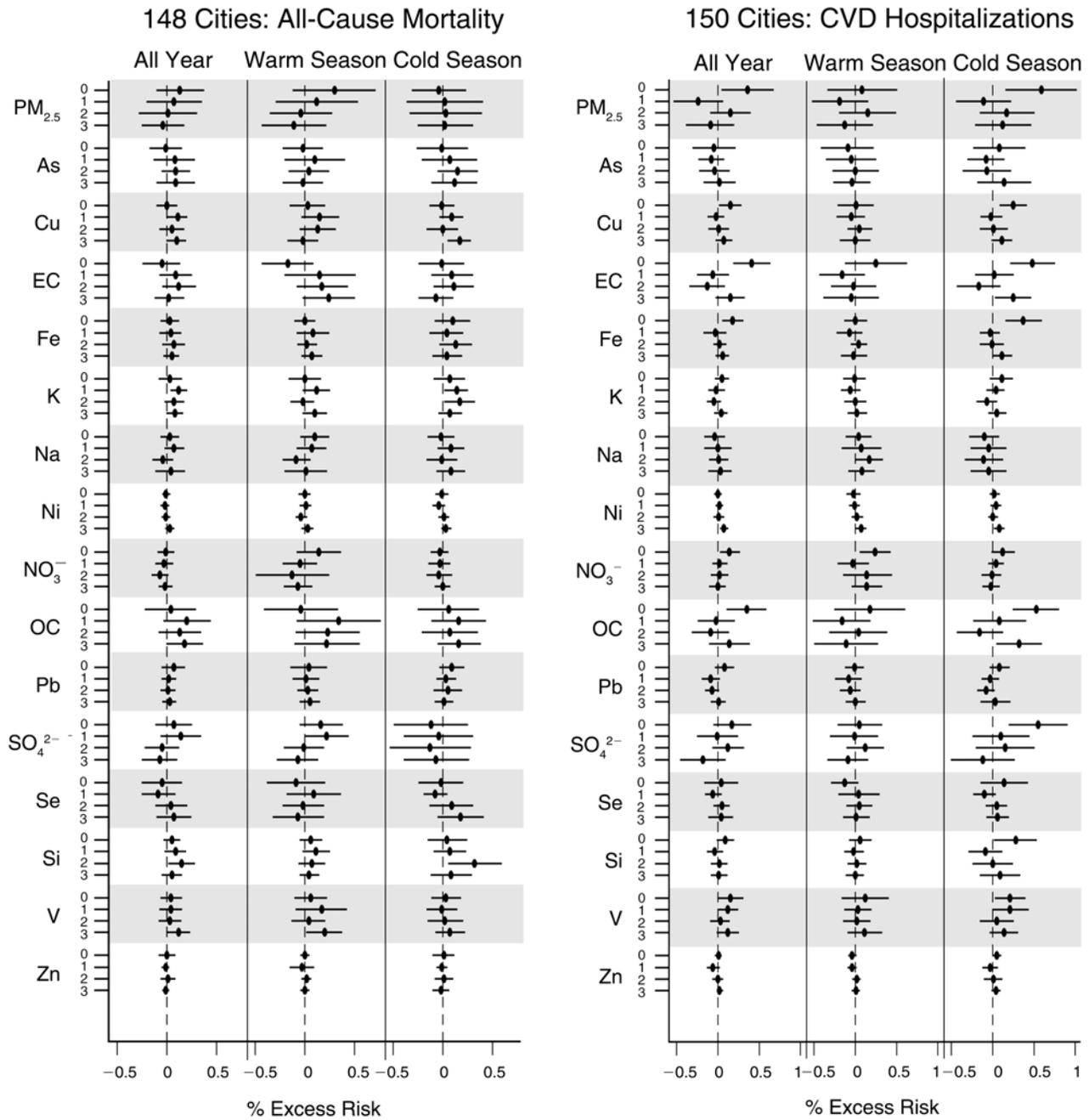


Figure B.4. Association of PM_{2.5} chemical components with all-cause mortality and CVD and respiratory hospitalizations. The estimated percentage of excess risk at 0- through 3-day lags for all-cause mortality (first panel), CVD hospitalizations (second panel), and respiratory hospitalizations (third panel) for PM_{2.5} chemical components is per the median IQR of the pollutants for 148 cities combined (all-cause mortality) or 150 cities combined (CVD and respiratory hospitalizations). (Figure continues next page.)

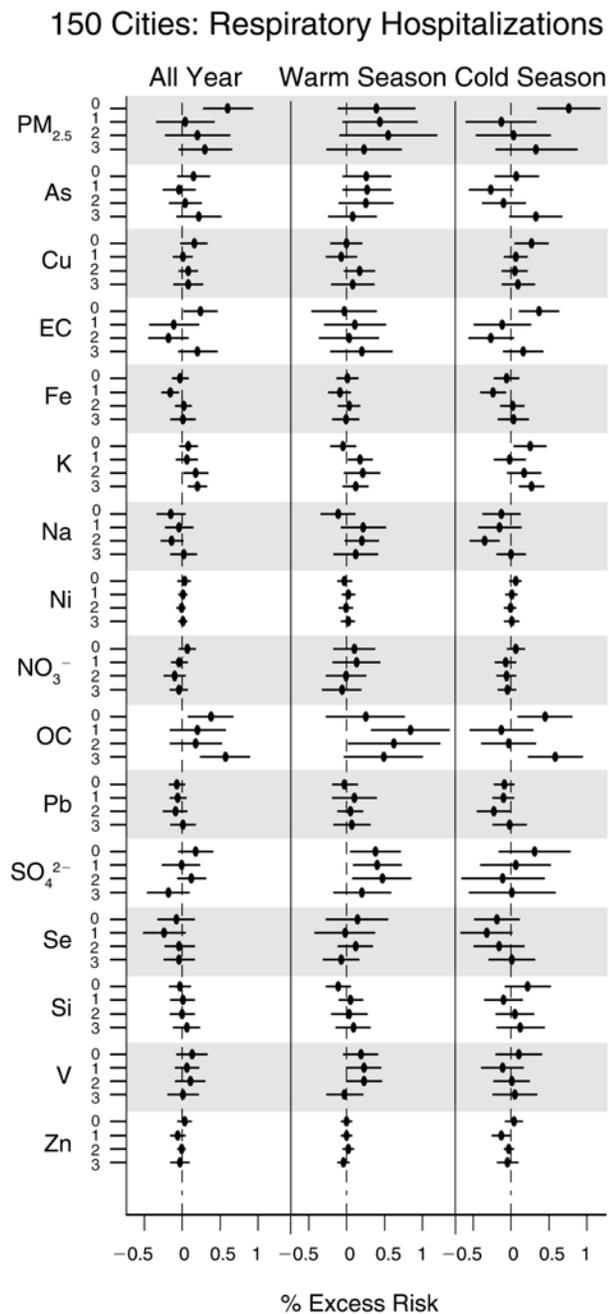


Figure B.4. (Continued)

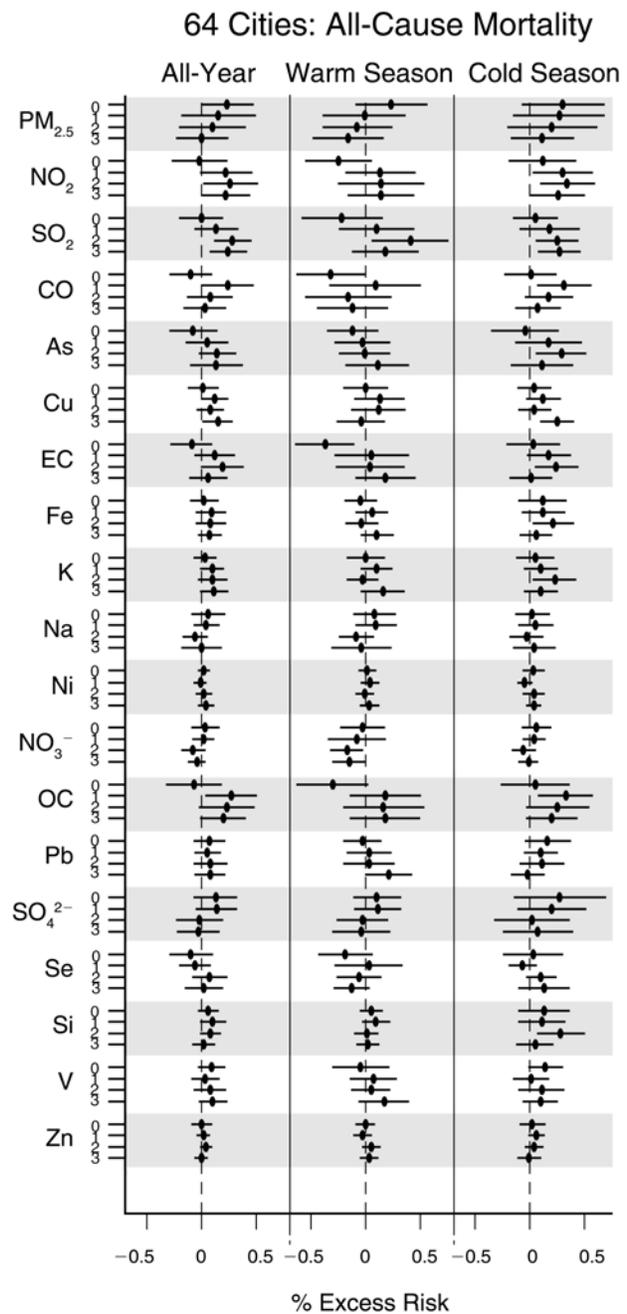


Figure B.5. Association of PM_{2.5}, gaseous pollutants, and PM_{2.5} chemical components with all-cause mortality and CVD and respiratory hospitalizations in 64 cities. The estimated percentage of excess risk at 0- through 3-day lags for all-cause mortality (first panel), CVD hospitalizations (second panel), and respiratory hospitalizations (third panel) is per the median IQR of the pollutants for the 64 cities combined. The results are based on the raw pollution variables (rather than the deviations from the monthly means). (Figure continues next page.)

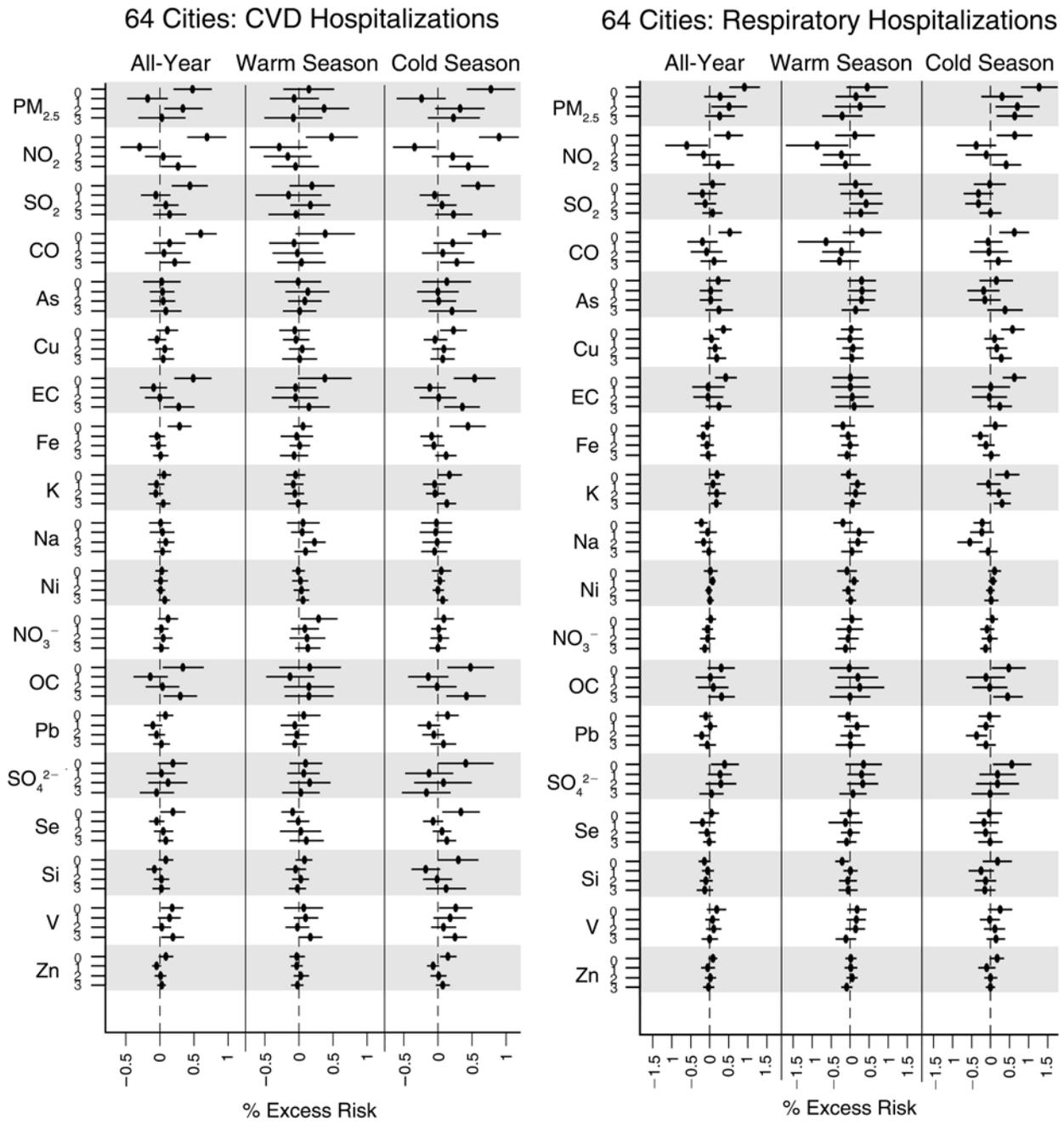


Figure B.5. (Continued)

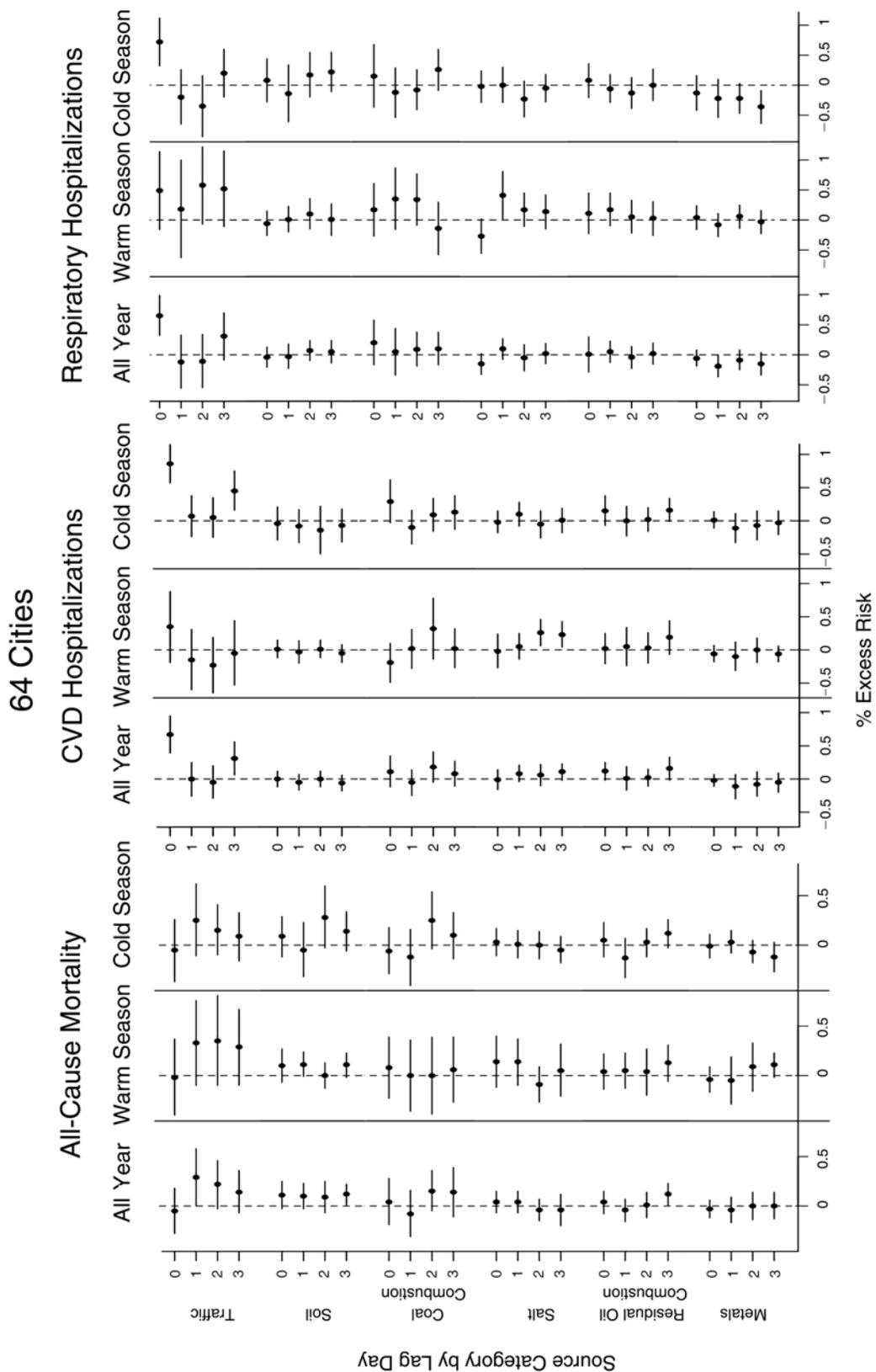


Figure B.6. The estimated percentage of excess risk for all-cause mortality (left panel), CVD hospitalizations (center panel), and respiratory hospitalizations (right panel) using factor scores from the 64-city factor analysis. The result is per the median IQR of the pollutants, at 0- through 3-day lags, for the 64 cities combined.

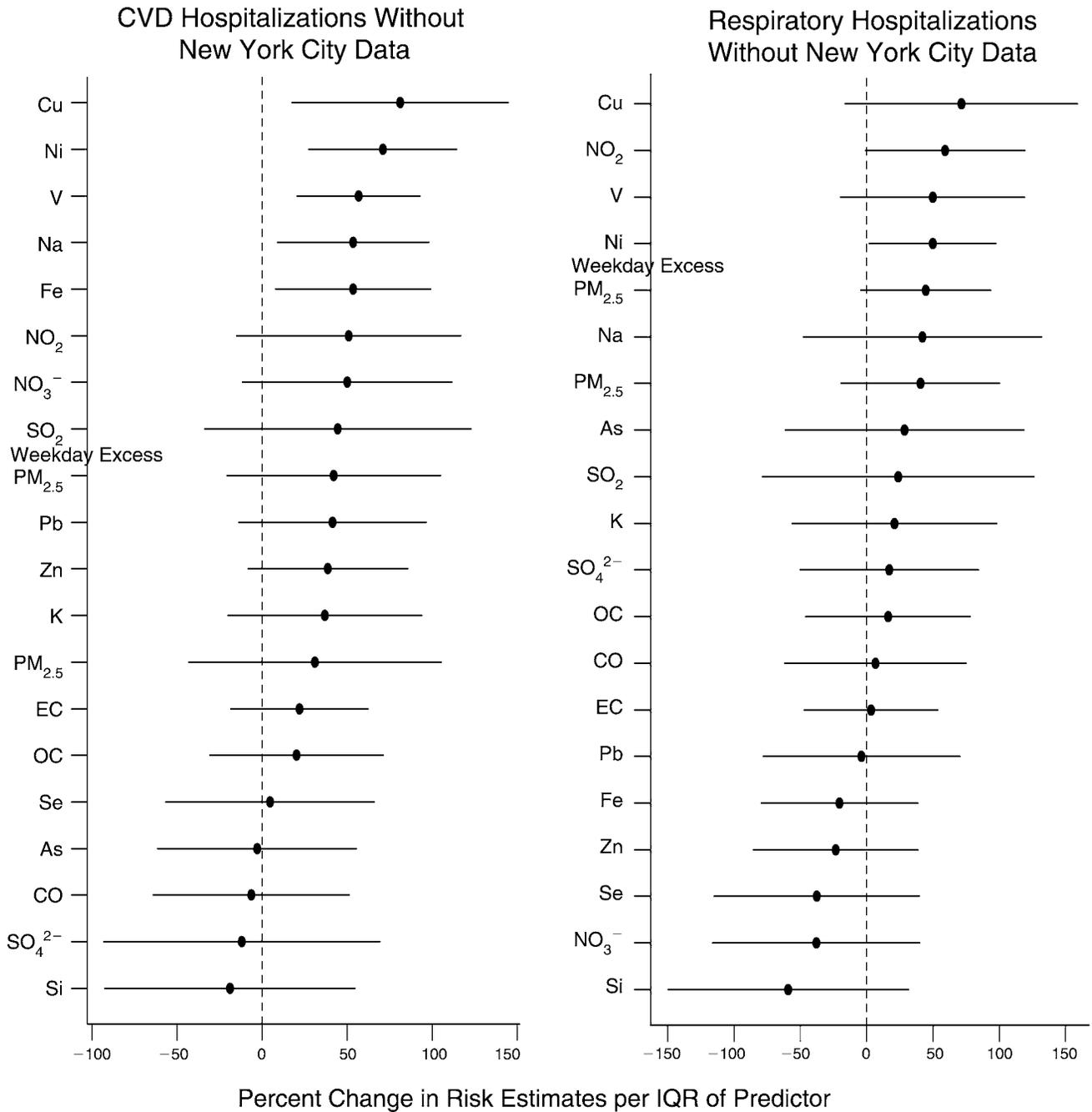


Figure B.7. Zero-day-lag risk estimates without New York City data for CVD hospitalizations (left panel) and respiratory hospitalizations (right panel) associated with city-specific average PM_{2.5} chemical components and gaseous pollutants. The percentage change in 0-day-lag PM_{2.5} risk estimates is per the IQR of the average air pollution indices across cities but without New York City: PM_{2.5} chemical species (147 cities), NO₂ (94 cities), SO₂ (105 cities), and CO (105 cities). (Note that data for the gaseous pollutants were not available for all of the 150 cities, whereas data for PM_{2.5} were available for most or all of the cities.) The PM_{2.5} chemical species except SO₄²⁻ are log-transformed.

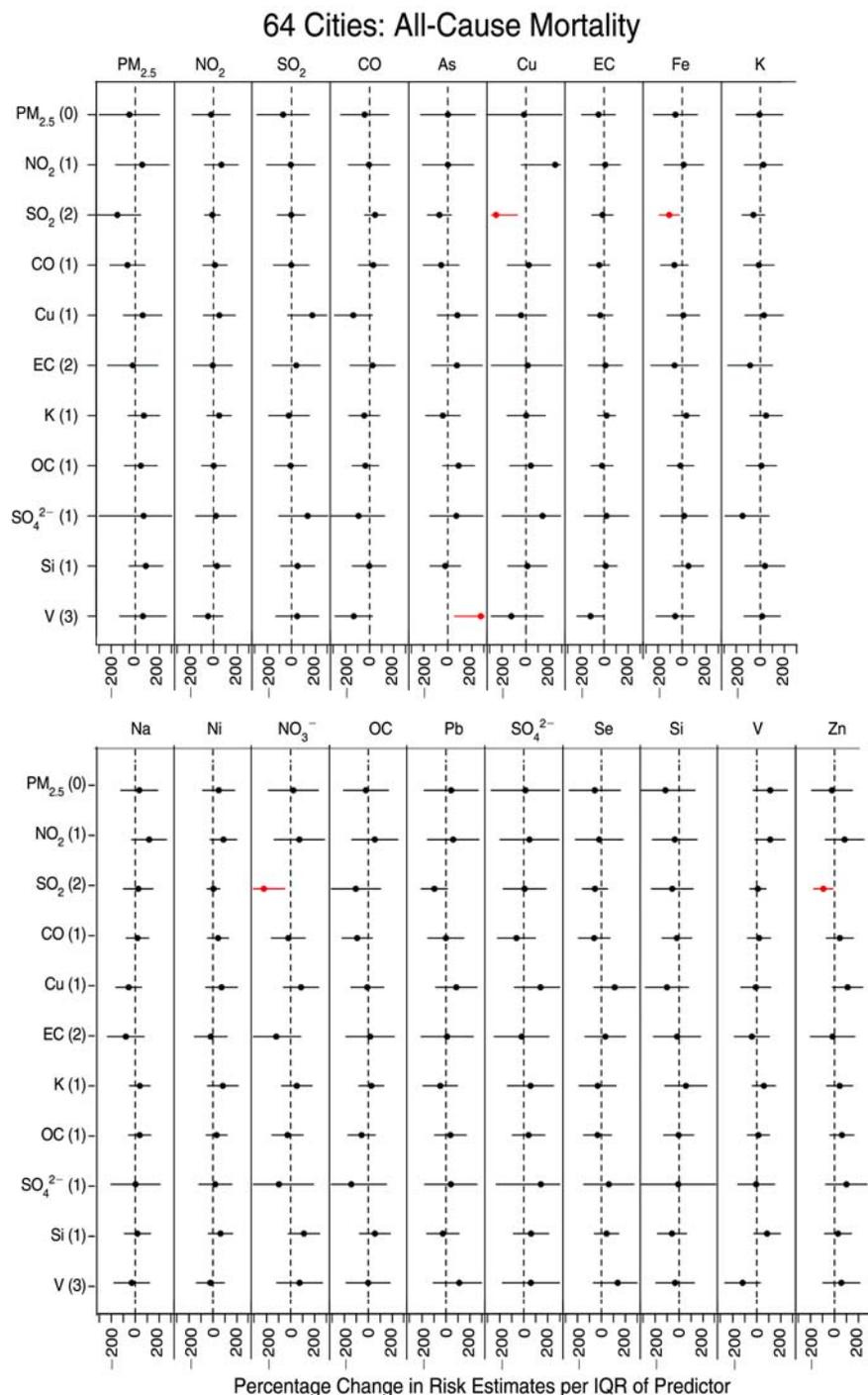


Figure B.8. Percentage change in all-year all-cause-mortality risk estimates associated with $PM_{2.5}$ mass, key $PM_{2.5}$ chemical components, and gaseous pollutants in the second-stage regression model. Risk estimates (regression coefficients) of the pollutants listed on the y-axis at the lags shown in parentheses from the 64 cities were regressed on the corresponding city-specific average values of the pollutants, shown at the top of each column, in the second-stage random-effects model (city-specific average values were used as covariates in the second-stage regression). Statistically significant estimates ($P < 0.05$) are shown in red. Results represent the changes in all-cause mortality risk estimates (the combined estimates across 64 cities without predictors as the baseline) for $PM_{2.5}$ mass, its key components, and gaseous pollutants per the IQR of the city-specific averages of the second-stage predictor variables across cities (all data are from Figure 7). For example, the point estimate for the row “ $PM_{2.5}$ (0)” and the column “ $PM_{2.5}$ ” in this figure, null in this case, suggests that the $PM_{2.5}$ risk estimate at 0-day lag is not influenced by the average level of $PM_{2.5}$ in these cities.

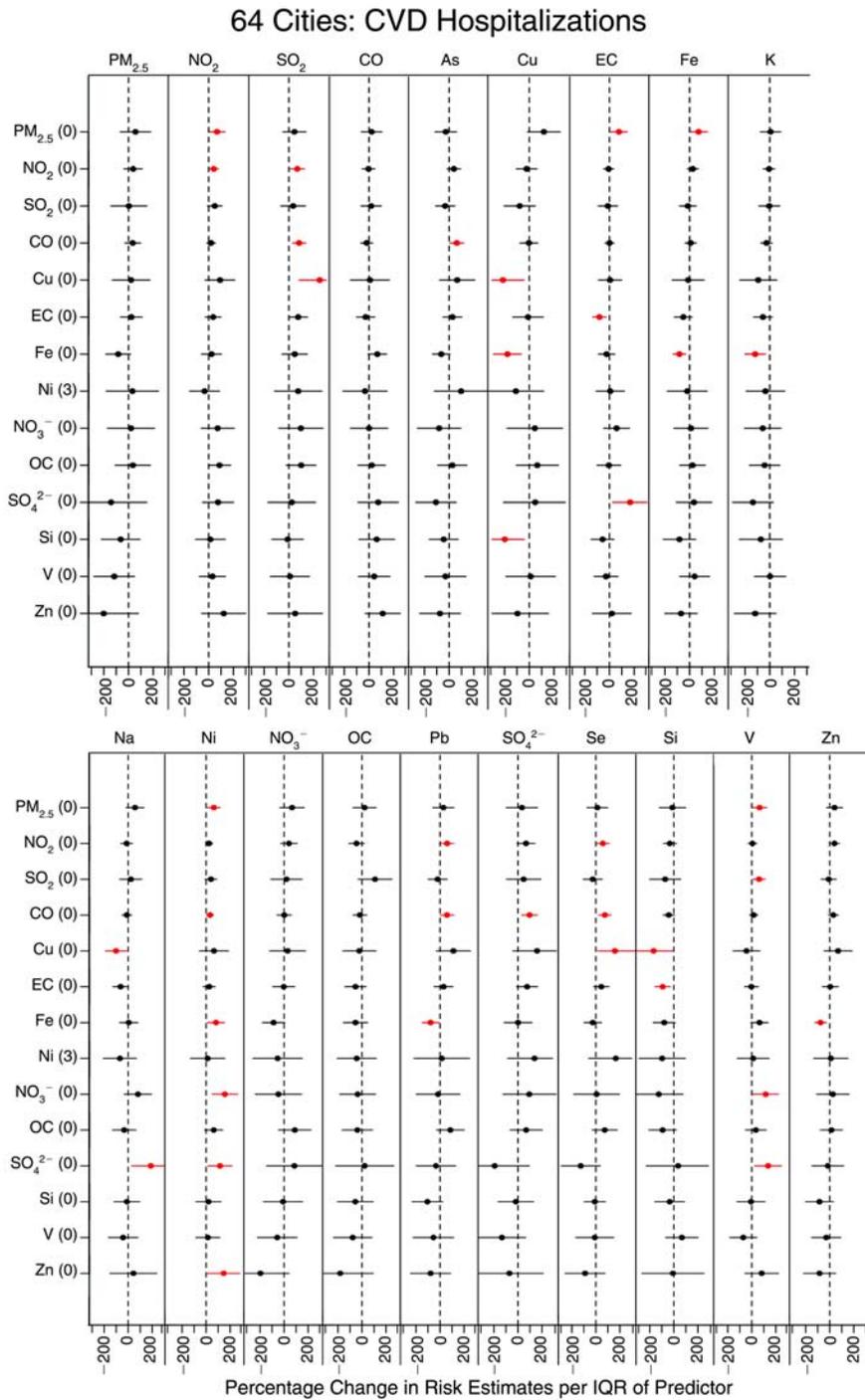


Figure B.9. Percentage change in all-year CVD hospitalization risk estimates associated with PM_{2.5} mass, key PM_{2.5} chemical components, and gaseous pollutants in the second-stage regression model. Risk estimates (regression coefficients) of the pollutants listed on the y-axis at the lags shown in parentheses from the 64 cities were regressed on the corresponding city-specific average values of the pollutants, shown at the top of each column, in the second-stage random-effects model (city-specific average values were used as covariates in the second-stage regression). Statistically significant estimates ($P < 0.05$) are shown in red. Results represent the changes in CVD hospitalization risk estimates (the combined estimates across 64 cities without predictors as the baseline) for PM_{2.5} mass, its key components, and gaseous pollutants per the IQR of the city-specific averages of the second-stage predictor variables across cities (all data are from Figure 9). For example, the point estimate for the row “PM_{2.5} (0)” and the column “PM_{2.5}” in this figure, null in this case, suggests that the PM_{2.5} risk estimate at 0-day lag is not influenced by the average level of PM_{2.5} in these cities.

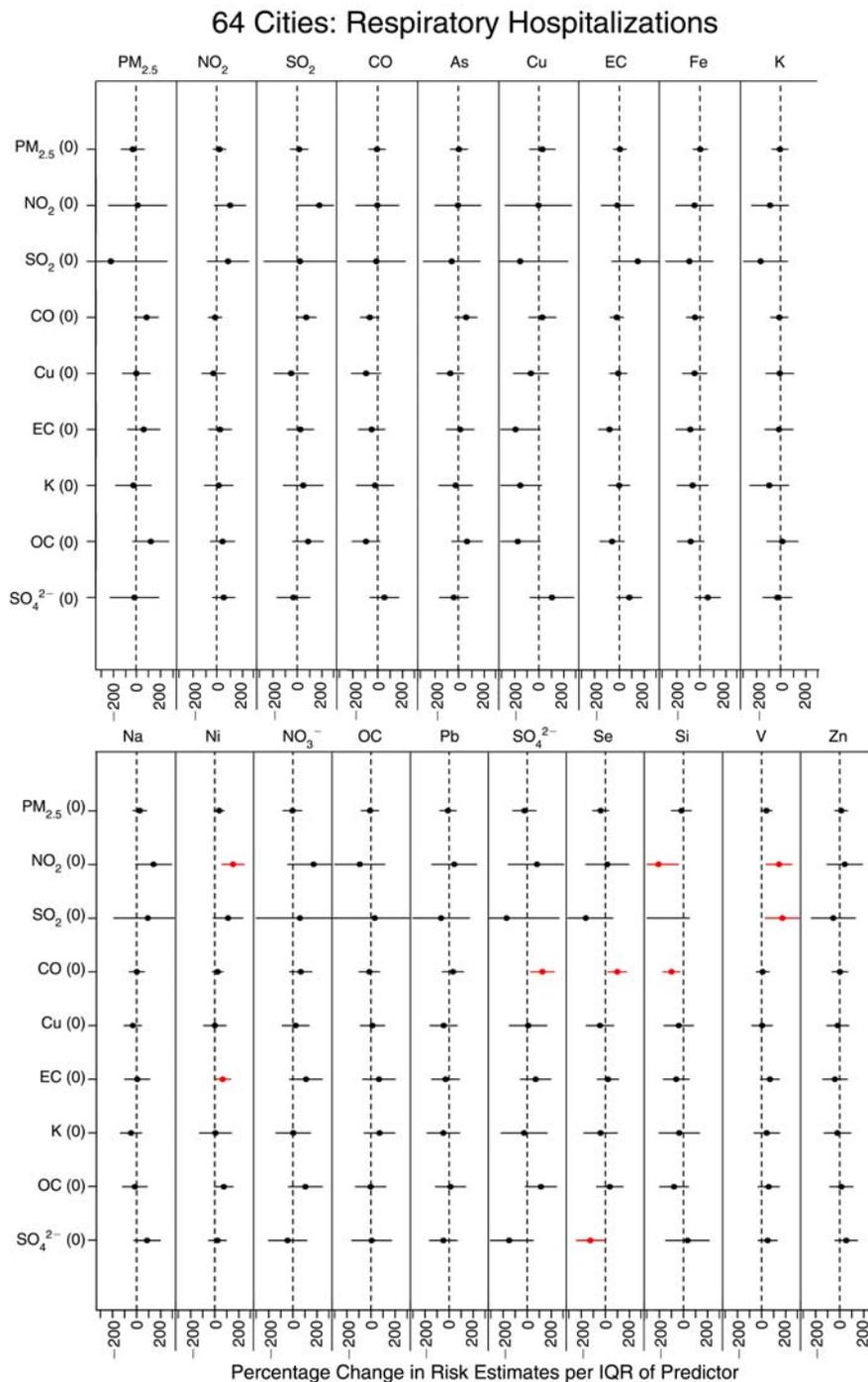


Figure B.10. Percentage change in all-year respiratory hospitalization risk estimates associated with $PM_{2.5}$ mass, key $PM_{2.5}$ chemical components, and gaseous pollutants in the second-stage regression model. Risk estimates (regression coefficients) of the pollutants listed on the y-axis at the lags shown in parentheses from the 64 cities were regressed on the corresponding city-specific average values of the pollutants, shown at the top of each column, in the second-stage random-effects model (city-specific average values were used as covariates in the second-stage regression). Statistically significant estimates ($P < 0.05$) are shown in red. Results represent the changes in respiratory hospitalization risk estimates (the combined estimates across 64 cities without predictors as the baseline) for $PM_{2.5}$ mass, its key components, and gaseous pollutants per the IQR of the city-specific averages of the second-stage predictor variables across cities (all data are from Figure 11). For example, the point estimate for the row “ $PM_{2.5}$ (0)” and the column “ $PM_{2.5}$ ” in this figure, null in this case, suggests that the $PM_{2.5}$ risk estimate at 0-day lag is not influenced by the average level of $PM_{2.5}$ in these cities. Note that the point estimate of the percentage change in the SO_2 respiratory hospitalization risk estimate for Si is -347% , but is missing because it is beyond the range chosen to display the other data.

APPENDICES AVAILABLE ON THE WEB

Appendices C through I contain supplemental material not included in the printed report. They are available on the HEI Web site at <http://pubs.healtheffects.org>.

Appendix C. Chen Study 1. Exposure Data and Factor Loadings

Appendix D. Chen Study 1. Frequency Domain Results

Appendix E. Chen Study 1. Ambient Particulate Air Pollution Induces Oxidative Stress and Alterations of Mitochondria and Gene Expression in Brown and White Adipose Tissues (Xu et al. 2011)

Appendix F. Ito Study 3. Seattle and Detroit

Appendix G. Ito Study 3. Supplemental Information

Appendix H. Thurston Study 4. Inhalable Particle Network

Appendix I. Thurston Study 4. Total Risk Index Measures to Assess Effects of Multiple Particulate and Gaseous Air Pollutants

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Medicine, The Lancet, and Proceedings of the National Academy of Science of the United States of America. Although he has investigated many health outcomes, he has focused on the long-term effects of air pollution on cardio-respiratory diseases. Over the decade, Dr. Jerrett has also studied the contribution of the built and natural environment to sedentary lifestyles and obesity. Dr. Jerrett is currently funded by the U.S. National Institutes of Health and the European Commission on studies that use cell-phone sensors to obtain personal monitoring information on physical activity, air pollution, and geographic location. He is also funded by the Centers for Disease Control and Health Canada to develop new methods for Spatial Epidemiology. In 2009, the United States National Academy of Science appointed Dr. Jerrett to the Committee on “Future of Human and Environmental Exposure Science in the 21st Century.” The Committee recently concluded its task with the publication of a report entitled *Exposure Science in the 21st Century: A Vision and a Strategy.*

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ABBREVIATIONS AND OTHER TERMS

ACS	American Cancer Society
AER	Atmospheric and Environmental Research
ANOVA	analysis of variance
APCA	absolute principal component analysis
APHENA	Air Pollution and Health: A European and North American Approach
ApoE ^{-/-}	apolipoprotein E-deficient [mice]
AQS	air quality system
BA	brachiocephalic artery
BC	black carbon
BEAS-2B	human bronchial epithelial cell line
bpm	beats per minute
CAPs	concentrated ambient particles
CMB	chemical mass balance
CO	carbon monoxide
COPD	chronic obstructive pulmonary disease
CPS-II	Cancer Prevention Study II
CSF	colony stimulating factor
CSN	Chemical Speciation Network
CV	coefficient of variation
CVD	cardiovascular disease
DCF	2',7'-dichlorofluorescein
DCFH-DA	2',7'-dichlorofluorescein diacetate
DMEM	Dulbecco's Modified Eagle Medium
DMEM/F12	Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12
EC	elemental carbon
ECG	electrocardiogram/electrocardiography
ELISA	enzyme-linked immunosorbent assay
FBS	fetal bovine serum

FIPS	Federal Information Processing Standard Codes	PCA	principal components analysis
FRM	Federal Reference Method	PFA	perfluoroalkoxy
GLM	generalized linear model	PM	particulate matter
GM-CSF	granulocyte-macrophage colony-stimulating factor	PM _{2.5}	particulate matter ≤ 2.5 μm in aerodynamic diameter
HBEpC	human bronchial epithelial cells	PM ₁₀	particulate matter ≤ 10 μm in aerodynamic diameter
HEPA	high-efficiency particulate air [filter]	PMF	positive matrix factorization
HF	high frequency	PUF	polyurethane foam
HO-1	heme oxygenase-1	qPCR	quantitative real-time polymerase chain reaction
HPMEC	human pulmonary microvascular endothelial cell line	RFU	relative fluorescence unit
HR	heart rate	ROS	reactive oxygen species
HRV	heart rate variability	R–R	R wave–to–R wave [intervals]
ICAM-1	inter-cellular adhesion molecule 1	RMSSD	root mean square of the successive differences [in beat-to-beat intervals]
ICD	International Classification of Diseases	RT-PCR	real-time polymerase chain reaction
ICP–MS	inductively coupled plasma mass spectroscopy	SDNN	standard deviation of normal-to-normal intervals
IHD	ischemic heart disease	SMPS	scanning mobility particle sizer
IL	interleukin	SO ₂	sulfur dioxide
IPN	Inhalable Particulate Network	SO ₄ ²⁻	sulfates
IQR	interquartile range	TNF-α	tumor necrosis factor-α
LAL	limulus amebocyte lysate	TRI	total risk index
LCCA	left common carotid artery	TXNRD1	thioredoxin reductase 1
LDH	lactate dehydrogenase	UK	United Kingdom
LDL	low-density-lipoprotein	U.S. EPA	United States Environmental Protection Agency
LF	low frequency	USGS	United States Geographical Survey
MSA	metropolitan statistical area	VEGF-A	vascular endothelial growth factor A
MSD	Meso Scale Discovery	XRF	x-ray fluorescence
NAAQS	National Ambient Air Quality Standards	ZCA	Zip Code Area
NCHS	National Center for Health Statistics		
NH ₄ ⁺	ammonium		
NMMAPS	National Morbidity, Mortality, and Air Pollution Study	Elements	
NO ₂	nitrogen dioxide	Ag	silver
NO ₃ ⁻	nitrates	Al	aluminum
NO _x	nitrogen oxides	As	arsenic
NPACT	National Particle Component Toxicity Initiative	Ba	barium
NRC	National Research Council	Be	beryllium
OC	organic carbon	Br	bromine
O ₃	ozone	Ca	calcium
PBS	phosphate-buffered saline	Cd	cadmium
		Cl	chlorine

Co	cobalt	P	potassium
Cr	chromium	Pb	lead
Cs	cesium	S	sulfur
Cu	copper	Sb	antimony
Fe	iron	Sc	scandium
Ge	germanium	Se	selenium
Hg	mercury	Si	silicon
K	potassium	Sn	tin
La	lanthanum	Sr	strontium
Mg	magnesium	Ti	titanium
Mn	manganese	Tl	thallium
Mo	molybdenum	V	vanadium
Na	sodium	Zn	zinc
Ni	nickel		

Research Report 177, *National Particle Component Toxicity (NPACT) Initiative: Integrated Epidemiologic and Toxicologic Studies of the Health Effects of Particulate Matter Components*, Morton Lippmann et al.

INTRODUCTION

Extensive epidemiologic evidence supports the association between air pollution exposure and adverse health effects worldwide (Dockery et al. 1993; Samet et al. 2000b; Aga et al. 2003; HEI 2003; Pope and Dockery 2006; Krewski et al. 2009; Brook et al. 2010). Exposure to particulate air pollution has been reported to increase the risk for a number of health outcomes, in particular cardiovascular diseases (CVDs*) (Pope et al. 2004; Miller et al. 2007). Hence, exposure to air pollution is currently regarded as an important, but modifiable, risk factor that could potentially affect large numbers of people around the globe (Lim et al. 2012). Although regional differences in the health effects of exposure to particulate matter of 2.5 μm or smaller in aerodynamic diameter ($\text{PM}_{2.5}$) have been observed, few data on the health effects of the components of PM are available, and it is unclear whether exposure to PM with different compositions is associated with different levels of risk, in particular cardiovascular risk. Additionally, the biological mechanisms underlying these associations are not well understood. Although regulations to address air quality over the past decades have focused on PM mass concentrations, scientists have hypothesized that PM composition and other characteristics, such as size or surface properties, are potentially important, as they may induce pathophysiological effects through different biological pathways. With a better understanding of the components of PM and their respective health impacts, it may be possible to more efficiently focus regulatory efforts on those sources that contribute the most toxic components.

Dr. Lippmann's study, "Characteristics of PM Associated with Health Effects," began in January 2007. Total expenditures were \$4,374,946. The draft Investigators' Report from Lippmann and colleagues was received for review in September 2011. A revised report, received in June 2012, was accepted for publication in August 2012. During the review process, the HEI NPACT Review Panel and the investigators had the opportunity to exchange comments and to clarify issues in both the Investigators' Report and the Review Panel's Commentary.

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* A list of abbreviations and other terms appears at the end of the Investigators' Report.

As outlined in the Preface, HEI funded the National Particle Component Toxicity (NPACT) Initiative to provide more insight into which components of the PM mixture may be responsible for its toxicity and human health effects. The Initiative was composed of coordinated epidemiologic and toxicologic studies conducted in multiple cities to evaluate the toxicity of different chemical and physical properties of PM and their associated health effects, while taking into account the contribution of gaseous copollutants. Given the strong associations between ambient PM concentrations and cardiovascular mortality and morbidity, and the need to better understand the mechanisms underlying those associations, the NPACT studies focused primarily on CVD outcomes. For more information on the companion NPACT study by Vedal and colleagues, see the Preface and Research Report 178 (Vedal et al. 2013).

In the research project described in this report, Morton Lippmann and colleagues conducted four separate studies that used toxicologic and epidemiologic methods to determine short- and long-term cardiovascular health effects and mortality associated with exposure to $\text{PM}_{2.5}$ and its components. One toxicologic study (Study 1, led by Lung-Chi Chen) exposed mice by inhalation to concentrated ambient particles (CAPs) in five different geographic regions in the United States; it used a mouse model of atherosclerosis to assess the effects of CAPs and its components and source categories on heart rate variability (HRV) and atherosclerosis. A second toxicologic study (Study 2, led by Terry Gordon) used PM collected in more or less the same five locations to measure oxidative stress associated with PM components and to compare the toxicity of coarse, fine, and ultrafine particles in vitro and in vivo. One of the two epidemiologic studies (Study 3, led by Kazuhiko Ito) used data from the U.S. Environmental Protection Agency (EPA) Chemical Speciation Network (CSN) in a time-series analysis of all-cause mortality and cardiovascular and respiratory hospital admissions associated with specific source categories of $\text{PM}_{2.5}$ in 150 U.S. cities or a subset of 64 cities. The second epidemiologic study (Study 4, led by George Thurston) estimated source contributions based on $\text{PM}_{2.5}$ speciation data from the CSN and evaluated associations of long-term average concentrations of $\text{PM}_{2.5}$ components and source categories with mortality from all causes, CVD, respiratory disease, and lung cancer

for subjects in the long-running Cancer Prevention Study-II (CPS-II) funded by the American Cancer Society (ACS) (Pope et al. 1995, 2002, 2004).

This Commentary is intended to aid the sponsors of HEI and the public by highlighting both the strengths and limitations of the study and by placing the Investigators' Report (IR) into scientific and regulatory perspective.

SCIENTIFIC AND REGULATORY BACKGROUND

In 1997, the U.S. EPA issued National Ambient Air Quality Standards (NAAQS) for PM_{2.5}, or fine PM, based on epidemiologic evidence that particles in this size fraction were associated with adverse human health effects (U.S. EPA 1996). Soon afterward, the U.S. Congress directed the EPA to undertake a major research program to answer key scientific questions, relevant to regulatory decisions, about the basis for the toxicity of PM. At that time, the Committee on Research Priorities for Airborne Particulate Matter, established by the National Research Council (NRC), reviewed the evidence and identified where research was critically needed to assess the contribution of

PM and its components to adverse health effects (NRC 1998). Further reviews conducted by HEI (2002) and the NRC (2001, 2004) found that progress had been made in understanding the role that PM characteristics might play in explaining health effects, but that progress had been uneven among technical disciplines. Toxicologic evidence from animal and in vitro studies was predominant, with a strong focus on metals and a growing emphasis on the ultrafine fraction of PM. Some components (e.g., organic compounds) had received less research attention than others (NRC 2004).

In 1999, the EPA created the Speciation Trends Network (currently known as the CSN) to monitor the mass and chemical composition of PM_{2.5} routinely across the United States. Over the subsequent years, the network was rapidly expanded to include more than 200 PM_{2.5} speciation monitoring sites across the continental United States and Puerto Rico. This network made it possible to conduct larger-scale epidemiologic studies of associations between PM composition and health effects, including the studies that were part of the NPACT Initiative and other studies that were started at around the same time (Franklin et al. 2008; Zanobetti et al. 2009; Ostro et al. 2009, 2010; Bell 2012).

Source Apportionment

As part of their research, both the Lippmann and Vedal teams used source apportionment, a method for quantifying how individual sources (or groups of sources) of pollution contribute to concentrations of air pollutants at a certain location. Typically, researchers apply source apportionment techniques to investigate how emissions from specific sources and source categories contribute to PM in the atmosphere, although such techniques have also been applied to gaseous pollutants. The techniques generally focus on pollution from combustion (from both mobile and stationary sources), other industrial activities, and dust (from natural soil or resuspension of road dust, which may include material from vehicle brakes and tires).

Most PM source apportionment techniques are "receptor oriented" and use observed concentrations of PM components measured at a monitoring station (the receptor) to calculate how much of the total PM can be attributed to specific sources. Generally, the underlying assumption is that the composition of different PM emissions can be used to trace them back to their sources because the sources have unique emissions "fingerprints". In particular, most of the methods assume a chemical mass balance (i.e., the mass of all chemical components combined is accounted for in the model) and

state that the observed PM concentration at a given location represents the sum of the contributions from individual sources. Thus:

$$c_i = \sum f_{ij} S_j,$$

where c_i is the concentration of the measured component i at the receptor; f_{ij} is the fraction of total PM emissions from source j that constitutes component i ; and S_j , the variable of interest, is the total PM concentration at the receptor coming from source j .

A second type of source apportionment technique is "source-based". Techniques in this category mathematically track emissions from sources in an air quality model, to estimate the contribution of the sources at one or more locations (e.g., a person's home). Such models typically do not use measured concentrations directly.

Given that it is impossible to measure all source contributions, neither approach can be directly evaluated for its accuracy, although the source-based methods can use measured concentrations to evaluate the model's performance and receptor

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Establishing the relative cardiovascular toxicity of different PM components might facilitate the identification of specific sources that contribute to the presence of these particles in ambient air. However, attributing cardiovascular toxicity to individual PM components is difficult because of the complexities associated with characterizing PM components and the often high correlations between components, as well as the fact that many components can be found in emissions from multiple sources. Lippmann and colleagues used source-apportionment techniques (see the Source Apportionment sidebar) to analyze data on PM_{2.5} components to identify specific source categories that might be contributing most to the health risks associated with exposure to PM (see Glossary of Statistical Terms).

The complexities associated with source identification are compounded by the numerous responses affected by PM exposure. For example, both epidemiologic and experimental studies have shown a range of both acute and chronic changes in cardiovascular function and health associated with exposure to PM. These include changes in HRV, thrombosis, endothelial function, atherogenesis, susceptibility to myocardial ischemia, and changes in the function of circulating cardiovascular progenitor cells (U.S. EPA 2009). Hence, it is difficult to evaluate the effect of the cardiovascular toxicity of a specific PM component on one specific cardiovascular function or disease state. In addition, such evaluations are made more difficult by the incomplete understanding of mechanisms that elevate cardiovascular risk and lead to adverse cardiac events.

models can be compared with other models and estimated emissions. Various hybrids of source- and receptor-based methods are being developed. Both the Lippmann and Vedal studies used receptor modeling approaches — in particular, factor analysis.

Solving the chemical mass balance model using measured concentrations (c_i) to find source contributions (S_j) requires either knowing the compositions of the various source emissions (f_{ij}) or being able to estimate them from the data. The latter approach typically relies on factor analysis (receptor modeling), a method that calculates the source fingerprints and source contributions together. According to this model, the source fingerprints are called “factors” and the source contributions are more appropriately referred to as “factor contributions” because the factors do not necessarily correspond to a specific source. Instead, the characteristics of the factors (i.e., which PM chemical components comprise a given factor) are associated with sources by comparing the composition of the factors (e.g., the dominant chemical components) with what is known about the composition of various source emissions that may be present and may affect the concentrations at the receptor site.

Factor analysis approaches are applied widely because they do not make assumptions about which actual sources contribute to a factor, and they are able to address the issue that source composition may vary spatially and often changes between the source and the receptor. On the other hand, it should be understood that factor analysis results are based on interpretations of how specific factors relate to sources (or to atmospheric formation processes, in the case of secondary PM components) and on operational judgments, such as how many factors to include in an analysis and how to treat

uncertainties and detection limits. Some studies have used multiple source apportionment methods side by side and have generally found them to produce similar results (Thurston et al. 2005; Hopke et al. 2006; Sarnat et al. 2008), even when the various source apportionment outputs were used to estimate exposures for epidemiologic analyses (Thurston et al. 2005; Sarnat et al. 2008).

Three of the Lippmann studies (Chen Study 1, Gordon Study 2, and Ito Study 3) used basic factor analysis methods to estimate source contributions from component concentration data. Thurston Study 4 further apportioned PM_{2.5} mass using absolute principal component analysis (APCA). APCA is a factor analysis technique that assesses portions of the mass associated with the identified factors that can then be regressed on the concurrent PM_{2.5} concentrations to apportion PM_{2.5} mass to source categories. This makes it possible to determine the fraction of mass attributable to the individual factors and the identified source categories that they are assumed to be associated with (Thurston and Spengler 1985; Hopke et al. 2006).

Vedal and colleagues used positive matrix factorization (PMF), a method that is widely used with software available from the EPA. PMF employs regression methods to constrain all factors to be positive and takes into account uncertainty in the measurements for each chemical component in the data set, which allows for weighting measurements that may have less measurement error (Hopke et al. 2006). In contrast with the Lippmann team, Vedal and colleagues did not use the source apportionment results directly in their health analyses. Instead, they used source apportionment to support their hypothesis that their selected indicators (EC, OC, silicon, and sulfur) are associated with their assumed sources, such as EC and OC with traffic-related emissions.

PM CHARACTERISTICS, COMPONENTS, SOURCES, AND EXPOSURE ASSESSMENT

Ambient PM is a complex mixture of solid and liquid particles suspended in air. The size, composition, and other physical and biological properties of particles vary with location and time. This variability in pollutant characteristics derives from differences in pollutant sources. The sources may be natural, such as forest fires, or the result of human activities, such as driving vehicles and operating manufacturing or power generating facilities. Reactive chemical species in the ambient atmosphere can also combine with PM to form secondary particles, such as sulfates from sulfur dioxide gas and secondary organic aerosols from volatile hydrocarbons, which may comprise a significant fraction of total PM.

Ambient PM concentrations in any particular location are affected by local ambient mixtures of gaseous pollutants and meteorology, and vary by geographic region and season. Many gaseous pollutants (ozone [O₃], carbon monoxide [CO], sulfur dioxide [SO₂], and nitrogen oxides [NO_x] in particular) derive from the same sources as PM, and they can have health effects on their own as well as in concert with PM. Also, any investigation of the health effects of different components and sources of PM should consider how gaseous pollutants may affect the associations; evaluating the role of gases was considered important for the studies funded under RFA 05-1A.

Human exposure assessment for large studies of PM_{2.5} is often carried out based on data from central monitors, and

exposure is assigned according to city of residence. This approach typically results in relatively low exposure misclassification, because PM_{2.5} concentrations in many cities tend to be relatively uniform across the metropolitan area (relative to pollutants such as nitrogen dioxide [NO₂], which exhibit high spatial variation). PM_{2.5} concentrations also commonly exhibit similar hour-to-hour and day-to-day variations across a metropolitan area. For time-series studies of acute effects, which compute effect estimates based on differences in exposure and outcomes on different days, PM_{2.5} concentrations measured at a central monitor are usually sufficient to represent time-based fluctuations in concentration across an urban area. For studies of chronic effects, this spatial uniformity in most cities allows studies to estimate associations based on differences in PM_{2.5} concentrations among cities.

For PM_{2.5} components, some evidence indicates that concentrations of some components are more spatially and temporally variable than others, and more variable than PM_{2.5} mass itself (Thurston et al. 2011; Bell et al. 2011), but this was not well understood when the NPACT Initiative commenced. More sophisticated exposure assessment methods, such as land-use regression, kriging, and inverse-distance weighting, which attempt to resolve within-city exposure contrasts, were available in 2005. However, at the time, these approaches would have been very difficult to implement and the results difficult to validate using the additional measurements for all of the components, gaseous variables, and source categories required in studies of 100 or 150 cities. Thus, the current NPACT studies are largely

Glossary of Statistical Terms

Component Chemical species present in PM_{2.5} mass and quantified in the EPA's Chemical Speciation Network data (e.g., Ni, V, EC).

Factor A group of components identified via factor analysis.

Factor Analysis A mathematical process used to group components based on the degree to which their concentrations in the atmosphere are correlated with each other.

Factor Loading The numerical value indicating the magnitude of the contribution of a component to a factor identified through factor analysis.

Factor Score The total numerical value indicating the magnitude of a factor based on the combined magnitude of the factor loadings.

Secondary Aerosols Components that are not directly emitted by sources, but form in the atmosphere as a result of physical and chemical reactions among primary pollutants (e.g., sulfates, nitrates, organic carbon). Secondary aerosols often comprise a high percentage of total atmospheric PM_{2.5} mass.

Source Apportionment The process through which factors are correlated with source emissions (based on source emissions or other source-related data) and named accordingly.

Source-Appportioned Mass PM_{2.5} mass that has been attributed to each source category based on the factor score and total measured PM_{2.5} mass concentrations (e.g., PM_{2.5} mass attributed to Traffic, Coal Combustion).

Source Category The name of the type of source a factor is identified with (e.g., Traffic, Coal Combustion).

based on differences in exposures and outcomes between cities, rather than within them.

Because PM_{2.5} components were the main focus of the NPACT Initiative, only one of the seven NPACT studies (Gordon Study 2) addressed the question about relative toxicity of PM sizes. Gordon and colleagues evaluated the subsets of PM between 2.5 and 0.2 µm in aerodynamic diameter (PM_{2.5-0.2}, or fine PM) and PM between 2.5 and 10 µm in aerodynamic diameter (PM_{10-2.5}, or coarse PM) *in vitro*; those size classes were of interest because of the different sources and health effects associated with them. Coarse particles tend to derive from resuspension or mechanical processes, whereas PM_{2.5} tends to be formed primarily from combustion and secondary formation that occurs while PM ages and is transported over quite long distances. Some scientists have hypothesized that even smaller particles (≤ 0.1 µm in aerodynamic diameter, or ultrafine PM), which dominate in terms of numbers of particles in ambient air, may be particularly toxic (Utell and Frampton 2000; Oberdörster 2001), but to date, no definitive answer about the relative toxicity of ultrafine PM compared to fine PM has emerged (HEI 2013).

Specific components of PM that were a focus of both Lippmann and Vedal NPACT studies include the following: trace elements (including metals); organic compounds; ions, such as sulfate (SO₄²⁻), nitrate (NO₃⁻), and ammonium (NH₄⁺); and elemental carbon (EC). In addition, an initial goal of the NPACT studies was to include gaseous copollutants, such as NO₂, O₃, and SO₂, to attempt to differentiate PM-related health effects from those related to gases. However, because of the limited number of cities for which data on both PM components and gaseous pollutants were available and the potential for high correlations between concentrations of gases and some PM components and sources, the NPACT investigators were less able to explore this line of research; only some of the NPACT studies looked specifically at gaseous components.

EPIDEMIOLOGIC EVIDENCE

Prior epidemiologic efforts in U.S. and European cities have contributed valuable insights to the general understanding of health effects associated with PM and its components (e.g., Özkaynak and Thurston 1987; Schwartz et al. 1996; Laden et al. 2000; Samet et al. 2000b; Metzger et al. 2004; Peel et al. 2005). However, various study limitations — for example, relatively short study periods, modestly sized study populations, and high correlations among pollutants in any one city — have limited the ability of the studies to either detect statistically significant pollution effects associated with specific PM components or discriminate among the effects of different pollutants. Furthermore, major questions remained about

the specificity of the markers used to define particular pollutant sources.

At the time the Lippmann NPACT study was funded in 2006, time-series studies had found associations with PM mass that varied seasonally and regionally (Samet et al. 2000b; Peng et al. 2005). A number of studies found stronger associations for fine or ultrafine particles than for coarse particles or for PM₁₀ (e.g., Schwartz et al. 1996). In addition, the Aerosol Research and Inhalation Epidemiology Study (ARIES) in Atlanta, Georgia, found PM_{2.5}, PM₁₀, NO₂, and CO to be associated with emergency room visits related to respiratory health effects (Peel et al. 2005) and PM_{2.5}, NO₂, and CO to be associated with cardiovascular endpoints (Metzger et al. 2004). Some epidemiologic studies found no differences in the associations of different size fractions with mortality (Wichmann et al. 2000) or with respiratory effects in asthmatic children (Pekkanen et al. 1997; Peters et al. 1997; Lippmann et al. 2000).

Prior to the NPACT Initiative, two key cohort studies found associations between cardiovascular mortality and long-term exposure to fine particulate; these associations were stronger for PM_{2.5} than for PM₁₀ or PM₁₅. Analyses of the CPS-II cohort data found all-cause, cardiovascular, and lung cancer mortality to be more strongly associated with long-term exposure to SO₄²⁻ and PM_{2.5} than with coarse PM (HEI 2000; Pope et al. 2002). Similar associations between PM_{2.5}, SO₄²⁻ particulate, and mortality were reported in a reanalysis of the Harvard Six Cities Study, which studied a cohort with long-term exposure to pollution, with the specific goal of evaluating the effects of pollution on health (HEI 2000). A sensitivity analysis of the CPS-II cohort also reported regional differences in the magnitude of risk estimates associated with a 10 µg/m³ increase in PM_{2.5} concentrations, implying that differences in the composition of PM_{2.5} might result in different PM_{2.5} toxicity.

Other studies attempted to associate health effects directly with source-related components of PM_{2.5} mass (Özkaynak and Thurston 1987; Clarke et al. 2000; Laden et al. 2000; Riediker et al. 2004). The statistical approaches in these studies, which included factor analysis, principal component analysis, and tracer methods (see the Source Apportionment sidebar), were based on assumptions about the groups of elements and compounds that characterize an emission source. For example, Laden and colleagues (2000) used atmospheric markers of different sources to examine relationships between source-related categories and all-cause mortality. They found no evidence of associations with crustal sources and more robust associations with markers for coal combustion, motor vehicle exhaust, and residual oil combustion.

Further analyses by Lippmann and colleagues found that the city-to-city variation in PM_{10} excess daily mortality risk estimates reported in the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) (Samet et al. 2000b) was significantly explained by the city average levels of Ni and V measured in $PM_{2.5}$ samples from the corresponding cities (Lippmann et al. 2006). Additionally, reductions in concentrations of SO_2 , Ni, and V were associated with decreased monthly mortality counts in studies conducted in Hong Kong after the introduction of low-sulfur fuel (Hedley et al. 2002). By the time NPACT was initiated, several studies attributed both acute and chronic cardiovascular and respiratory health effects to motor vehicle-related pollution (Künzli et al. 2005; Schwartz et al. 2005). The adverse effects associated with exposure to traffic were summarized by the WHO (2005) and, more recently, HEI (2010).

TOXICOLOGIC EVIDENCE

At the time the current study was funded, many toxicologic studies had investigated the types of particles that may cause adverse health effects, and much of the evidence suggested that exposure to several kinds of PM triggers acute events such as oxidative stress, inflammatory events, and cell injury both *in vitro* and *in vivo* (U.S. EPA 2004a). In an effort to understand the potential underlying mechanisms for the long-term adverse cardiovascular outcomes observed in epidemiologic cohort studies, Lippmann and colleagues conducted animal studies in which mice were exposed by inhalation to fine CAPs. They reported effects on heart rate (HR) and HRV measures in normal and atherosclerotic mice exposed to CAPs for several months in Tuxedo, New York; source apportionment of collected CAPs pointed toward secondary sulfate, resuspended soil, and residual oil combustion as possible source categories that contributed to the observed toxicity (Lippmann et al. 2005). Lippmann and colleagues also conducted a more detailed analysis of the source of Ni from CAPs; it included back trajectory analysis, which implicated an upwind Ni smelter as the likely largest Ni source contributing to the animal exposures (Lippmann et al. 2006). For the same subchronic CAPs inhalation study, Sun and colleagues (2005) showed that the subchronic CAPs exposures were associated with inflammation and increased atherosclerosis. In addition, *in vitro* exposures of lung cells to extracts from archived samples collected during the inhalation exposures of these subchronically exposed mice affected certain blood markers of inflammation and oxidative stress, such as nuclear factor kappa B (NF κ B). The strongest associations were with a Ni–V source

category that was identified in source apportionment of the CAPs (Maciejczyk and Chen 2005).

Geographic and seasonal differences in the toxicologic effects of PM and its components have been assessed in few previous studies. Given the variation in ambient aerosols within and between locations and time periods, some researchers focused on studies of animals that were exposed to specific source mixtures — such as diesel exhaust, gasoline exhaust, and emissions from wood and coal combustion (McDonald et al. 2004). At the time NPACT was initiated, one such study had found that the effects on vasoconstriction and inflammation may be driven by the gaseous rather than the particulate components of diesel exhaust (Campen et al. 2005), whereas a subsequent subchronic inhalation study at New York University (NYU) found that gaseous components in diesel engine exhaust did not enhance atherosclerosis produced by CAPs (Quan et al. 2010). Inflammatory effects of diesel exhaust were also found in human controlled-exposure studies (Salvi et al. 1999; Holgate et al. 2003); animal studies reported that exposure to wood smoke had similar inflammatory effects (Tesfaigzi et al. 2002). Prior work by Campen and colleagues (2001) also showed some immediate and delayed cardiac effects, including arrhythmias, in mice exposed to vanadium sulfate and nickel sulfate particles, with time course of response varying by element. Additionally, based on mass, smaller particles induced more inflammatory effects than did larger particles (Oberdörster et al. 2000; Li et al. 2003). Properties other than size, such as solubility, are also likely to play an important role in particle effects (Leikauf et al. 2001). These and similar studies have provided information about the physicochemical characteristics of particles that may induce adverse effects (U.S. EPA 2004a).

INTEGRATING EPIDEMIOLOGIC AND TOXICOLOGIC APPROACHES IN NPACT

Despite a large body of epidemiologic and toxicologic evidence on the effects of PM on health, few studies have combined both types of evidence in a systematic way. The NPACT Initiative was designed to provide a systematic approach to the study of PM components, size fractions, and source categories by using complementary epidemiologic and toxicologic approaches to evaluate related cardiovascular and respiratory endpoints and disease pathways. The designs anticipated points of comparison both among studies conducted at each research center (the Lippmann study as described in this report, and the Vedal study [Vedal et al. 2013] as described in the companion report) and across the Lippmann and Vedal studies. Dr. Lippmann's study proposed to systematically evaluate the health and toxicologic

effects of components of PM that are associated with likely sources, applying a similar source-apportionment approach to each of the parallel epidemiologic and toxicologic evaluations, with some adaptations to the approach warranted by the respective study designs.

OVERALL SPECIFIC AIMS AND APPROACH

Lippmann and colleagues included the following specific aims and approaches for the entire NPACT study:

1. To relate the differences in short-term responses to the local daily variations in ambient PM_{2.5} composition at each site, and relate the differences in the long-term responses to variations in the mean PM_{2.5} compositions among the five sites.

The investigators evaluated apolipoprotein-E (ApoE) knockout mice that were exposed for 6 months to fine CAPs at the five selected sites, using the same experimental protocols, and conducted elemental speciation analyses on filter samples collected daily during the exposure period. They hypothesized that biological responses to PM_{2.5} exposure were driven by specific chemical components rather than by overall PM_{2.5} mass concentration (Chen Study 1).

2. To identify biological responses and their daily and long-term variations by particle-size range, collection site, and season, and relate the observed responses to PM elemental composition.

The investigators collected high-volume samples of ambient air PM in three different particle-size ranges (PM_{10-2.5} [coarse], PM_{2.5-0.2} [fine], and PM_{0.2} [ultrafine]) in summer and winter at the same five sites as above and conducted elemental speciation analyses on the samples they collected. They administered aliquots of the samples to cells in vitro (epithelial cells, endothelial cells, and cardiomyocytes) and to mice in vivo by aspiration. They hypothesized that the biological responses were driven by particle-size range and local variations in climate, as well as by specific components, rather than by overall PM_{2.5} mass concentration (Gordon Study 2).

3. To estimate short-term risk of mortality and hospital admissions and to model city-to-city variation in risk as a function of city-specific characteristics.

The investigators conducted time-series studies in 150 U.S. cities with PM_{2.5} compositional data from the CSN (available every third or sixth day), and in 64 of these cities that also had data on the gaseous NAAQS pollutants. They hypothesized that the daily mortality and morbidity rates

were driven by specific chemical constituents rather than by overall PM_{2.5} mass concentration (Ito Study 3).

4. To investigate the association between mortality and long-term exposure to components of PM_{2.5} in the United States.

The investigators evaluated associations of daily mortality and hospital admissions, by cause, with the daily concentrations of PM_{2.5} mass and its elemental components, using a time-series approach. They hypothesized that an increase in mortality was driven by specific components (Thurston Study 4).

5. To evaluate (1) short-term outcomes, i.e., the influence of PM_{2.5} and its elemental components on short-term biological responses of cells in vitro and of organ systems in animals in vivo, and on daily morbidity and mortality data for human populations; and (2) long-term outcomes, i.e., the influence of PM_{2.5} and its elemental components on long-term plaque progression in mouse aorta and on mortality data for a human cohort.

The investigators stated that they tested the following overall hypotheses: (1) that the increase in mortality from CVD is related to plaque progression, and that both plaque progression and CVD are driven by specific chemical components rather than by overall PM_{2.5} mass concentration; (2) that the PM_{2.5} components that drive short-term responses may differ from those that drive long-term responses; and (3) that the components of coarse, fine, and ultrafine PM may differ in their capacity to produce short-term responses.

In the following sections, each of the four studies is described and discussed separately. An overview of the study designs is provided in Commentary Table 1. In addition, an evaluation of the entire research project is presented at the end of the Commentary.

STUDY 1. LUNG-CHI CHEN AND MORTON LIPPMANN

GENERAL APPROACH

In their study, Chen and Lippmann stated the following hypotheses related to overall Specific Aim 1:

1. PM_{2.5} is capable of producing acute health effects of public concern but the effects might differ according to the chemical composition of the PM_{2.5}.
2. Long-term PM_{2.5} exposures are closely associated with chronic health effects.
3. The source-apportionment techniques that have been developed and refined in recent years provide a useful

Commentary Table 1. Overview of Study Designs

	Principal Investigator			
	Chen	Gordon	Ito	Thurston
Study type	Toxicology	Toxicology	Epidemiology	Epidemiology
Study design	In vivo inhalation	In vitro; in vivo aspiration	Time series	American Cancer Society CPS-II cohort
Species	ApoE knockout mice	Human cells; FVB/N mice	Humans	Humans
Geographic locations	Tuxedo, Manhattan, East Lansing, Seattle, Irvine	Tuxedo, Manhattan, Ann Arbor, Seattle, LA area	150 or 64 U.S. cities	100 U.S. cities
Exposure duration	Acute (0- to 2-day lags); subchronic (up to 6 months)	Acute (same day)	Acute (0- to 3-day lags)	Chronic (average of all available 24-hr concentrations 2000–2005)
PM exposures	CAPs (VACES concentrator)	Resuspended PM collected with high-volume cascade impactor	Ambient PM	Ambient PM
Exposure modeling	—	—	CSN central monitors; city averages	CSN central monitors; city averages
PM size classes	Fine	Coarse, fine, ultrafine	Fine	Fine
PM characterization	34 Elements by XRF; PM number and size distribution by scanning mobility particle sizer	27 Elements by inductively coupled plasma mass spectroscopy; endotoxin content by limulus amebocyte lysate assay	CSN database: PM _{2.5} mass, As, Cu, EC, Fe, K, Na, Ni, NO ₃ ⁻ , OC, Pb, Se, Si, SO ₄ ²⁻ , V, Zn ^a Where available: NO ₂ , SO ₂ , CO	CSN database: PM _{2.5} mass, As, Ca, Cl, EC, Fe, K, Na, Pb, Mn, Ni, OC, S, Se, Si, V, Zn ^a
Source apportionment	Factor analysis with oblique rotation	Factor analysis with varimax rotation	Factor analysis with orthogonal rotation and automated assignment via algorithm to six possible source categories for each city	Factor analysis with absolute principal component analysis followed by mass regression modeling for source-apportioned PM _{2.5} mass
Endpoints	Acute — ECG measures; markers of systemic and vascular inflammation Chronic — ECG measures; atherosclerotic plaque progression	Acute, in vitro — ROS generation; markers of inflammation; cardiomyocyte beat frequency Acute, in vivo — Differential cell count in lavage fluid; protein concentrations	Acute — All-cause mortality; respiratory and CVD mortality and hospitalizations	Chronic — All-cause, ischemic heart disease, respiratory, and lung cancer mortality

^a Components used for the health outcomes analyses only.

basis for identifying the principal PM_{2.5} air pollution source categories and specific chemical components of PM_{2.5} that have the greatest impacts on a variety of acute and chronic health issues.

4. The health effects caused by ambient PM_{2.5} exposures are more likely to be observed in animal models that represent sensitive subgroups within overall human populations.

Chen and Lippmann performed mouse inhalation studies to evaluate the role of PM components on cardiovascular endpoints *in vivo*. They selected ApoE knockout mice because these mice are particularly susceptible to the development of atherosclerosis, the underlying cause of most CVD. The mice were exposed to fine CAPs in five different cities across the U.S., to capture ambient pollutant mixtures from a variety of sources, including coal-fired power plants, wood smoke, and traffic. Control groups of mice were exposed simultaneously to air filtered with a high-efficiency particulate air (HEPA) filter under the same conditions. For their comprehensive assessment of the cardiovascular toxicity of PM_{2.5} components, Chen and Lippmann chose two commonly measured cardiovascular endpoints, atherosclerotic plaque progression and HRV, as well as a number of additional markers of inflammation, oxidative stress, and cardiovascular changes.

To capture the chronic effects of CAPs exposure, Chen and Lippmann measured changes in atherosclerotic plaque progression in ApoE knockout mice exposed for 6 months. There has been increasing evidence showing that cumulative exposure to PM is associated with increased atherosclerotic plaque progression in mice (Sun et al. 2005; Araujo et al. 2008; Araujo and Nel 2009; Quan et al. 2010) as well as increased intima-media thickness — a surrogate marker of atherosclerosis progression — in humans (Araujo and Nel 2009; Bauer et al. 2010; Tonne et al. 2012). (Note that in the companion NPACT study, Vedal and Lippmann included an evaluation of intima-media thickness in humans; see Vedal et al. 2013.)

Because the mice were implanted with transmitters that captured daily information on HR and other related electrocardiogram (ECG) measures, Chen and Lippmann were also able to evaluate both acute and chronic effects of CAPs exposure on HRV, which has been shown to be affected by PM exposure both in animals (Wellenius et al. 2002; Chen and Hwang 2005; Corey et al. 2006) and humans (Creason et al. 2001; Magari et al. 2002; Pieters et al. 2012). In humans, high HRV is associated with good cardiovascular health, whereas low HRV values are indicative of poor prognosis (Brook et al. 2010). Thus, the study by Chen and

Lippmann provided a comprehensive evaluation of both chronic and acute CVD effects in the same mice.

METHODS

CAPs Exposures and PM Composition

Chen and Lippmann exposed male ApoE knockout mice to fine CAPs for 6 hours per day, 5 days per week, for a total of 6 months. Mice had unrestricted access to normal chow except during the 6-hour exposures.

To obtain a wide range of exposures in terms of PM composition, the investigators exposed mice to CAPs in five different locations: Mount Sinai hospital in New York City, New York (referred to as Manhattan); Tuxedo, New York; East Lansing, Michigan; Seattle, Washington; and Irvine, California. Tuxedo was selected as a semirural area with regional background pollution representative of the area; Manhattan as a location with a combination of traffic sources, residential residual oil combustion, and the same background air pollution observed at Tuxedo; East Lansing as an area with coal combustion; Seattle as an area with port emissions as well as wood smoke in winter; and Irvine as a location at which traffic sources predominate and that has relatively high levels of secondary aerosol.

The exposures at Manhattan and Tuxedo were conducted simultaneously from July through November 2007, at Seattle from January through July 2009, at East Lansing from April through November 2010, and at Irvine from September 2010 through March 2011. Exposure atmospheres were generated using a versatile aerosol enrichment system originally developed by Dr. Constantinos Sioutas at the University of Southern California and adapted by Drs. Polina Maciejczyk and Chen at NYU. The ambient PM was always concentrated 8 to 10 times (regardless of the ambient concentration that day), and gaseous copollutants were not intentionally scrubbed from the exposure atmosphere (but were not concentrated). Control groups of mice were exposed under the same conditions to HEPA-filtered air. At Manhattan and Tuxedo, CAPs- and air-exposed mice were analyzed after 3 and 6 months of exposure. Based on the results, the investigators decided to analyze mice at the three other locations after 2, 4, and 6 months of exposure.

The investigators collected PM_{2.5} on filter samples during each 6-hour exposure and analyzed them for 35 elements by x-ray fluorescence (XRF). They also measured black carbon (BC) semicontinuously, using an aethalometer, and organic carbon (OC) and elemental carbon (EC) on quartz filters. Particle number and size distributions were obtained once per month using a scanning mobility particle sizer. The compositional data were then used in a

factor analysis with oblique (oblimin) rotation (conducted by Drs. Ramona Lall and George Thurston) to identify major PM sources in each location. Because PM composition differed depending on location, the number of factors identified and their associations with source categories were not identical. It should be noted that the factor analysis and the source categories identified are unique to the Chen study and are different from those in the studies led by Gordon, Ito, and Thurston (Commentary Table 1; see the Source Apportionment sidebar for more details).

Measuring Health Endpoints

Cardiac Measures Mice were implanted with ECG telemetry devices for collection of HR and HRV data, three weeks before the start of exposures. HRV data included time-domain measures, such as the standard deviation of normal-to-normal intervals (SDNN) and the root mean square of successive differences in beat-to-beat intervals (RMSSD), and frequency-domain measures, such as low frequency (LF), high frequency (HF), and the ratio of HF to LF (HF/LF) (see the sidebar Measuring Heart Rate Parameters). ECG data were collected continuously during exposure and nonexposure periods. The investigators measured additional cardiac function parameters, such as ejection fraction, fractional shortening, and cardiac wall thickness, using ultrasound biomicroscopy in mice exposed at Manhattan and Tuxedo. These additional measurements were not taken at the other locations because no significant changes were observed in mice exposed at Tuxedo and at Manhattan. For the ECG analyses, groups of 8 to 12 mice

were exposed, but not all mice completed the experiments. Some animals had to be killed because of skin lesions, which are common in ApoE knockout mice because of subcutaneous cholesterol deposits. The result was that some experimental groups were reduced to as few as 4 mice (a control group) or 6 mice (a CAPs-exposed group).

Atherosclerosis The progression of atherosclerotic lesions was measured at 3 and 6 months at Manhattan and Tuxedo, and at 2, 4, and 6 months at the other locations. The investigators used different groups of mice than for the ECG analyses (at least 10 per group, except for the group analyzed at Seattle after 4 months of exposure, which had only 6 mice). They placed anesthetized mice on their backs and recorded high-resolution ultrasound video images of the brachiocephalic and left common carotid arteries at 10 locations about 330 μm apart. They then took three representative still pictures of the second, third, and fourth locations (nine total) and measured plaque area using an imaging program and freehand drawing. Data were expressed as the percentage of plaque area relative to the cross-sectional area of the vessel cavity; data were then averaged in each of the three locations.

Additional plaque verification was conducted in animals that were killed after the exposures. Plaque verification was performed on groups of 7 mice exposed to CAPs or filtered air for 6 months at Seattle and to groups of 12 CAPs-exposed and 17 filtered air-exposed mice that were exposed for 6 months at Irvine. The mouse aortas were removed, cut open, and pinned on a board. Tracings of the

Measuring Heart Rate Parameters

An ECG measures the electric potential of cells in the heart and shows real-time peaks and troughs that illustrate the heart's electrical signals that initiate contraction of the atria and ventricles. Computer programs analyze the different waves in the ECG traces to detect the onset and offset and amplitude of the wave forms and to also detect abnormal sinus waves. Only normal-to-normal heart beats are included in the analyses. HRV is the conventionally accepted term to describe the considerable long- and short-term fluctuations in HR that occur in normal individuals. A substantial body of evidence indicates that, in humans, reduced HRV is associated with cardiac mortality after myocardial infarction.

The most established and simplest HRV measures to obtain are the *frequency-domain* HRV measures: the low frequency

(LF) and high frequency (HF) components of HRV, which describe short- and long-term fluctuations in HR, respectively. In addition, the LF/HF ratio has been proposed as an index of the balance between the regulatory influences of the sympathetic and parasympathetic nervous systems. In addition, *time-domain* HRV parameters can be measured, such as the standard deviation of normal-to-normal intervals (SDNN), a broad measure of HF and LF oscillations that reflects changes in autonomic tone, and the square root of the mean of the squared differences between adjacent normal-to-normal intervals (RMSSD), a measure that corresponds to HF variability and reflects changes in cardiac vagal tone. These are common parameters that are reported in the scientific literature. An ECG can be obtained in humans as well as animals, and the parameters reported are the same across species.

plaques were used to make semiquantitative assessments of the plaque progression over time.

Serum Biomarkers Chen and Lippmann collected serum samples at each time point at which atherosclerosis was evaluated to measure concentrations of markers of systemic and vascular inflammation and oxidative stress, including C-reactive protein, interleukin (IL)-6, IL-10, IL-12, IL-13, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF- α), and vascular endothelial growth factor A (VEGF-A). Samples collected at Manhattan and Tuxedo were analyzed using enzyme-linked immunosorbent assays. Because marker levels were very low, samples collected at Seattle and Irvine were analyzed using a more sensitive method, electrochemiluminescence assays.

Statistical Approaches

ECG data were analyzed during four daily time periods: 9 AM–2 PM (during CAPs exposures), 7 PM–10 PM, 10 PM–1 AM, and 1 AM–4 AM (during quiet hours, when ECG measures were less likely to be disrupted by environmental factors). Data were summarized over 5-minute intervals and then averaged over each period. The period data were adjusted for the daily average across all four periods. Data were analyzed using a mixed-effects time-series approach that included evaluations of chronic effects (i.e., gradual changes in ECG measures over the 6-month period) as well as acute effects associated with daily exposure concentrations. Data were analyzed for associations of cardiac measures with CAPs concentrations, with and without source categories as covariates, and also with concentrations of CAPs components at each location. The investigators evaluated associations of cardiac measures with CAPs exposures on the current day (lag 0), previous day (lag 1), and two days earlier (lag 2) as well as across those three days (distributed lag).

Significant results ($P < 0.05$) for the source categories and for the components were tabulated across the six cardiac measures and evaluated based on the number of changes, regardless of the direction or magnitude of the changes. Differences in plaque progression in CAPs-exposed compared to filtered air-exposed mice were analyzed using one-way analysis of variance (ANOVA) followed by the Dunnett test.

KEY RESULTS

Exposure Measurements and Source Categories

Mean CAPs exposure concentrations over 6 months ranged from approximately 60 to 70 $\mu\text{g}/\text{m}^3$ at East Lansing and Seattle to 120 to 140 $\mu\text{g}/\text{m}^3$ at Manhattan, Tuxedo, and

Irvine (IR Figure 2, top panel, Study 1), with variations across the seasons (IR Figure 2, bottom panel, Study 1). Locations with higher local traffic volumes — Manhattan, Irvine, and Seattle — showed higher BC concentrations. The investigators did not correct their analyses for differences in CAPs concentrations across the locations. Analyses of CAPs elements collected on filters showed that each location had a unique pattern of factor loadings (IR Figure 4, Study 1), and therefore each location had different definable source categories.

Using factor analysis, the investigators identified nine source categories at Manhattan, four source categories at Tuxedo, five source categories at East Lansing, and six source categories at Seattle and at Irvine (IR Tables 3–7, Study 1; Commentary Table 2). Note that the investigators did not attempt to define source categories using consistent component clustering across locations. Thus, source categories with the same name may represent somewhat different groups of elements and underlying sources. For example, all locations had a source category identified as Soil, which consistently included strong contributions from Al and Si; however, contributions of more weakly associated components, such as S, Ca, K, Fe, and other elements to the Soil source category varied across locations. At the same time, specific elements contributed to more than one source category; for example, Mn contributed to a Traffic and Road Dust source category at Seattle, a Traffic source category at Irvine, a Sulfur–Coal source category at East Lansing, Sulfur–Coal and Ni Refinery source categories at Tuxedo, and a Steel source category at Manhattan. An overview of the source categories identified for each location is provided in Commentary Table 2, which indicates the two or three elements contributing the most to each source category.

As expected, a Traffic source category was identified at Manhattan and Irvine, a combined Traffic and Road Dust source category at Seattle, and an OC–EC source category at East Lansing; however, no traffic-related source category was identified at Tuxedo. Pollutants transported over long ranges were identified as contributing to a Sulfur–Coal source category at East Lansing, a Sulfates source category at Seattle, Sulfur–Coal as well as Secondary Aerosols source categories at Manhattan, and Sulfur–Coal and Ni Refinery source categories at Tuxedo; no source category at Irvine was said to include long-range transported particles. A Residual Oil Combustion source category was identified at East Lansing, Manhattan, Irvine, and Seattle; contributions of Ni and V defined the category at each location. A Biomass Combustion source category was identified only at Irvine and Seattle, and a Fireworks source category only at Manhattan, mainly attributed to the 4th of July holiday.

Commentary Table 2. Source Categories and Selected Factor Loadings Identified at Each Location in Chen Study 1^{a,b}

Factor	Manhattan	Tuxedo	East Lansing	Seattle	Irvine
1	Incineration Zn 0.90, Cl 0.72, Pb 0.61	Soil Al 1.01, Si 0.68, Pb 0.44	Soil Si 0.91, Ca 0.91, Al 0.7	Salt Na 0.85, Cl 0.85, Mg 0.71	Residual Oil Combustion V 0.89, Ni 0.86, S 0.64
2	Steel Mn 0.95, Fe 0.62	Sulfur-Coal Br 0.80, Se 0.76, S 0.57	Sulfur-Coal S 0.77, Se 0.59, Br 0.53	Soil Al 0.82, Si 0.83	Soil Si 0.75, Al 0.72, Ca 0.68
3	Soil Al 0.71, Si 0.59	Ni Refinery Fe 0.69, Zn 0.65, Ni 0.64	Residual Oil Combustion V 0.66, Ba 0.60, Ni 0.56	Traffic and Road Dust Mn 0.83, Ca 0.53, Cu 0.53	Traffic Mn 0.67, Ba 0.67, Fe 0.48
4	Residual Oil Combustion V 0.74, Ni 0.71	Salt Na 0.75, Cl 0.73, Mg 0.54	Zn-Cl Cl 0.84, Zn 0.50	Biomass Combustion K 0.63, EC 0.58, Cu 0.47	Biomass Combustion K 0.44, Br 0.45
5	Sulfur-Coal Br 0.74, Se 0.57, S 0.46	—	OC-EC OC 0.77 EC 0.7	Residual Oil Combustion Ni 0.79, V 0.76	Salt Cl 0.62, K 0.43
6	Fireworks Ba 0.77, K 0.70	—	—	Sulfates S 0.69, Br 0.67	Metals Ba 0.59, Zn 0.58
7	Salt Na 0.71, Mg 0.37, Cl 0.31	—	—	—	—
8	Traffic ^c NO ₂ 0.61, EC 0.48	—	—	—	—
9	Secondary Aerosols OC 0.70, S 0.31	—	—	—	—

^a The source categories in each location are listed in the order in which the factor analysis identified them (i.e., strongest correlations listed first). Only the top two or three elements are shown. Elements with higher correlations contributed more strongly to a particular source category.

^b — indicates that no further source categories were identified.

^c The investigators used NO₂ gaseous concentrations obtained from the EPA CSN database to help identify a Traffic source category at Manhattan. NO₂ data were not used at the other locations.

Heart Rate and Heart Rate Variability

Using time-series analyses, Chen and Lippmann observed that CAPs exposures were associated with acute increases in HR and decreases in HRV (SDNN, RMSSD, LF, HF, and HF/LF ratio). Most associations were observed at lag-0 exposures and to a lesser extent at lag 1; few associations were observed at lag 2. These associations were observed at Manhattan and, to a lesser extent, at Tuxedo;

very few significant associations were seen at the other locations (Commentary Figure 1 and Commentary Table 3). Effects were seen during both the exposure period (9 AM–2 PM daily) and the nonexposure hours.

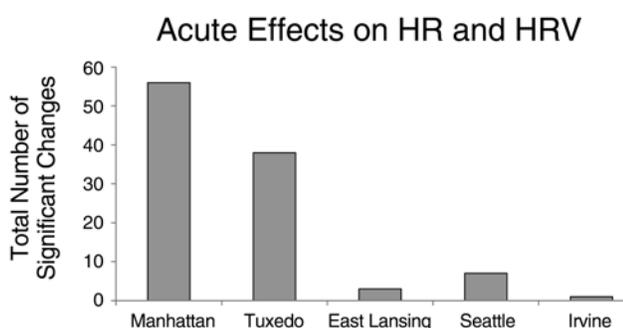
Chen and Lippmann conducted additional analyses to investigate associations of cardiac measures with source categories at each location. The highest number of significant changes was observed at Manhattan and the lowest number

at East Lansing (IR Table 8, Study 1; data were tabulated irrespective of the direction of the changes). The investigators concluded that at Manhattan the highest number of significant changes was associated with Residual Oil Combustion, Secondary Aerosols, and Sulfur–Coal source categories, followed by Salt and Traffic source categories. Fewer changes were associated with the Steel and Soil source categories, and the lowest number with Fireworks and Incineration. At Tuxedo, the highest number of significant changes was associated with Sulfur–Coal, followed by the Salt source category. Soil was the source category with the highest number of associations with cardiac measures at the three

other locations, with some effects attributed to the Traffic, Biomass Combustion, and Salt source categories.

Given that the strongest associations of cardiac measures with source categories were observed at Manhattan and Tuxedo, but not at other locations, the investigators concluded that residual oil and coal combustion as well as traffic pollution contributed most to the observed acute cardiac effects associated with CAPs exposures.

Because the animals were exposed for 6 months, the investigators were also able to evaluate HR and HRV changes over time (i.e., chronic effects) associated with CAPs exposures. HR measured from 7 PM to 10 PM each day was elevated at Manhattan during the first 50 days in CAPs-exposed mice relative to control mice, but it decreased gradually thereafter until the HR of the exposed mice was the same as that of the control group. No chronic changes were seen in HRV measures at Manhattan. At Tuxedo, HR measured from 10 PM to 1 AM every day decreased gradually and reached significance after 75 days; SDNN measured during the same period every day increased gradually and reached significance after 75 days. No consistent changes were seen at the other locations (see IR Figures 5–10, Study 1, and Commentary Table 3).



Commentary Figure 1. Effects of acute exposure to CAPs on heart rate and heart rate variability parameters (Chen Study 1). The data represent the total number of significant changes observed across four daily periods (i.e., CAPs exposure, 9 AM–2 PM, and three nonexposure periods between 10 PM and 4 AM) in CAPs-exposed mice compared with filtered air-exposed controls. (Based on data in IR Table 2.)

Atherosclerotic Plaque Progression

Chen and Lippmann observed that subchronic exposure (for 6 months) was associated with greater plaque progression in the brachiocephalic arteries CAPs-exposed compared

Commentary Table 3. Summary of Significant Changes in Cardiovascular Outcomes Observed in Mice After CAPs Exposures at Five Locations in Chen Study 1^a

Endpoint	Manhattan	Tuxedo	East Lansing	Seattle	Irvine
Acute Changes					
HR ^b	9	6	1	3	1
HRV parameters ^b	47	32	6	0	0
Chronic Changes					
HR	Increase ^c	Decrease ^d	n.s.	n.s.	n.s.
HRV (SDNN)	n.s.	Increase ^d	n.s.	n.s.	n.s.
Atherosclerotic plaque progression ^e	Increase	Increase	Increase	n.s.	n.s.
Serum biomarkers	Increase (IL-10)	n.s.	n.s.	n.s.	Increase (GM-CSF); Decrease (IL-6, IL-10)

^a n.s. indicates no significant results.

^b Number of significant changes for the four daily analysis periods (during CAPs exposure as well as nonexposure periods). The number of significant changes is the sum of the results for lag days 0, 1, and 2. See IR Table 2.

^c Observed for the first 50 days, for the nonexposure part of the day (7 PM–10 PM). See IR Figure 5.

^d Starting after a few days and reaching significance at 75 days, for the nonexposure part of the day (10 PM–1 AM). See IR Figures 5 and 6.

^e Significantly larger increase than that observed in the control group exposed to filtered air.

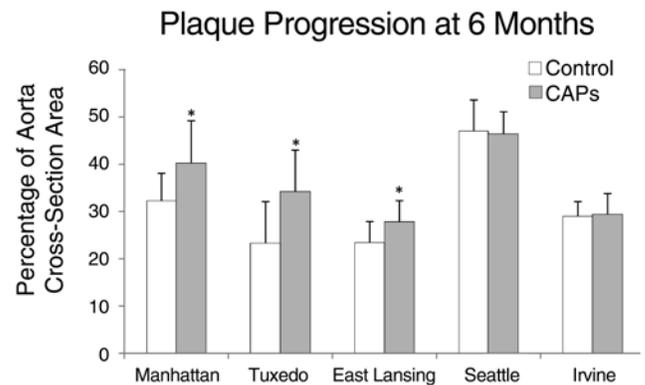
with filtered air–exposed ApoE knockout mice at Manhattan after 6 months, at Tuxedo after 3 and 6 months, and at East Lansing after 6 months (Commentary Figure 2 and Commentary Table 3). Plaque progression in the left common carotid artery was more pronounced in control mice; thus fewer CAPs-related effects were observed (at Tuxedo after 6 months but also at Irvine after 2 months). The investigators concluded that the effects were most likely attributable to a Coal Combustion source category. The data at Irvine and East Lansing were verified using visual maps of the plaque area in mice that were killed after 6 months of exposure. The investigators concluded that the histological data confirmed the data obtained by ultrasound.

Serum Biomarkers

Few consistent changes were evident in serum biomarkers following CAPs exposures either by location or by exposure duration. Some biomarkers were upregulated compared to those of the control groups: for example, IL-10 after 3 months of exposure at Manhattan, and granulocyte-macrophage colony-stimulating factor (GM-CSF) after 6 months at Irvine. In addition, levels of IL-12 tended to increase after 3 and 6 months at both Manhattan and Tuxedo. Other biomarkers were downregulated compared to those of the control groups: IL-6 and IL-10 after 6 months at Irvine. No changes were observed in the remaining biomarkers that were evaluated: C-reactive protein, IL-13, MCP-1, TNF α , and VEGF-A. The investigators concluded that these small changes were probably spurious events and were of unclear biological significance, indicating that no systemic inflammation was found.

EVALUATION OF CHEN STUDY 1

In its independent review of the study by Chen and Lippmann, the HEI NPACT Review Panel noted that the study represents the first comprehensive assessment of the effect of intercity variation in PM composition on HRV and atherosclerosis in experimental animals. Chen and Lippmann chose two commonly evaluated, appropriate cardiovascular endpoints. HRV has been previously shown to be particularly sensitive to pollutant exposure. Many epidemiologic studies have shown that high HRV is associated with good cardiovascular health, whereas low HRV values are indicative of poor prognosis (Brook et al. 2010). Additionally, extensive literature has shown that CAPs exposures increase atherogenesis in mice in a manner that is similar to how air pollution affects intima-media thickness — a surrogate marker of atherosclerosis progression — in humans (Sun et al. 2005; Araujo et al. 2008; Araujo and Nel 2009; Bauer et al. 2010; Quan et al. 2010; Tonne et al. 2012). In addition, the study design using CAPs exposures in different locations to



Commentary Figure 2. Atherosclerotic plaque progression in the brachiocephalic artery of ApoE knockout mice after 6 months of exposure to CAPs or filtered air (Chen Study 1). Asterisks indicate a significant difference ($P < 0.05$) between CAPs-exposed mice and the control group at that location. Each bar represents 10 to 13 animals. (Based on data in IR Table 12.)

capture different source mixtures was well thought out. However, the interpretation of the results obtained, within the context of cardiovascular toxicity, is complicated by several methodologic issues, discussed below.

Choice of Animal Model and Evaluation of Plaque Progression

Normal wild-type mice or rats do not spontaneously develop advanced atherosclerotic plaques, even when placed on a high-fat diet. Although the exact reasons are unclear, it has been suggested that this resistance may be related to higher high-density lipoprotein and lower low-density-lipoprotein (LDL) levels in rodents compared to humans. However, deletion of the ApoE gene or the gene for the LDL receptor, which leads to increased LDL and very-low-density lipid levels in mice, induces abnormal lipid levels (dyslipidemia) and promotes the formation of advanced vascular lesions that resemble human atherosclerotic plaques. Such models have been criticized because they are associated with supraphysiological levels of cholesterol that are rarely seen in humans. However, in the absence of other relevant models, they have been widely used to study mechanisms underlying the progression of atherosclerotic disease.

ApoE knockout mice have also been used to demonstrate that CAPs exposure accelerates atherogenesis. While the results of such studies are not all consistent, previous studies in Los Angeles and New York have shown consistent exacerbation of atherosclerotic lesion formation (Sun et al. 2005; Araujo et al. 2008; Quan et al. 2010). In addition, increases in plaque inflammation and changes in plaque composition were reported in ApoE knockout mice exposed to diesel exhaust (Campen et al. 2010; Quan et al.

2010). However, in the current study, exposure of this type of mouse to pollutants in locations with varying source profiles produced different results, with a lack of plaque progression in some locations. Although the authors attribute this to variations in the composition and concentrations of CAPs in different cities, this does not explain all of the findings. For example, ApoE knockout mice, by design, show plaque progression during aging. Thus, it is surprising that the investigators did not observe such progression in the brachiocephalic artery or the left common carotid artery of mice that were exposed to filtered air at Seattle and Irvine. This lack of progression in control mice complicates the interpretation of the changes (or lack of changes) observed in CAPs-exposed mice. It should be pointed out that in the current study mice were fed a normal diet, which results in lower levels of atherosclerosis as compared to feeding mice a high fat diet, the approach that is more common and that was used in the NPACT study by Campen and colleagues (2013). It remains unclear to what extent differences in baseline levels of atherosclerosis may have played a role in the lack of atherosclerosis progression observed in mice exposed to CAPs at Seattle and Irvine.

In this study, the investigators used a relatively novel method to measure the progression of atherosclerotic lesions: noninvasive ultrasound biomicroscopy. The advantage of this technique is that repeated measurements can be made over time in the same mice. While previously validated (Chen et al. 2010; Quan et al. 2010; Wang et al. 2011), this is a new technique that is not (yet) widely used. It is therefore unclear whether some of the reported variations in lesion sizes might be due to the insensitivity of the technique or other reasons such as differences in how the operators performed the ultrasounds. Finally, it is possible that plaques might have progressed in vascular beds that were not evaluated. For example, increases in plaque progression in the aortic root that were evaluated in this study frequently do not translate to changes in the dorsal aorta, indicating that some vascular effects may have been missed. The reason for such site-specific susceptibility is not clear, but might be related to differences in sheer stress and local responses of the vessel wall. Plaque progression in ApoE knockout mice showed strong geographic variation. However, such geographic inhomogeneity makes interpretation of negative results difficult. Even though no significant increases in plaque progression in brachiocephalic or carotid arteries were observed in mice exposed to CAPs at Seattle or Irvine, this does not necessarily mean that these exposures were innocuous and that plaque formation at other vascular beds was not affected. Such uncertainty underscores the complexity associated with studying atherosclerosis in mice and highlights the need

for simpler, surrogate measures or biomarkers to assess the impact of environmental exposures on atherosclerosis.

Evaluation of Cardiac Function

Similarly, methodologic issues could not be ruled out in interpreting changes in cardiac function. The investigators measured parameters related to cardiac function (fractional shortening and cardiac-wall thickness) but did not observe changes after 6 months of exposure at Manhattan and Tuxedo and subsequently discontinued taking these measurements. As a result, the only cardiac measure that could be analyzed at all locations was HRV. However, variable results were obtained. In some cities, HRV increased after long-term exposure, whereas in others a decrease in HRV was observed. The reasons for such diametrically opposed effects are unclear, and they make consistent interpretation of the findings difficult. Moreover, even though HRV and HR have been used as surrogate markers of cardiac function, they reflect changes in the autonomic nervous system and cannot be directly related to changes in the contractility of the heart or in its chamber properties. As an alternative, one could analyze spontaneous dysrhythmias to assess changes in myocardial excitability. Adult, healthy C57BL/6 mice, the strain from which ApoE knockout mice are derived, spontaneously undergo three to six arrhythmic events (e.g., supraventricular tachycardia, ventricular premature beats, atrioventricular block, or sinus pause) per day, and changes in the frequency of those events have been observed when the mice are exposed to pollutants (London et al. 1998; Remme et al. 2006). Therefore, it would be of interest in future studies (or in future analysis of the current data) to see how different PM exposures affect myocardial rhythm disturbances.

The Panel noted that a relatively high number of mice evaluated for cardiac function had to be killed because they developed skin lesions. Although ApoE knockout mice commonly develop skin lesions because of excessive cholesterol deposition, this problem is usually not severe enough to require the killing of a substantial number of mice in a colony over the course of 6 to 8 months. In the current study, this complication led to the elimination of a fairly large number of mice from the experimental groups. The number of animals per group was reduced to as few as four mice (a control group) or six mice (a CAPs-exposed group), which is 50% of the originally exposed mice in some cases. This may have affected the statistical power of the study to find an effect and could have caused bias if loss of animals was different in the exposure and control groups. The investigators mentioned that the skin lesions were randomly distributed among experimental groups (Chen, personal communication, August 2013), making a systematic bias unlikely.

Defining Source Categories

Study 1 used a factor analysis approach that was similar to those of Studies 2 and 3 (see a detailed evaluation of this method in the discussion of Ito Study 3 later in this Commentary). Although Study 1 provides sparse details about how the method was applied, the evaluation of how it was applied to Study 3 is helpful in understanding Study 1.

The investigators identified between four (Tuxedo) and nine (Manhattan) source categories across the five locations. The top factors identified were Soil (East Lansing and Tuxedo), Residual Oil Combustion (Irvine), Incineration (Manhattan), and Salt (Seattle), which is thought to represent ocean spray. It was surprising that a Traffic source category was not higher on the list at Irvine and Manhattan, which were specifically selected to study the effects of traffic-related pollution as part of the ambient pollution mixture. Each location had a unique source profile because each one had unique data sets for PM components and other pollutants, and also because data were collected during different time periods (more or less at the same time as the CAPs exposures, which were performed in different years and seasons).

One of the challenges in interpreting the source categories is that many have similar sounding names that may or may not represent similar sources. For example, the investigators identified a Residual Oil Combustion source category at East Lansing (based on V, Ba, and Ni), at Manhattan (based on V and Ni), at Irvine (based on V, Ni, and S), and at Seattle (based on Ni and V). In contrast, Ni at Tuxedo was attributed to long-range transport of particles from a nickel smelter in Canada, similar to what was observed by the NYU team in previous studies in Tuxedo (Lippmann et al. 2006). It is unclear, however, why those earlier studies (Maciejczik and Chen 2005) identified "oil-fired power plant emissions" in Tuxedo that were not identified in the current study. One possible explanation is that some power plants in the region were converted to natural gas since the 2005 study and, if that indeed happened, it could have accounted for this difference.

The investigators identified a Fireworks source category (based on Ba and K) at Manhattan only; it was observed during the 4th of July holiday and surrounding week. The Review Panel thought that it would have been preferable to remove the related data points from the analyses, given that they were clear outliers that could have affected the time-series results. The investigators and the Review Panel differed on this issue and it was not resolved; one possible solution would have been to conduct sensitivity analyses while including or excluding those data points.

Conclusions

The study by Chen and Lippmann represents the first comprehensive assessment of the effect of intercity variation in PM composition on HRV and atherosclerosis in experimental animals, two commonly evaluated, appropriate cardiovascular endpoints. Their results are consistent with earlier reports that exposure to CAPs leads to adverse cardiovascular events, including acute changes in HR and related ECG measures, as well as chronic changes in atherosclerotic plaques and serum biomarkers of vascular and systemic inflammation. The investigators concluded that residual oil and coal combustion, which are primarily observed in the eastern United States and observed less in the western United States, as well as traffic pollution (based on the fact that some changes were observed at East Lansing in the Midwest) contributed most to the observed acute cardiac effects associated with CAPs exposures. The Review Panel was not persuaded by the investigators' interpretation that residual oil and coal combustion were the most important contributors to health effects, however, and thought that several issues warranted more cautious interpretation of the results:

1. Large differences in CAPs concentrations were found among locations. It is thus possible that larger and more numerous changes in biological markers were observed in some locations because animals were exposed to higher concentrations of CAPs, rather than because of differences in PM composition. The investigators did express the changes in ECG measures as changes per $\mu\text{g}/\text{m}^3$ of CAPs, but they did not adjust for CAPs concentrations in the analysis of plaques. It therefore remains unclear to what extent the differences in plaque progression were due to differences in PM composition or PM mass concentrations.
2. Various uncertainties are associated with assigning source categories in the factor analyses, as discussed in more detail in the evaluation of Thurston Study 4. It remains unclear why few changes were observed in mice exposed at Seattle and Irvine, where the air pollution mixture is dominated by traffic and other combustion sources. (See the companion NPACT report by Vedal and colleagues [2013]; they found much stronger evidence for traffic-related health effects.)
3. It remains unclear why there was no plaque progression in mice exposed to filtered air at Seattle and Irvine (this finding was confirmed by additional histologic analyses of aortic tissues). It is possible that plaques might have progressed in other vascular beds that were not evaluated. Also, the mice at Seattle were older and therefore had larger plaques at the beginning of the

exposures than mice at other locations. It also remains unclear whether the fact that mice were fed a normal rather than high-fat diet may have played a role.

4. The investigators used a relatively novel method to measure the progression of atherosclerotic lesions: noninvasive ultrasound biomicroscopy. The advantage of this technique is that repeated measurements can be made over time in the same mice, but it is not (yet) widely used in other laboratories, and the Review Panel felt that, to ensure its accuracy, further validation is needed by other researchers.
5. The investigators observed acute as well as chronic changes in HRV, but the changes went in opposite directions at different locations, and the chronic changes were not consistent in direction or when they occurred (early on or later during the 6-month exposure period). This makes it difficult to interpret the ECG results. There was also high mortality from skin lesions in these mice, which may have affected the power of the study to find significant effects.

The Review Panel thought that the toxicity of source categories other than Residual Oil Combustion, Coal Combustion, and Traffic could not be ruled out and remain important from a regulatory perspective. For example, a series of animal studies involving subchronic exposure by inhalation of fresh emissions resulting from coal combustion, diesel exhaust, gasoline exhaust, and wood smoke (using identical study designs) found that the relative toxicity of the simulated downwind coal combustion was less compared to those of the other complex mixtures (Mauderly et al. 2011). Detailed analyses of those comparative studies, using compositional data of the various exposure atmospheres, pointed toward SO₂, ammonia, NO_x, and CO as most predictive of changes in inflammatory and vascular markers (Seilkop et al. 2012). Thus, the Panel felt that there is some evidence that specific sources, such as residual oil and coal combustion and traffic, play an important role in PM toxicity, but that other sources cannot be ruled out.

STUDY 2. TERRY GORDON AND COLLEAGUES

GENERAL APPROACH

In their study, Gordon and colleagues tested the following hypotheses, which center around overall Specific Aim 2:

1. Coarse PM, fine PM, and ultrafine PM are each capable of producing acute health effects of public health

concern, but the effects might differ according to particle-size range and particle composition within each size range.

2. The source-apportionment techniques that we have developed and refined in recent years provide a useful basis for identifying major PM air pollution source categories as well as specific chemical components having the greatest impacts on a variety of acute and chronic health effects.
3. The acute health effects caused by short-term exposures to PM samples in various particle-size ranges collected at multiple sites can usefully be studied in cells *in vitro* and in animal models treated *in vivo* by aspiration.

Gordon and colleagues used a combined *in vitro* and *in vivo* approach to analyze acute toxicity of a large number of PM samples collected in five locations comparable to the locations for CAPs exposures in the study by Chen and Lippmann. The overall goal was to examine how PM of varying composition and size classes affected toxicity. At each location, daily PM samples were collected on filters in three size fractions (coarse, fine, and ultrafine PM) over a 2-week period (12-day study) during two seasons. Each sample was tested in a cell culture or administered to mice by aspiration into the lung. In addition, the investigators conducted a longer-term study in two of the five locations, in which 100 daily samples were collected, extracted, and then resuspended for administration to mice by aspiration (100-day study).

In vitro experiments consisted of administering reconstituted PM filter extracts to cell cultures, including human airway epithelial cells, human pulmonary microvascular endothelial cells, and mouse cardiomyocytes. Biological responses in these cells that were evaluated included cell viability, production of reactive oxygen species (ROS), gene expression of inflammatory markers, as well as beat frequency and electrical conductivity (cardiomyocytes only). *In vivo* experiments consisted of administering PM samples by intratracheal aspiration followed by lung lavage 24 hours later. Given the very large number of samples collected, the investigators selected a subset of PM samples that produced the highest and lowest ROS responses *in vitro*. They measured differential cell counts and production of inflammatory markers.

All PM filter samples were analyzed by size fraction for detailed chemical composition and endotoxin content. Concentrations of individual components and endotoxin were then correlated with the biological responses. In addition, the investigators conducted a factor analysis to group components by source categories and correlate source categories with biological responses.

METHODS

PM Sample Collection and Analysis

The investigators collected PM samples in five locations that more or less overlapped with the locations in the study by Chen and Lippmann: Tuxedo, New York; Manhattan in New York City, New York; Ann Arbor, Michigan (65 miles southeast of East Lansing, Michigan, the location used in the Chen study); Seattle, Washington; and the Los Angeles area, California. The 100-day-study samples at Manhattan were collected at Hunter College (five miles south of the Mt. Sinai School of Medicine, where the Chen study was conducted) because the original sampling location at Mt. Sinai was no longer available. The investigators used two locations in California: The 12-day study samples were collected at the LA basin (Anaheim), and the 100-day study samples at the University of California–Irvine campus, 15 miles southeast of Anaheim, the same place that Chen and Lippmann conducted their CAPs study.

For the 12-day study, PM samples were collected for 2 weeks at the five locations during two seasons. Weekend days were combined, thus yielding 12 samples per collection period. For the 100-day study, PM samples were collected for 100 consecutive days at two locations (Manhattan and Irvine). PM was collected using a Harvard high-volume cascade impactor. Particles larger than 10 μm were excluded. Coarse particles ($\text{PM}_{10-2.5}$) and fine particles ($\text{PM}_{2.5-0.2}$) were collected on a polyurethane foam substrate; ultrafine particles ($\text{PM}_{0.2}$) were collected on a polypropylene substrate. The nominal size cut-off for ultrafine PM was 0.15 μm , but the ultrafine fraction is referred to in the IR as $\text{PM} < 0.2 \mu\text{m}$, indicating that some fine particles were included in this size fraction.

PM samples were extracted from the filter substrate using sonication in ethanol and ultrapure water and subsequently lyophilized (i.e., dehydrated by freeze-drying) and weighed. Extraction efficiency was about 80% for the coarse and fine fractions, and about 64% for the ultrafine fraction. Samples were resuspended in ultrapure water at a concentration of 250 $\mu\text{g}/\text{ml}$ for in vitro experiments and 1 mg/mL for in vivo experiments. A subset of samples was analyzed for endotoxin content. Analysis of the PM samples for composition included acid digestion followed by inductively coupled plasma–mass spectrometry to measure 27 different elements. The samples were not analyzed for EC or OC. A factor analysis with varimax rotation was applied to the compositional data (conducted by Drs. Kazuhiko Ito and Ramona Lall and similar to the factor analysis performed in Study 3; see also the Source Apportionment sidebar) to identify major PM source categories in each location. Varimax rotation is a type of rotation that maximizes the sum of the variances of the squared loadings

(squared correlations between variables and factors). It should be noted that the source categories identified are unique to this study and are somewhat different from those in the studies led by Chen, Ito, and Thurston. This is in part because Study 2 used inductively coupled plasma mass spectroscopy for chemical speciation, which yields better signal-to-noise ratios for some of the elements (e.g., antimony) than the XRF method used for Studies 1, 3, and 4. It is also likely different because Study 2 analyzed different PM size fractions (Studies 1, 3, and 4 analyzed only $\text{PM}_{2.5}$). The investigators used a metal-rich sample from fireworks smoke as a positive control for some experiments.

Measuring Biological Responses in Vitro

Cell Cultures For the main sets of in vitro experiments, the investigators used an immortalized human bronchial epithelial cell line (BEAS-2B), an immortalized human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R), and primary cultures of cardiomyocytes derived from transgenic mouse embryonic stem cells. They also used primary human bronchial epithelial cells and primary human lung microvascular endothelial cells for validation of results obtained with the immortalized cell lines, using a subset of the PM samples. All cell cultures were exposed to aliquots of reconstituted PM samples, and experiments were run in triplicate. Because cardiomyocytes are unlikely to be confronted with particles directly, those cells were exposed to only the soluble components of PM.

Cell Viability Cell viability was assessed in epithelial and endothelial cells by the release of lactate dehydrogenase (LDH) after exposure to PM, and in cardiomyocytes by spontaneous beat frequency. Additional validation of the LDH assay was conducted using a clonal survival assay in BEAS-2B cells.

ROS and Markers of Inflammation Intracellular production of ROS was measured using a fluorescent 2',7'-dichlorofluorescein-diacetate (DCFH-DA) assay before and after the cells were exposed to the PM solution. A subset of 60 PM samples (see Measuring Biological Responses in Vivo, below) was used to measure oxidative capacity in cell-free ascorbic acid and dithiothreitol assays; this work was performed by Dr. Flemming Cassee and colleagues at the National Institute for Public Health and the Environment in the Netherlands. The same 60 samples were evaluated for messenger ribonucleic acid (mRNA) expression of inflammatory markers: CSF-2, heme oxygenase-1 (HO-1), IL-6, IL-8, and VEGF-A in epithelial cells; and HO-1, intercellular adhesion molecule-1, IL-8, and thioredoxin reductase-1 in endothelial cells.

Beat Frequency and Signal Conduction in Cardio-

myocytes Beat frequency was assessed by an observer counting beats before and after the cells were exposed to soluble PM extracts. A microscope-mounted camera combined with a software-capture system was used for quality control. In addition, conduction velocity and action potential duration at 50% and 70% repolarization were measured using optical mapping. This method visualizes electrical signals in stimulated cells with fluorescence lighting and analyzes pixels recorded with a microscope-mounted camera.

Measuring Biological Responses in Vivo

For the 12-day study, Gordon and colleagues selected a subset of 60 samples (collected in summer) that had shown the two highest and two lowest ROS responses in the DCFH-DA assay *in vitro*. This set included all three size fractions at all five locations. In a second set of experiments, samples from the 100-day study collected at Manhattan and Irvine were evaluated. The number of samples tested (67 and 93, respectively) was smaller than 100 because collection equipment malfunctioned.

The investigators used 6- to 10-week old male and female FVB/N mice, three per sex per group for the 12-day study, and three per group (random sex) for the 100-day study. The mice were anesthetized with isoflurane and administered water or 50 µg resuspended PM (in a 1 mg/mL suspension) by oropharyngeal aspiration. After 24 hours, the mice were killed and the investigators obtained serum and lung lavage fluid to assess numbers of macrophages, neutrophils, eosinophils, and epithelial cells as well as concentrations of markers of inflammation, such as total protein. (These analyses were not completed when the Investigators' Report was written and the authors did not specify what other markers they measured.)

Statistical Analyses

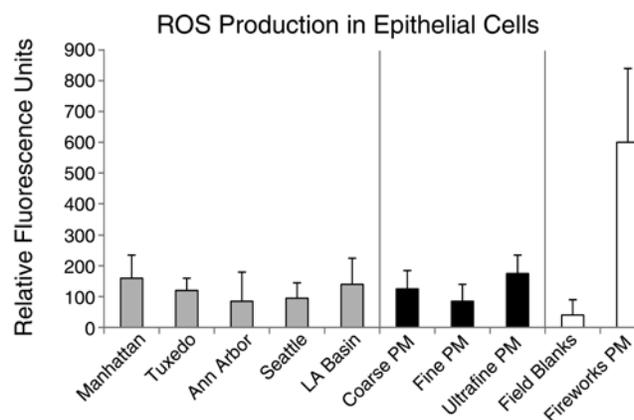
All biological data were presented as the mean of three replicates. Data for ROS and markers of inflammation were analyzed by ANOVA followed by the Wilcoxon rank-sum test; beat frequencies were analyzed by ANOVA followed by the Dunnett test. Results were considered statistically significant at $P < 0.05$. Correlations between biological endpoints and PM components were analyzed by linear regression. Correlations between biological endpoints and source categories were analyzed by varimax rotation.

KEY RESULTS

12-Day Study

PM Composition Large variations were observed in metal composition across locations, seasons, and size fractions. The concentrations of a majority of the metals were higher in samples collected in summer than in those collected in winter. The compositions of the fine and ultrafine PM fractions were more similar to one another than they were to the coarse fraction. The coarse fraction showed less seasonal variation. Some of the highest levels of metals were observed at the LA Basin and Manhattan. Endotoxin levels were generally low, with higher concentrations in the coarse PM fraction compared to those in the fine and ultrafine fractions.

ROS Production in Vitro Because ROS production in epithelial BEAS-2B and endothelial HPMEC-ST1.6R cells was highly correlated, the investigators focused the presentation of results in their report mostly on the experiments with BEAS-2B cells. There were small but significant differences in ROS production by location, season, and size fraction (see IR Figure 3, Study 2, and Commentary Figure 3). The highest ROS production — averaged across all samples — was observed at Manhattan and the LA Basin, followed by Tuxedo, with the lowest production at Seattle and Ann Arbor. ROS production observed for ultrafine PM samples was higher than that for coarse and fine PM samples (when analyzed using equal mass concentrations; this



Commentary Figure 3. ROS production in BEAS-2B epithelial cells exposed to resuspended PM *in vitro* (Gordon Study 2). Grey bars indicate median values across samples collected for 12 consecutive days at each of the five locations. Black bars indicate median values for the coarse, fine, and ultrafine fractions across samples collected at all locations. Error bars indicate 75% confidence limits. See IR Figure 3. For comparison purposes, blank bars on the right indicate ROS production in BEAS-2B cells exposed to field blanks (negative control) or metal-rich fireworks PM (positive control). (See IR Figure 1.)

effect was tempered when the samples were evaluated based on percentages of total mass because the ultrafine portion made up much less of the total PM mass than did the fine and coarse PM). Samples of both the fine and ultrafine fractions collected in summer had slightly higher ROS production than samples collected in winter; this held true for all five locations. However, for the coarse PM fraction, samples collected in winter produced more ROS than samples collected in summer at Tuxedo, the LA Basin, and Manhattan (IR Figure 4, Study 2). Daily variation in ROS production was more pronounced at some locations (e.g., the LA Basin) than others (e.g., Tuxedo).

Additional experiments with primary bronchial epithelial cells exposed to a subset of PM samples showed that the water-soluble fraction of PM was responsible for approximately 55% of the ROS production by the total PM. However, for some of these samples, the ROS production for the water-soluble fraction was similar to that for the total PM. Tests with another subset of 60 PM samples showed that their oxidative potential as measured in acellular assays was correlated with the level of ROS production by vascular endothelial cells treated with the same PM samples.

Correlation Between PM Composition and ROS

Production Correlations between concentrations of PM components and ROS production were stronger in endothelial cells than in epithelial cells. Strong correlations ($P < 0.001$) in both cells lines were observed for Cu, Sb, V, Co, Be, and Ni. Some of the correlations were size dependent. For example, correlations with Cu concentrations were seen with the coarse and fine, but not ultrafine, PM fractions, whereas correlations with V were observed with the ultrafine fraction. The investigators reported the largest number of significant correlations between elements and ROS production at Seattle, even though the ROS production at Seattle was not the highest compared to other locations. The investigators noted that some of the strongest correlations for Cu and K — at Ann Arbor, the LA Basin, and Manhattan — could point toward a Traffic source category, whereas correlations with Ni at Manhattan and Tuxedo could point toward a Residual Oil Combustion source category. Endotoxin was detected in the PM samples but was not correlated with ROS production.

Correlation Between Source Categories and ROS

Production The investigators identified five source categories when they included all 27 elements in the analyses. However, they could not identify clear source categories in the analyses and therefore selected a subset of 15 elements for a more focused factor analysis. This second analysis

identified the following source categories: Traffic/Brake Wear (Cu/Fe/Ti/Sb), Cr/Ni/Zn, Residual Oil Combustion (V/Ni/S), Coal Combustion (K/As/Se/S), and Soil Dust (K/Ca/Mn/Fe). Slight variations in source categories were identified when the size fractions were analyzed separately; for example, a Fireworks source category was identified for fine PM. The Traffic/Brake Wear category was most strongly (and significantly) correlated with ROS production; the Residual Oil Combustion category was also strongly correlated but with a lower r value (IR Figure 18, Study 2).

Gene Expression for Inflammatory Markers in Vitro

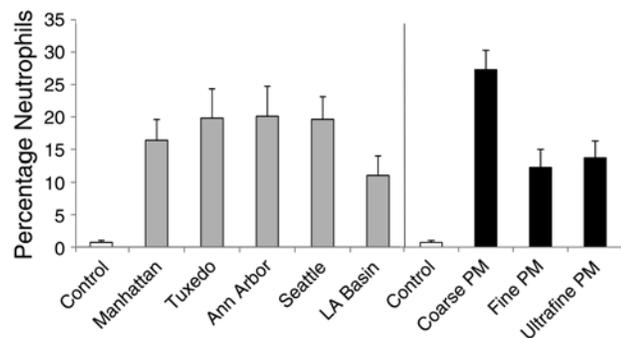
When responses across all samples were averaged, the most consistent effect was increased HO-1 mRNA expression in epithelial and endothelial cells. In addition, there was increased IL-8 mRNA expression in epithelial cells (IR Figure 8, Study 2). A few samples showed significant increases in mRNA expression across four or five markers of inflammation: four ultrafine PM samples (one at the LA Basin and at Seattle, two at Manhattan) and three fine PM samples (one at the LA Basin, at Seattle, and at Manhattan). More responses were observed in endothelial compared to epithelial cells, but there was no clear pattern in the gene expression of the five markers of inflammation by size fraction, location, or season (IR Tables 6 and 7, Study 2).

Correlation Between PM Composition and Gene

Expression Several elements were correlated with the expression of HO-1 mRNA in both endothelial and epithelial cells: Cu, Sb, Fe, Ti, Mn, and Cr. The strongest correlation was with Sb ($r = 0.73$). IL-8 mRNA levels in epithelial cells were correlated with Cu, Sb, Sr, Fe, Ti, Mn, and Cr. The investigators noted that these results may point to a similar mechanistic pathway underlying the HO-1 response in the two cell lines (IR Tables 12 and 13, Study 2).

Electrophysiologic Responses in Vitro The spontaneous beat frequency of untreated and PM-treated cardiomyocytes did not differ, except for two samples collected in winter at Manhattan (fine PM: significant decrease compared to untreated samples; ultrafine PM: significant increase). A decrease in conduction velocity was also observed with the Manhattan winter fine PM sample. The investigators noted that this sample had very high levels of Zn.

Lung Inflammation in Vivo The investigators did not observe changes in total protein content in lavage fluid, a sign of lung injury, in mice exposed to resuspended PM samples by aspiration. However, there was an increase in neutrophils, a sign of inflammation; this effect was smaller at the LA Basin than at the four other locations (Commentary Figure 4). Averaged across all locations, a larger response was associated with exposures to the coarse



Commentary Figure 4. Percentage of polymorphonuclear neutrophils in lavage fluid of FVB/N mice exposed by intratracheal aspiration to resuspended PM (Gordon Study 2). Grey bars indicate mean values across samples collected for 12 consecutive days at each of the five locations. Black bars indicate mean values for the coarse, fine, and ultrafine fractions across samples collected at all locations. Error bars indicate 95% confidence limits. (See IR Figure 12.)

fraction PM compared with the fine and ultrafine PM fractions. A similar response was also observed at Ann Arbor, Seattle, and Tuxedo individually. At Manhattan and the LA Basin, however, the neutrophil responses to the coarse and ultrafine fractions were the same. Changes in neutrophils did not correlate well with ROS production for the same PM sample (IR Figure 1, Study 2). Similarly, there was only partial overlap of the correlations of concentrations of elements with ROS responses and with neutrophil responses (IR Table 15, Study 2). Endotoxin and phosphorus concentrations correlated with neutrophil responses, but the correlation with endotoxin levels was difficult to explain given the low endotoxin concentrations in PM.

100-Day Study

Neutrophil responses were largest for the coarse PM fraction, intermediate for ultrafine PM, and smallest for fine PM. This pattern was observed both at Manhattan and Irvine, with the difference that at Irvine the response to fine PM was not significantly different from that of the control samples. The investigators also noted an increase in total protein content in lavage fluid of exposed mice, which followed patterns for the different size fractions that were similar to those observed for neutrophil responses at Manhattan and to a lesser extent at Irvine. These findings were different from the observations in the 12-day study, for which the *in vitro* responses to the ultrafine and fine fractions of PM differed less. Because PM compositional data were not yet available, the investigators could not evaluate correlations between biological endpoints and PM composition or source categories.

EVALUATION OF GORDON STUDY 2

In its independent review of the study by Gordon and colleagues, the Review Panel noted that the investigators had conducted a large and systematic evaluation of the toxicity of PM samples by size fraction as well as by season and location. They used valid approaches to evaluate a range of responses in pulmonary and vascular cells as well as cardiomyocytes *in vitro*, complemented by *in vivo* experiments in which mice were treated with PM samples that were aspirated directly into the airways. The Review Panel thought that this was an ambitious study of an array of outcomes meant to provide an understanding of differences among the collected samples. The study compared ROS and other *in vitro* and *in vivo* parameters in response to a reasonable concentration of PM, while controlling for the PM mass collected on the filters. The investigators did commendable work in carrying out this study carefully with much attention to detail. They compared different airsheds, different particle sizes, and focused on metals as a means to identify sources; they adequately assessed the role of endotoxin.

The investigators chose locations for the collection of PM samples similar to those selected for the CAPs exposures in Study 1; this would allow for useful comparisons even though the timing of sample collections was different. Note that the experiments using cardiomyocytes for assessing direct toxicity of PM are of interest, but it remains unclear how well the changes in beat frequency of those cells might reflect effects *in vivo*. Thus, the cardiovascular measurements in this study bear little relationship to those made in the other studies, led by Chen, Ito, and Thurston, described in this report.

Gordon and colleagues concluded that the toxicity of PM was driven by a complex interaction of particle size, site, and season in which the PM samples were collected. This is a reasonable conclusion. They suggested that PM components, as dictated by particle source, were responsible for the adverse effects of ambient PM. However, they did not conduct analyses of multiple PM components, so it remains unclear whether this general conclusion is supported by the data. Further, the investigators concluded that PM composition pointed in the direction of Traffic and Residual Oil Combustion source categories as contributors to the observed effects. Although the investigators emphasized the differences among their outcomes, the Review Panel found the similarities of the results across outcomes striking: The Panel noted that very similar biological responses were reported for the particle-size fractions and samples from the different airsheds. The findings about the role of metal elements in toxicity confirmed findings from a number of previous studies (see review by Chen and Lippmann 2009). The

Panel noted the absence of source-apportionment data for the 100-day study, which would have allowed for a more thorough evaluation of PM components.

Overall, the biological responses observed *in vitro* exhibit a level of consistency that is important. However, the results of the final analyses of correlations of PM components with sources do not add much new knowledge to the general understanding of health effects associated with PM. The study was somewhat limited in its use of correlation coefficients with individual components, which did not do justice to the complexity of the compositional data and did not address correlations among PM components. However, the findings that ROS formation and inflammatory markers were correlated with concentrations of specific metals in PM are consistent with the findings of other studies (Kodavanti et al. 2005; Duvall et al. 2008; Happo et al. 2008) and add further support for the hypothesis that the cardiovascular toxicity of PM may be mediated in part by metals.

The Review Panel noted that the study's approach to PM sample collection — using a high-volume cascade impactor — is state-of-the-art. While this is a powerful approach for the direct assessment of molecular and cellular mechanisms of PM toxicity, there are some caveats that should be kept in mind. The first is that the extraction of PM from filters leaves out those compounds that do not easily dissolve in water and ethanol, such as many organic compounds. Thus, the experiments could only capture and evaluate a portion of components of PM. In addition, the composition of PM may have changed during the process of collecting the PM on the filter substrates, extracting it, and reconstituting it for application to cell cultures and mice. PM chemical composition may have changed even further when it translocated through alveolar walls into the blood stream. However, this is a limitation of any study that requires extraction of PM components from filters and uses a highly unnatural route for delivery of PM concentrations to cells. The authors adequately defend and provide the rationale for such *in vitro* studies.

The *in vitro* experiments used a dose of 50 µg/mL based on preliminary studies of dose ranges; the Review Panel judged the dose to be reasonable for *in vitro* toxicologic comparisons. In the case of cardiomyocytes, the investigators used only the soluble fraction of PM, but it is unclear how representative this may be of what heart cells inside the body may encounter. A large body of current evidence supports the notion that autonomic nervous system signals and inflammatory mediators (such as ROS or proinflammatory cytokines) generated systemically or in the lung following exposure are responsible for downstream cardiovascular adverse effects; that is, the effects are via indirect pathways and are not direct effects of PM components on the heart (Brook et al. 2010). Similar caveats apply to

the exposure of endothelial cells, as the choice of direct dose to cells did not consider how the dose may have related to inhaled PM and the amount that may have translocated to the circulation. The amount, type, and size of PM that may reach the systemic circulation remain controversial topics. Therefore, conservative conclusions are appropriate from *in vitro* experiments in which cells, other than those from the lung, are directly exposed to ambient PM.

The Review Panel thought that the report could have more fully distinguished the relevance of *in vitro* versus *in vivo* studies in studying the biological plausibility of PM toxicity based on PM composition. A key feature of the study by Gordon and colleagues lies in what cells may encounter *in vitro* compared to *in vivo*. Observed differences in response could be based on the relative dose, the route of exposure, and PM composition. Although the authors focused on PM composition, they did consider the other variables.

An important finding of this study is the lack of correlation between the results of the *in vitro* and *in vivo* studies, suggesting caution in the use of *in vitro* studies to predict *in vivo* effects; it also suggests caution in extrapolating the results to long-term effects of PM exposure. Gordon and colleagues concluded that ROS production may not be a good indicator of *in vivo* PM toxicity, and stated as possible reasons that the pathways for ROS formation and lung inflammation are not strongly linked in mice, or that the responses are affected differently by different PM components. The investigators pointed to the possible role of endotoxin, although it was present only in very small amounts; treating mice with endotoxin by itself at higher doses than those reported here did not result in an inflammatory response (data not shown). The Review Panel agreed that the correlation between ROS production and lung inflammation was poor; however, they thought it may be possible that better correlation with ROS production might be observed in different cell types, such as lung macrophages. The investigators did not show correlations of lung inflammatory markers with ROS production in epithelial cells, although presumably the correlation would have been as poor as it was with ROS production in endothelial cells (IR Figure 14, Study 2).

The investigators acknowledged the limitations of *in vitro* studies and were appropriately conservative in interpreting their data throughout most of the Investigators' Report. However, in the final paragraph of the discussion, they noted that their data "suggest that a NAAQS for coarse PM should be considered and that it would also be important to acquire speciation data for coarse PM." Regarding some of the findings for ultrafine PM, they also suggested "that it would be useful to consider implementing a separate nationwide monitoring program for

ultrafine PM, at least for initial research purposes.” The Review Panel thought those suggestions were premature based on the limitations of the findings of the *in vitro* and *in vivo* studies described here.

Conclusions

In summary, this is a high-quality report with useful information on the toxicity of various PM components and size classes. It should be noted that the study did *not* rule out the possible toxicity of any particular components or size classes. The strength of the study lies in the care in which it was done, using reasonable doses for lung cell exposures and a focused group of well-defined outcomes to compare equal concentrations of particulate matter from different size fractions and airsheds. As discussed by the investigators, a limitation of the study is that it only focused on inorganic components of PM in its use of both water-soluble and insoluble size fractions for its *in vitro* experiments. The study did not evaluate OC, EC, or other organic components of PM. Although the companion NPACT study by Campen and colleagues (2013) has provided additional insights on specific source mixtures (including PM and gases from mixed-vehicle emissions as well as inorganic PM), further research is needed into the relative toxicity of combinations of PM of various composition with gaseous or semivolatile compounds. Also needed is an investigation of the role of atmospheric aging of complex mixtures, to mimic real-world conditions.

STUDY 3. KAZUHIKO ITO AND COLLEAGUES

GENERAL APPROACH

Study 3 examined associations of short-term exposure to ambient air pollution with effects on mortality for all ages and hospital admissions among people 65 years of age and older. The study used the well-established, multicity two-stage time-series study design employed by such large projects as HEI’s NMMAPS (Samet et al. 2000b, HEI 2003), APHENA (Katsouyanni and Samet et al. 2009), ESCALA (Romieu et al. 2012), and PAPA (Wong et al. 2010). The main difference between the current study and previously published studies is that Ito’s team estimated exposure to ambient concentrations of PM_{2.5} and individual components of PM_{2.5} as well as factor analysis–based pollutant indicators, instead of to PM₁₀, PM_{2.5}, or total suspended PM alone.

Ito and colleagues collected data from 150 cities in the U.S. (and in a subset of 64 cities where gaseous pollutant data were also available), including concentrations of PM_{2.5} mass, criteria air pollutant gases (NO₂, SO₂, and CO), and

PM_{2.5} components from the CSN, which began measuring PM_{2.5} components in the year 1999. The investigators also conducted source apportionment, using factor analysis to partition temporal variations of the daily PM_{2.5} mass and the mass of PM_{2.5} components and gases into separate factors assigned to specific source categories. Mortality data were available for the years 2001 through 2006, and hospitalization data for the years 2000 through 2008.

The investigators conducted city-specific analyses and combined the results using a second-stage random-effects model. This two-stage method allowed the investigators to control for potential confounding by city-level variables that affect health outcomes, both measured and unmeasured. They also conducted analyses that examined factors responsible for heterogeneity of effects between cities. Because of the less-than-daily collection schedule for PM_{2.5} components (sampling occurred every third or sixth day), the investigators could not conduct the technically preferred distributed lag analyses; instead they presented the results for only single-day pollutant lags (0–3 days), as has been done in most of the multicity studies conducted to date.

Specific Aims

In Study 3, the investigators’ main hypothesis was that one or more components of PM_{2.5} or source-related components, or both, are more strongly associated than PM_{2.5} mass concentrations with the health effects associated with PM_{2.5} exposure previously reported for CVD and respiratory morbidity and mortality. The specific aims for this study were to:

1. Characterize PM_{2.5} components to help interpret the results of time-series analyses of health effects data and these air pollution indices.
2. Conduct factor analyses of the components of PM_{2.5} and local gaseous pollutants to characterize local-pollution source types and to reduce the dimensionality of the large number of air pollution indices examined.
3. Conduct time-series analyses of mortality and elderly hospital admissions, to estimate short-term risk.
4. Model the city-to-city variation of risk estimates (using the analyses of aim 3) as a function of city-specific characteristics including pollution levels, land use, and other exposure-related information.
5. Evaluate the consistency of results, (based on the analyses and models from aims 3 and 4), about which PM_{2.5} components and source types are associated with the health outcomes.

METHODS

Data on Health Outcomes

This study used counts of daily events (deaths or hospitalizations) for each of the 150 metropolitan statistical areas (MSAs) for which exposure data were available. MSAs are geographic areas defined by the U.S. Census Bureau that include cities and their surrounding suburbs (for simplicity, we use “city” in the Commentary). Mortality data for the years 2001 through 2006 were obtained for 148 of the 150 cities through a U.S. EPA research agreement with the National Center for Health Statistics (NCHS). Honolulu and Boise were not included, because data for Hawaii and Idaho were not available through the NCHS program. Using the *International Classification of Diseases, 10th Revision* (ICD-10) codes (NCHS 2008), the investigators analyzed daily counts of all deaths classified as nonaccidental (A00–R99), and, to a lesser extent, deaths from CVD (I01–I79) and respiratory disease (J00–J99). Hospitalization data from the 150 cities for the years 2000 through 2008 were obtained through the Medicare system from the Center for Medicare and Medicaid Services. These data included admissions of persons aged 65 and older through hospital emergency departments for CVD (ICD-9 codes: 410–414, 427–428, 431–437) and respiratory conditions (ICD-9: 480–486, 490–496) (NCHS 2010).

Exposure Assessment

Data Sets Several different data sets were used to estimate the exposure concentrations and source categories used in the statistical models for assessing the effects of short-term exposures and to provide data on variables that could modify or confound the associations between measured concentrations and health outcomes. The primary exposure data set was obtained from the CSN, which provides data on concentrations of ambient PM_{2.5} and its components. Ito's team obtained CSN data from the U.S. EPA Air Quality System (AQS) archive.

The AQS data used for the statistical analyses included 24-hour average concentrations for PM_{2.5} mass for the 150 cities in the study, calculated per the Federal Reference Method. Particle component concentrations were measured every third or sixth day. Gaseous pollutant data were available from either the same monitoring sites as the CSN sites or from “nearest-neighbor” sites in 64 of the 150 cities. In these 64 cities, hourly data were available for NO₂, CO, and SO₂. For the statistical analyses, the investigators calculated the maximum 8-hour average concentrations for a given study day for CO, and 24-hour average concentrations for NO₂ and SO₂.

In the second stage of their analysis, in which they combined the estimates for all of the cities, Ito's team included additional variables to account for factors that could modify or confound the associations between the PM_{2.5} or PM_{2.5} component exposures and the observed health effects. For example, they incorporated satellite data for each city from the Landsat Thematic Mapper, with land-use categories defined according to the National Land Cover Data system (Homer et al. 2007; United States Geological Survey 2007). Their primary source of traffic data was the Highway Performance Monitoring System, and they acquired information on emissions from industrial sources from the U.S. EPA's National Emissions Inventory. Data on the location and size of port facilities, a known source of emissions from ships and transport activities, were obtained from the U.S. Army Corps of Engineers.

PM_{2.5} Components and Gaseous Pollutants Through the CSN, data were available for PM_{2.5} and 55 different components of PM_{2.5} that were measured in the 150 cities during the study period. Ito's team first evaluated the quality of the available data for each of these components for the percentage of readings below the limit of detection (LOD) and the fraction of zeros in the reported values, choosing 27 components for further analysis. They also looked for monitor-to-monitor temporal correlations in the 21 cities with multiple monitors (a measure of the spatial and temporal stability of city-wide averages). In addition, they wanted to check the data for potential correlations with temperature variables used in the time-series model — to control for the influence of weather on health effects — and with the day-of-the-week variable (because two or more highly correlated variables in the analysis could cause statistical instability of time-series model estimates). After assessing the data, the investigators included PM_{2.5}, CO, NO₂, NO₃⁻, SO₂, SO₄²⁻, EC, OC, As, Cu, Fe, K, Na, Ni, Pb, Se, Si, V, and Zn in the statistical models applied to assess associations between pollutants and health outcomes.

The investigators estimated the daily exposures of the residents of each city to PM_{2.5}, gaseous pollutants, and PM_{2.5} components by calculating the difference between the measured daily concentration of the pollutants for the city and day of interest and the calculated monthly mean concentration for that city. For the 21 cities that had more than one monitor, values were averaged to produce a city-wide mean value for a given day. This “deviation from monthly mean” approach was used to reduce the effect of seasonality in the factor analysis and statistical analysis while retaining the daily differences in concentration values necessary for the time-series analysis.

Source Apportionment The investigators performed factor analysis to reduce the number of exposure variables in the analysis, and to apportion measured levels of components to likely source categories (see the Source Apportionment sidebar). Because the analyses for the time-series study were based on counts of health events or deaths and pollutant concentrations for each of the cities, they focused on those primary PM_{2.5} components that are most related to source emissions and form local aerosols whose levels vary between cities, whereas they excluded secondary PM_{2.5} components, such as S, SO₄²⁻, NO₃⁻, and NH₄⁺, that are transported and vary on a regional or national scale. They included NO₂, SO₂, and CO in the analysis of the 64 cities for which data were available, excluding O₃ because it tends to vary on a regional level. Using the SAS PROC FACTOR procedure, Ito's team set parameters to the equivalent of a varimax rotation, to produce factors that were not correlated with one another. They then named and identified the factors as major source categories according to their correspondence with source categories and factors identified in past source-apportionment studies (Coutant et al. 2003; Desert Research Institute 2005) and during a workshop at which multiple research groups conducted and discussed parallel analyses of data from two cities (Thurston et al. 2005).

The investigators first conducted a nationwide factor analysis, followed by a city-specific analysis. For the nationwide analysis, the investigators analyzed combined data for the 64 cities for which gaseous pollutant data were available. Here, as in the health outcomes analysis, they used the daily deviations from monthly city means as input variables to reduce the influence of seasonal variation on the factor analysis. They performed five sets of analyses, with the number of factors varying from four to eight. They then selected the optimum number of factors as the number that minimized the "mixing and splitting" of component concentrations across factors and that maximized their ability to confidently identify component clusters that were representative of known source emissions. The investigators identified six source categories corresponding to source emission profiles for PM_{2.5} components and gaseous pollutants: Traffic, Soil, Coal Combustion, Residual Oil Combustion, Salt, and Metals. The resulting factor loadings from this analysis are presented in IR Figure 3, Study 3.

For the factor analysis of data for each individual city, the investigators used the six source categories identified in the nationwide factor analysis to define source categories for each city based on the factor loadings for the pollutants. This process yielded values for two to six named categories per city that could be matched to the nationally

derived source categories; however, the use of the nationally identified factors meant that this analysis could not identify potentially locally important sources that were unique to a particular city.

To analyze the associations between source categories and health effects, the investigators used the factor scores (i.e., estimated day-to-day fluctuations of pollution levels associated with each source category) for each day when PM_{2.5} component concentrations were available — one day in three for some cities, one day in six for others. For the nationwide analysis, data on health outcomes and factor scores were pooled across the 64 cities for which NO₂, SO₂, and CO data were available on the same days as PM_{2.5} component data. For the city-specific analysis, the investigators used the daily factor scores and health outcome data to calculate excess risks for each city.

Statistical Analyses

The statistical analysis of associations between health and pollution data was conducted first at the individual city level and then at the national level (i.e., across all 148, 150, or 64 cities). The investigators calculated associations between pollutant concentrations and outcomes for full-year, warm-season (April–September) and cold-season (October–March) data sets.

City-Specific Analyses In the first stage, the investigators fit Poisson regression models to the air pollution and mortality time-series data in each city to develop city-specific estimates of the effect of PM_{2.5}, PM_{2.5} components, pollutant gases, and source categories on mortality. They used the Poisson generalized linear model (McCullagh and Nelder 1989) to evaluate the association between daily pollution concentrations and daily time-series data for mortality and hospitalizations, estimating health effects while controlling for other factors that might also explain the temporal patterns of mortality in individual cities. In the first-stage analyses, the investigators modeled the daily counts of deaths or hospitalizations as a function of these variables: pollutant concentration (or factor score); a categorical variable for day of the week; and natural cubic splines of study days, same-day temperature, and average temperature for the previous 3 days. The investigators did not include humidity variables because they were highly correlated with temperature. They used the same models for each city, consistent with the methodology used in the NMMAPS (Samet et al. 2000a; HEI 2003), APHENA (Katsouyanni and Samet et al. 2009), and PAPA (Wong et al. 2010) multicity studies. Estimated effects were presented as the percentage change in risk of mortality per interquartile range (IQR) increment in concentrations of PM_{2.5}, PM_{2.5} components, or pollutant

gases, or per factor scores, referred to in the report as “percentage excess risk.”

The investigators explored the relationships between hospitalizations or deaths and exposures estimated on the same day (lag 0) and up to 3 days before (lag 3). Because PM_{2.5} components were measured only every third or sixth day in most locations, analyses for each lag were limited to the set of observations for cities for which pollutant concentrations were available on the appropriate day (the day of, or 1–3 days prior to the occurrence of the death or hospitalization). Data sets limited to every-third-day or every-sixth-day measurements restricted the size of the data set available for the analyses and also prevented the investigators from constructing distributed lag models.

National-Level Aggregation and Second-Stage

Analyses In the second stage, the investigators combined the individual effect estimates to provide mean estimates of the effects of air pollution on mortality across cities (DerSimonian and Laird 1986). They used a random-effects model to combine the individual city results for excess risk of death by all nonaccidental causes, and for cardiovascular and respiratory hospitalizations associated with an IQR increment in the concentration of a pollutant. The results were thus aggregated — for all-year, warm-season, and cold-season analyses — into summary estimates of nationwide effects for the 148 and 150 cities and for the 64 cities with gaseous pollutant data.

Ito's team applied a second random-effects model that aggregated the city-specific results and included a suite of city-level traffic and land-use variables. These variables were included in order to investigate which variables could modify the relationship between air pollution and health in ways that could have led to observed differences in the results calculated for the different cities. Additional city-level variables used in the second-stage analysis included the city-specific mean pollutant concentrations (PM_{2.5} components were log-transformed because the distributions of average concentrations of the components across cities were highly skewed) and weekday “excess” PM_{2.5} (weekday averages minus weekend averages).

Sensitivity Analyses In the first-stage models described above, the investigators used the deviations from monthly means for pollutant-concentration variables and used the deviation pollution variables in the time-series models to be consistent with the factor scores that were derived using the deviation variables. For comparison purposes, Ito's team conducted sensitivity analyses in which they recalculated their models using the “raw” (as-measured)

values of temperature and pollutant concentrations in the 64-city data set.

The investigators calculated two sets of daily factor scores for all locations: a national-level set and a city-level set based on the nationally identified source categories (described above). They calculated nationwide effect estimates based on the city-specific factor scores and the nationwide analysis using combined pollution data from all 64 cities.

Because one goal of this project was to determine whether concentrations of any of the measured PM_{2.5} components demonstrated stronger associations with health effects attributed to PM_{2.5} than PM_{2.5} mass itself did, the HEI NPACT Review Panel suggested that the investigators construct two-pollutant models. In these models, concentrations of a few selected PM_{2.5} components that exhibited strong associations with health effects in the single-pollutant models were analyzed in the same model with PM_{2.5} mass concentrations. The Review Panel believed that this analysis would allow the investigators to assess the effects of these selected components relative to the effects of PM_{2.5} mass concentrations because the risk estimates of the components were adjusted for the effects of the PM_{2.5} mass concentrations as a whole.

KEY RESULTS

Study 3 investigated whether the short-term associations between concentrations of specific components of PM_{2.5} (and source categories derived from them) and selected health effects were stronger than the corresponding associations between PM_{2.5} concentrations and health effects.

Commentary Table 4 lists the PM_{2.5} components and source categories for which Ito's team found positive and statistically significant associations with the health effects studied. Ito's team reported specific results for associations between each component or source category and total mortality but not cardiovascular or respiratory mortality (presumably due to the smaller numbers of deaths for these subcategories). However, they did report results for cardiovascular and respiratory hospitalizations. Although the findings were somewhat consistent across the different models for each season and health outcome, the associations of individual PM_{2.5} components with mortality and hospitalizations were not consistent. However, PM_{2.5} itself frequently exhibited positive associations with the analyzed health outcomes and the most frequently appearing source category was Traffic. Of the PM_{2.5} components, OC presented the most consistent patterns of positive associations with health outcomes, although the seasonal pattern differed from that of PM_{2.5}. The PM_{2.5} and OC concentration data were more frequently detected

(concentrations above the detection limit were recorded on all study days) and had a wider range of concentrations than most other PM_{2.5} component data (the median IQR was 7.89 µg/m³ for PM_{2.5} and 2.04 µg/m³ for OC), which resulted in good statistical power. In addition, a Traffic source category was identified in nearly all cities. Therefore, the more consistent significant relationships of PM_{2.5}, OC, and the Traffic source category with daily mortality and hospitalizations may have been a result of their having higher-quality data sets than the individual PM_{2.5} components and the other source categories did.

For the national analysis, the investigators plotted the percentage change in excess risk for each pollutant that resulted from the addition of city-level confounding variables to the model, as shown in IR Figure 14, Study 3. The effect estimates that were most dramatically increased by the inclusion in the analysis of such variables (at 0-day lag) were for the following pollutants: SO₄²⁻, weekday excess PM_{2.5}, Pb, and V for all-cause mortality; Cu, Ni, V, SO₂, NO₂, and Fe for CVD hospitalizations; and Cu, NO₂, and V for respiratory hospitalizations. In some cases, the addition of these confounding variables substantially reduced the effect

Commentary Table 4. Significant Positive Short-Term Associations of PM_{2.5}, PM_{2.5} Components, Gases, or Source Categories with Selected Health Effects in Ito Study 3^a

Outcome	Components (148 / 150 Cities)			Components (64 Cities)			Source Categories (64 Cities)		
	All Year	Warm Season	Cold Season	All Year	Warm Season	Cold Season	All Year	Warm Season	Cold Season
All-Cause Mortality									
	Cu (1,3)	—	Cu (3)	SO ₂ (2,3)	PM _{2.5} (0)	SO ₂ (3)	Traffic (1)	Soil (1)	—
	K (1,3)		K (1,2)	CO (1)	NO ₂ (3)	Cu (3)	Soil (1)	Metals (3)	
	Si (2)			K (1)	SO ₂ (2,3)	K (2)	Coal Com-		
	V (3)			OC (1)	EC (3)	OC (1)	bustion (3)		
					Fe (3)	Si (2)			
					OC (1,2)				
					Pb (3)				
					Si (1)				
CVD Hospitalizations									
	PM _{2.5} (0)	NO ₃ (0)	PM _{2.5} (0)	PM _{2.5} (0)	Na (2)	PM _{2.5} (0)	Traffic (0)	Salt (3)	Traffic (0)
	EC (0)		EC (0,3)	NO ₂ (0)	NO ₃ ⁻ (0)	NO ₂ (0)	Coal Com-		
	Fe (0)		Fe (0)	SO ₂ (0)		SO ₂ (0)	bustion (2)		
	NO ₃ ⁻ (0)		OC (0,3)	EC (0)		CO (0,1,3)	Salt (3)		
	OC (0)		SO ₄ ²⁻ (0)	Fe (0)		Cu (0)			
	V (0,1)		Si (0)	OC (0,3)		EC (0,3)			
			V (0,1)	Si (0)		Fe (0)			
				V (0,1,3)		OC (0,3)			
						SO ₄ ²⁻ (0)			
						Se (0)			
						Si (0)			
						V (1,3)			
						Zn (0)			
Respiratory Hospitalizations									
	PM _{2.5} (0)	K (1)	PM _{2.5} (0)	PM _{2.5} (0)	PM _{2.5} (0)	PM _{2.5} (0)	Traffic (0)	Coal Com-	Traffic (0)
	EC (0)	OC (1,2)	Cu (0)	CO (0)	CO (0)	CO (0)		bustion (1)	
	K (2,3)	V (1,2)	EC (0)	Cu (0)	K (1)	Cu (0)			
	OC (0,3)		K (0,3)	EC (0)	OC (1,2)	EC (0)			
			OC (0,3)	K (0,3)	SO ₄ ²⁻ (0,1,2)	K (0,3)			
				OC (0,3)		Si (0)			
				SO ₄ ²⁻ (0,1)					

^a Numbers in parentheses represent the lag days for which significant associations were observed.

estimates by as much as 50% to 100%, although the change for CO with all-cause mortality and the negative changes for Si with CVD and respiratory hospitalizations were not significant (i.e., the confidence interval included zero).

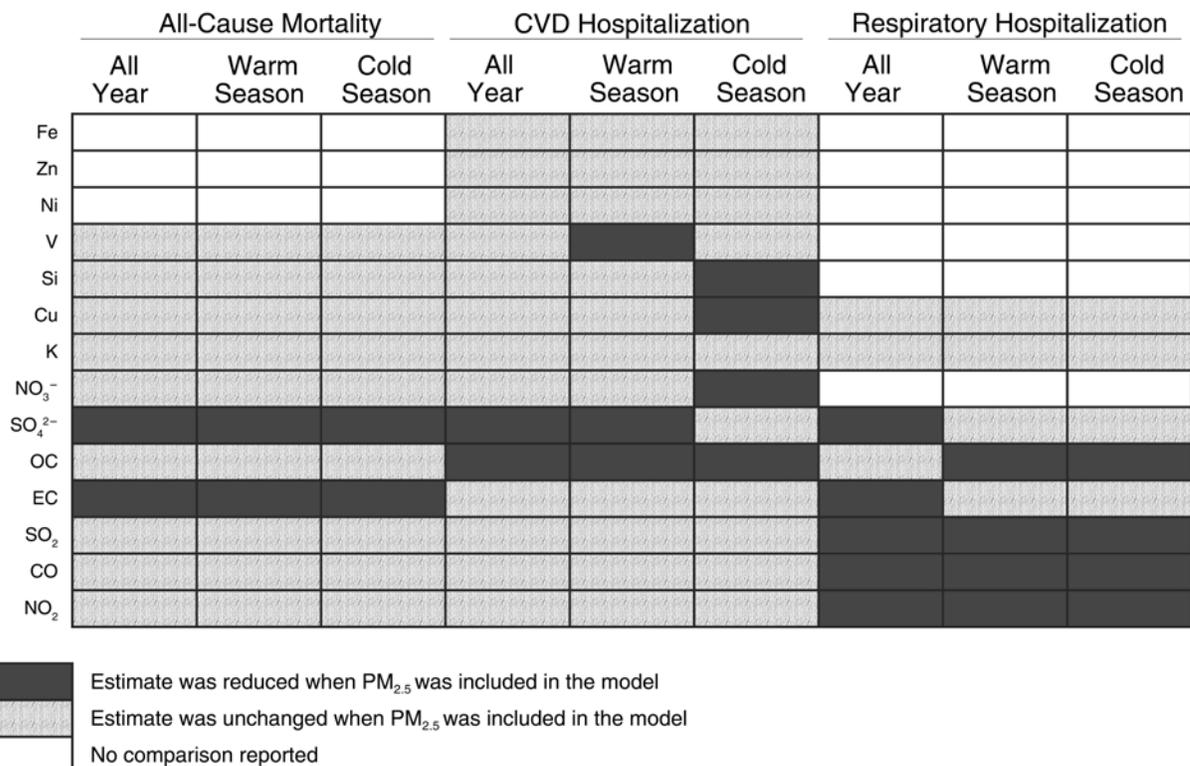
Sensitivity Analyses

The investigators reported that the results from analyses including the measured variables for pollution concentrations were similar to the results for models that used the values for the deviations from monthly means, particularly for the analyses of daily hospitalizations. They speculate that their smoothing of the study day variable used in the first-stage models resulted in adequate control of the known effect of season on mortality and morbidity without the additional transformation of the pollution variables.

A national-level analysis of pooled data from 64 cities using the national-level factor scores yielded results that were similar to those for the aggregation of individual city results using the city-level factor scores. The investigators noted that the results show similar patterns across the lags

and seasons, particularly for the Soil and Traffic source categories (see IR Figure 8 and Appendix Figure B.6, Study 3).

Two-pollutant models that included individual PM_{2.5} components and PM_{2.5} were used to analyze those components that were associated with health outcomes in the single-pollutant models. In IR Figure 13, Study 3, the investigators plotted the estimated percentage of excess risk for each component in the models, with and without the inclusion of PM_{2.5}. As shown in Commentary Figure 5, including PM_{2.5} in the models appears to have decreased the all-cause mortality effect estimates for EC and SO₄²⁻ concentrations for the all-year, cold-season, and warm-season results; the most severe decrease was in the cold-season results. In the two-pollutant models for CVD hospitalizations, including PM_{2.5} in the models reduced the effect estimates for OC and SO₄²⁻ for the all-year results; SO₄²⁻, V, and OC for the warm-season results; and OC, Si, Cu, and NO₃⁻ for the cold-season results. For respiratory hospitalizations, including PM_{2.5} in the two-pollutant models reduced effect estimates



Commentary Figure 5. Short-term changes in effect estimates resulting from adding a PM_{2.5} mass variable to the time-series analytic model (Ito Study 3). The investigators reported decreases or no changes, but no increases in effect estimates. (Based on data in IR Figure 13.) Dark grey: estimate was reduced when PM_{2.5} was included in the model; light grey: estimate was unchanged when PM_{2.5} was included in the model; white: no comparison reported.

for CO, NO₂, and SO₂ for the all-year, cold-season, and warm-season results and also reduced effect estimates for EC and SO₄²⁻ for the all-year results, and OC for both the warm- and cold-season results. As the investigators noted, the reduction of effect estimates for SO₄²⁻, OC, and EC in the two-pollutant models that included PM_{2.5} mass concentrations is not surprising, because each of these components explains a major fraction of PM_{2.5} mass.

EVALUATION OF ITO STUDY 3

The Model

In its independent review of the study, the Review Panel noted that the time-series regression model for investigating city-specific associations of daily concentrations of single-pollutant components, gases, and factors with health outcomes is a fairly standard and a well-respected approach. Residual confounding (for example by more complex dependence of outcomes on weather than allowed for in the model) is always possible, but no more so for this study than for other major, multicity time-series studies of pollution that have been published.

However, the Review Panel was not persuaded that considering deviations of pollutant concentrations from monthly means provided any advantage over other commonly used methods of controlling for the effects of seasonal cycles. Ito primarily used deviation variables to control the effects of seasonality in the factor analysis, and then applied a similar approach to the component concentrations to make the results comparable with those from the factor analysis. The Panel thought this seemed an unnecessary complexity given that confounding by unidentified phenomena changing slowly over time was controlled for in the epidemiologic model, and that this transformation may have introduced undesirable noise. On the other hand, the close similarity of the results to those obtained with the more conventional approach of using raw concentrations was reassuring and suggested that the interpretation of the results was not likely affected by the more complex method used by Ito.

Approach to the Large Numbers of Associations Studied

The Review Panel was impressed with the comprehensiveness of the analyses reported and the number of associations considered ([4 lags] × [2 seasons + all year] × [15 particle components + 2 gases + 6 source categories] × [3 outcomes] — or more than 800 associations). However, the decision to consider this many associations on an equal footing made interpretation particularly challenging. Although the Panel appreciates that any approach to handling such a large number of analyses and results has advantages

and disadvantages, it was convinced that the rather informal approach adopted allowed only fairly tentative conclusions to be drawn.

Given the paucity of prior information, the study was designed to yield a plethora of results for different components, but the Panel thought that alternative approaches, such as highlighting results that were more informative than others a priori (for methodologic reasons), would have allowed for firmer conclusions about the highlighted results. Although the use of factor analysis to identify groups of covarying components helped reduce dimensionality, difficulties in establishing comparable factors across widely ranging cities and the complications of national and city-specific factor analyses suggest that caution in the interpretation of results is warranted.

Regarding the variety of lags analyzed, the Review Panel appreciated that it was not possible with the data available to evaluate summed effects from distributed lag models, which would otherwise have been an attractive way to reduce the multiplicity of the associations considered, given that component concentrations were not measured every day. However, the Panel thought that there was considerable prior information that could have been used to guide which lags to consider for associations of PM_{2.5} (aggregated and components) with the health outcomes. As these associations were what the study sought to elucidate, it would seem natural to focus primarily on those lags that were found to be most strongly associated with PM_{2.5} in previous studies. For all-cause mortality, for example, this points to 0- and 1-day lags rather than 2- or 3-day lags.

Classifying results as either significant or not also limited the ability to find patterns across associations by including results that were not significant but showed a trend in the same direction. The Review Panel appreciated the need to simplify but was disappointed that other approaches were not considered. Because of the large numbers of associations estimated, 5% of the results would be expected to be significant by chance alone; that is, false positive or false negative associations. If we add to this the percentage of false positive and negative associations that may be expected as a result of biases, it is clear that chance or bias may be a possible explanation for any significant association reported here. Compounding these issues is the fact that the investigators made little note of statistically significant associations that were negative while focusing on the statistically significant positive associations. The investigators' explanation that weekly patterns may produce the inverse associations observed for pollution at 1-day lag is not convincing and could indicate a problem with the statistical model or with the sparse data, available

only on one in three or six days. It could also illustrate that with so many tests, some associations (positive and negative) will be false. A clearer distinction between primary a priori hypotheses and a larger number of exploratory hypotheses would have helped reduce this problem.

The variation in the number of cities for which any one source category showed a significant association with health outcomes suggests particular caution in basing interpretations of results on tallying the numbers of significant associations. This is because analyses of source categories that were identified in more cities would have greater power to find effects than analyses of source categories that were identified in fewer cities; the chances of finding significant effects would be increased, even if the source categories identified more frequently were less toxic. This could be one reason the investigators found more significant associations with Traffic than with other source categories.

Single- and Multiple-Pollutant Analyses

Although the Review Panel again appreciated the need for reducing complexity, it was disappointed that the main focus of the analyses was on one-pollutant models of component concentrations (or single source categories). Although those models and a variety of possible multiple-pollutant models each have advantages and disadvantages (see the sidebar *What Different Statistical Models Can Tell Us About Associations Between Specific Components and Health Outcomes*), the Panel was unconvinced that the single-pollutant models were the best approach to addressing one of the overall objectives of the Lippmann project, that is, to identify whether health effects are driven by specific components rather than by overall PM_{2.5} mass concentration. While models with two or more pollutants have inherent and well-known limitations, they can provide insights about pollutant mixtures in ways not addressed by factor analysis.

The presentation of selected two-pollutant analyses showing excess risk for specific components with or without inclusion of total PM_{2.5} in the model was helpful (IR Figure 13, Study 3). These results clarify the extent to which associations identified in the single-pollutant models might be merely reflecting a generic PM_{2.5} effect or a correlation of PM_{2.5} with the component in question (i.e., because of confounding by other components). The Review Panel agreed that comparing the results from these models, particularly for the less abundant components, was broadly reassuring that these associations were indeed not a result of such confounding.

However, the Review Panel found the investigators' interpretation of the substantial reduction of risk estimates for some of the more abundant components — for example

SO₄²⁻ — when total PM_{2.5} was included in the model misleading. The investigators maintain that a bias in the two-pollutant analyses caused the coefficient to be decreased for such abundant components when PM_{2.5} mass concentrations were included in the model, but the bias may have depended on what the risk estimate was thought to represent (see the sidebar *What Different Statistical Models Can Tell Us About Associations Between Specific Components and Health Outcomes*). If the risk estimate was interpreted to be an estimate of *additional* risk associated with SO₄²⁻ compared to other components of PM_{2.5} — arguably the point in question here — the adjusted coefficient for SO₄²⁻ was not biased. The investigators may have sought to estimate the *total* effect of SO₄²⁻, which, if the effect were adjusted for PM_{2.5}, would have been biased downward. However, the coefficient from the single-pollutant analysis of SO₄²⁻ (the only other estimate presented) would have also been biased if SO₄²⁻ were correlated with other toxic particle components. Other multiple-pollutant models (such as model 3 in the sidebar referred to above) could provide relatively unconfounded risk estimates. Furthermore, the investigators seemed to have ignored the estimates for other components (e.g., EC, OC) that also appeared to be sensitive to the inclusion of PM_{2.5} mass.

The absence of a table of confidence intervals (CIs) or *P* values for the risk estimates that were adjusted for PM_{2.5} limited the Panel's ability to fully evaluate these results. For example, if the 95% CI for the excess risk for SO₄²⁻ adjusted for total PM_{2.5} includes the null — as seems likely — it would imply that the evidence is weak that SO₄²⁻ is more toxic than other PM_{2.5} components.

Interpretation of the Results

Although multiple studies conducting similar time-series analyses of PM_{2.5} mass have been published, those analyses necessarily assumed that PM_{2.5} components all have the same toxicity. Some evidence supports the hypothesis that this is not the case (e.g., evidence of the heterogeneity of effects across regions with varying relative and absolute concentrations of PM components, as well as toxicologic evidence); however, the evidence has been limited because of the lack of data on the concentrations of individual PM_{2.5} components. Because such data have now become available, a comprehensive analysis that included multiple PM components and source categories for a large number of U.S. cities was clearly justified.

The investigators posited multiple hypotheses for this study:

1. PM_{2.5} is capable of producing acute health effects of public health concern, but the effects may differ according to its composition.

2. The source-apportionment techniques that we have developed and refined in recent years are useful for identifying the categories of sources of PM_{2.5} air pollution and specific components that have the greatest impacts on a variety of acute health effects.
3. The acute health effects due to ambient PM_{2.5} exposures can best be seen in elderly populations (who are more likely to be sensitive to the effects of the pollution) within human populations.

Despite the clear strengths of the study, the Review Panel questioned whether it has been able to fully test these hypotheses. The primary conclusions from the investigators were the following: “This study used source-apportionment methods to reduce the dimensionality of multiple pollutant atmospheres and suggests that a major fraction of variation in multiple pollutants could be attributable to traffic-related sources and that the temporal variations in traffic are also associated with temporal

What Different Statistical Models Can Tell Us About Associations Between Specific Components and Health Outcomes

Different combinations of concentrations of total PM_{2.5} mass and mass of specific components can be included in regression models. Here are three possibilities for doing so, discussed in terms of the regression coefficient, β , and the independent variable, x . The first model is the primary approach used by the investigators in this study, and the second was used in some additional analyses. A more extensive discussion of models that might be used in a similar context is given in Mostofsky and colleagues (2012).

Model Terms

- x_s is the (daily) mass concentration of the component, s , under consideration
- x_{total} is the total concentration of PM_{2.5} mass
- $x_{\text{total}-s}$ is the total concentration of PM_{2.5} mass minus the mass concentration of the component under consideration

Model 1.

$$\text{Pollution term} = \beta_{s,1} x_s.$$

Here $\exp(\beta_{s,1})$ is interpretable as the relative risk increment per unit increase in the component concentration, if that component concentration is considered as an indicator of the pollution mixture present in the city or cities under investigation. It is an unconfounded estimate of the relative risk increment per unit increase of component s (specifically) only if daily concentrations of s (x_s) are not correlated with other pollutants (including other PM_{2.5} components) associated with the health outcome.

Model 2.

$$\text{Pollution term} = \beta_{s,2} x_s + \beta_{\text{total},2} x_{\text{total}}.$$

Here $\exp(\beta_{s,2})$ is interpretable as the *additional* increment in relative risk per unit increase in component s over and above

any increment due to a change in PM_{2.5} mass. Algebraically, this can be seen from the following expression:

$$\begin{aligned} & \beta_{s,2} x_s + \beta_{\text{total},2} x_{\text{total}} \\ &= \beta_{s,2} x_s + \beta_{\text{total},2} (x_s + x_{\text{total}-s}) \\ &= (\beta_{s,2} + \beta_{\text{total},2}) x_s + \beta_{\text{total},2} x_{\text{total}-s}. \end{aligned}$$

The estimate of this additional increment is unconfounded if x_s is uncorrelated with any gaseous pollutant modifying the outcome and with any variation in toxicity of the mixture of PM_{2.5} components not including component s . The model is equivalent to a model with a main effect of PM_{2.5} (x_{total}) and an interaction with the proportion of PM comprising component s (x_s/x_{total}), because

$$\begin{aligned} & \beta_{s,2} x_s + \beta_{\text{total},2} x_{\text{total}} \\ &= \beta_{s,2} (x_s/x_{\text{total}}) \times x_{\text{total}} + \beta_{\text{total},2} x_{\text{total}} \\ &= [\beta_{s,2} (x_s/x_{\text{total}}) + \beta_{\text{total},2}] x_{\text{total}}. \end{aligned}$$

The coefficient $\beta_{s,2}$ can thus also be interpreted as the degree to which the proportion of PM_{2.5} composed of component s modifies the incremental increase in log risk related to total PM_{2.5}.

Model 3.

$$\text{Pollution term} = \beta_{s,3} x_s + \beta_{\text{total}-s,3} x_{\text{total}-s}.$$

Here $\exp(\beta_{s,3})$ is interpretable as the increment in relative risk per unit increase in s specifically (not as an indicator of total PM_{2.5} toxicity). As with the (different) term $\exp(\beta_{s,2})$ in model 2, the estimate of this increment is unconfounded if x_s is uncorrelated with any gaseous pollutant modifying the outcome and with any variation in toxicity of the mixture of PM_{2.5} components not including component s .

variations in daily counts of all-cause mortality and CVD and respiratory hospitalizations. . . . [We] also found that the secondary aerosols were associated with these outcomes.”

The results of this study contribute to the existing evidence for the acute health effects of urban air pollution (see Levy et al. 2012; HEI 2003; Zanobetti and Schwartz 2005); although not the primary goal of the study, this may be an important result. For example, the findings support the growing body of literature that a range of traffic-related pollutants (e.g., PM_{2.5}, NO₂, CO, EC, and OC) are associated with CVD hospital admissions at very short time lags (i.e., same day). Overall, the results support associations of mortality and morbidity with both traffic-related pollutants and secondary aerosols (e.g., OC and SO₄²⁻).

However, several issues make further interpretation of these results challenging. For example, the investigators did not analyze certain PM_{2.5} components because of the proportion of data that were below the LOD or because of low monitor-to-monitor correlations within a particular city, but they did not specify the criteria for inclusion or exclusion. The Panel noted that several components with a high proportion of data that were below the LOD or that had low monitor-to-monitor correlations (e.g., Ni, As, Cu, and V) were included in the analysis nevertheless. Several of these PM_{2.5} components were among those associated with health endpoints in the various analyses.

The complicated patterns of correlations between pollutants (which were beyond the control of the investigators) were a challenge to summarize, and it was difficult to interpret the potential effects of such correlations on associations with health effects. The factor analyses and the correlations of factor scores with individual pollutants (i.e., factor loadings; see IR Appendix Table B.8, Study 3) were helpful, although simple pair-wise correlations would have added to the ability to appropriately interpret the results in the broader context of the multiple-pollutant atmosphere.

Conclusions

The investigators conducted a comprehensive and detailed analysis of a large amount of data, although their study was limited by the type and availability of data. Given these limitations and others discussed above, the Review Panel did not agree that this study provided evidence that certain PM_{2.5} components and source categories are more strongly associated with health outcomes than other components or source categories, or than PM_{2.5} mass.

Although the Review Panel did not think that this study provided clear evidence for differing effects on health outcomes of different particle components or pollution source categories, there are reasons to be cautious in coming to a

reverse negative conclusion — that no such differences exist. The errors in measuring population exposure to particle components, in particular the potential for misalignment between air quality data and health outcome data in the presence of sometimes substantial spatial heterogeneity (see, for example, Bell et al. 2011), reduces the chances of finding differences in toxicity among pollutants. The specific impacts of such errors would depend on their structures (e.g., whether of Berkson or classical type), but these errors would probably attenuate the effects of those components for which the estimated exposures showed the largest classical measurement error.

The Panel also noted that different biological mechanisms may operate at the short-term time scale of this study, as compared to the long-term time scales analyzed in Study 4 and the companion NPACT study by Vedal and colleagues (2013). This would add caution to extrapolating any differences in effects, even if they were found, to the larger public health impacts that have been estimated for exposure to PM_{2.5} based on the long-term studies.

As the field of research on the effects of PM_{2.5} components matures, the array of apparently disparate associations found in this study may eventually allow researchers to draw more detailed conclusions. The results of this study will certainly help generate hypotheses for future research. The research community should also consider whether the right questions are being asked, given the limitations of currently available data and methods of analysis.

STUDY 4. GEORGE D. THURSTON AND COLLEAGUES

GENERAL APPROACH

For the last 20 years, using the Cox proportional hazards model to analyze data from large cohort studies has allowed scientists to investigate and establish long-term associations between exposure to PM_{2.5} and mortality. In particular, the American Cancer Society's CPS-II cohort has been the subject of several studies since the original study of air pollution and mortality (Pope et al. 1995), including further analyses by Pope and colleagues (2002, 2004) and HEI-sponsored reanalyses and extensions (Krewski et al. 2000a,b, 2009). With each investigation, the study design was updated and refined by including potential personal-level (e.g., smoking and dietary habits) and community-level (e.g., neighborhood education levels) confounders, and through analytic refinements offered by extensions of the Cox model that incorporated a number of random effects variables.

In the current analysis, Dr. Thurston and colleagues used the CPS-II cohort data, from the study's inception in 1982 through 2004, to investigate associations of long-term exposure to PM_{2.5}, its components, and gaseous pollutants with long-term health effects — specifically, all-cause, CVD, pulmonary, and lung-cancer mortality. The current analysis included only the 445,860 members of the CPS-II cohort who were residing in the 100 metropolitan areas for which CSN monitoring data for PM_{2.5} components were available. The investigators' primary analyses averaged all available measurements of 24-hour concentrations of PM_{2.5} components obtained from the CSN for six years (2000–2005). They constructed factors and source categories from the PM_{2.5} component data for the initial analyses, and they applied a new approach — calculating the total risk index (TRI) — in order to determine the relative effects of mixtures of components in a multiple-pollutant environment.

Specific Aims

The overall goal of Study 4 was to investigate the associations between long-term exposures to source-related components of PM_{2.5} and mortality in the United States. The specific aims of the study were to (1) apply recently developed source-apportionment methods to the newly available PM_{2.5} component data from the CSN, and (2) combine those data with the extended CPS-II cohort data to test the following overall NPACT hypotheses, restated to reflect the focus on mortality as a response to air pollution exposure:

1. Associations between mortality and long-term PM_{2.5} exposure are stronger with certain components of PM_{2.5} than with others.
2. Associations between mortality and long-term PM_{2.5} exposure are stronger with some pollution source categories than with others.

The main approach was to apply source-apportionment techniques that Thurston's team had developed and refined through earlier research (Thurston and Spengler 1985; Thurston et al. 2005) in order to identify the major PM_{2.5} air pollution source categories associated with all-cause, ischemic heart disease (IHD), respiratory, and lung cancer mortality in the CPS-II cohort.

The primary goal of the supplemental TRI analyses was to test whether models that estimate the combined risk of mortality associated with a group of two or more pollutants analyzed as a single matrix variable would provide significantly different overall risk estimates for mortality than do models that use a single pollutant or multiple pollutants as predictive variables. The TRI approach was developed to overcome the limitations of other models in

which mortality risks associated with multiple-pollutant variables are considered simultaneously but estimated as if they were independent, despite their interdependence. For example, the Cox regression models that use two or more pollutant variables may not produce reliable individual pollutant effect estimates when the pollutant concentrations are highly correlated with each other. The investigators conducted a focused exploratory analysis to evaluate which combinations of pollutants (e.g., all the components) in a TRI analysis added significantly to estimates of total relative risk.

METHODS

Study Population and Mortality Data

In late 1982, volunteers from the ACS recruited participants in the 50 United States, the District of Columbia, and Puerto Rico for a large prospective cancer prevention study of 1.2 million adults. For the current analysis with PM_{2.5} components, the ACS — through the co-investigators at the University of Ottawa — provided the research team access to the cohort data. In this study, the cohort was restricted to CPS-II participants who resided in cities (and surrounding suburbs) in the contiguous 48 states and the District of Columbia for which speciated PM_{2.5} component concentrations were available from the CSN monitoring network. A total of 445,860 of the participants lived in these 100 cities; in their study, Thurston and colleagues used the data on participant deaths during the period of September 1982 through December 2004.

At enrollment in 1982, participants were at least 30 years of age and were members of households with at least one person 45 years of age or older. Participants filled out an extensive questionnaire that included personal demographic characteristics, personal habits, occupational history and exposures, tobacco and alcohol use, and other factors possibly related to mortality from cancer. These questionnaires provided data for the 42 individual-level covariates (see details in IR Table 3, Study 4) used in the current and earlier analyses of the cohort.

The ACS volunteers contacted participants in 1984, 1986, and 1988, and gathered data through the National Death Index thereafter. They obtained death certificates for participants who were known to have died and compiled cause-of-death information. Causes of death analyzed in the CPS-II fell into these categories: all causes, IHD, respiratory disease, and lung cancer.

In addition to the individual-level covariate information gathered with the CPS-II enrollment questionnaires, Thurston's research team also applied data for six contextual ecologic covariates that were intended to represent

local neighborhood and city-level conditions or factors known or suspected of influencing mortality. They used ecologic covariates for the Zip Code area and city of residence for each participant based on demographic data from the 1980 U.S. Census. The neighborhood factors included percentage of residents who completed high school; percentage of residents who were black, percentage who were Hispanic; percentage who were unemployed; median household income; and relative income disparity (how income was distributed within neighborhoods and cities) (see details in IR Table 4, Study 4). These ecologic covariates were used in the analytic models at the Zip Code area level for the residence of each participant, and as the difference between the values for the Zip Code area and the city-wide average.

Air Pollutants

Air pollutant exposures for the CPS-II mortality analyses were estimated from data from several air pollution monitoring systems in the continental United States. The investigators compiled data on PM_{2.5} mass and component concentrations that were obtained via the HEI-funded Web site maintained by Atmospheric and Environmental Research (AER), which provides access to data from 273 U.S. EPA state, local, and tribal air monitoring stations and the CSN in the contiguous 48 states. Of these sites, 114 monitors in the 100 cities met the quality control criteria of the study. In 55 of these cities there was a within-city monitor that collected NO₂ data from 2000 through 2006; data from those monitors were obtained via the AER Web site and included in the analyses as additional markers for emissions from traffic sources. The investigators compiled data for PM_{2.5} mass, 19 of its different components (As, Ca, Cl, Cu, EC, Fe, K, Mg, Mn, Na, Ni, NO₃⁻, OC, Pb, Se, Si, SO₄²⁻, V, and Zn), and NO₂ (IR Table 2, Study 4). Of these, they used PM_{2.5} mass and 16 components for the mortality analyses. Although most of the component measurements were collected only every third or sixth day, enough readings were taken during the 6-year measurement period to construct a variable representing long-term average concentrations for each component.

Thurston's team constructed long-term averages of pollutant concentrations for each city by averaging all of its available daily measurements for PM_{2.5} and PM_{2.5} components, as well as NO₂, for the entire period for which data were available. They included measurements below the LOD in these averages, as well as levels recorded as zeros or nondetectable. They stated that any such measurements below the LOD on a given day would not overly influence long-term average concentrations given that there were several hundred measurements for each city. For the cities with multiple monitors, the daily measurements from those monitors were averaged to produce a single value for the city.

Source Apportionment

Thurston's team selected 17 input variables (components) consistent with those used in previous source-apportionment studies (Thurston et al. 2005). These components were considered to be potential tracers of specific source categories of pollutants. The investigators used the daily measured values for each monitoring site so that day-to-day and site-to-site variability in concentrations of the selected components were reflected in the apportionment results.

The initial source-apportionment factor analysis was conducted using the PROC FACTOR procedure in SAS statistical software. Using the available software options, the researchers specified that the output from this process would produce eight distinct factors using a varimax rotation. The model specifications were designed to produce factor scores that were not correlated with one another and that were likely to be interpretable in terms of real-world pollution sources. The investigators then named the eight factors for source categories based on key pollutants as follows: Soil (Ca, Si), Metals (Pb, Zn), Traffic (EC, NO₂, Cu), Salt (Na, Cl), Residual Oil Combustion (V, Ni), Steel Industry (Fe, Mn), Coal Combustion (As, Se), and Biomass Combustion (K). The resulting eight source categories, their components, and their respective factor loadings appear in IR Table 5, Study 4.

Subsequently, Thurston's team created absolute principal component analysis (APCA) scores, in which the mass of the measured PM_{2.5} concentrations was apportioned to the factors using the factor analysis scores. This resulted in a set of source-apportioned mass variables in which exposure to each category was expressed as a mass concentration in µg/m³, which was based on that category's proportion of total PM_{2.5} mass (Thurston and Spengler 1985). Source-apportioned mass variables were analyzed separately from the factors in the Cox models. (This process was unique to the Thurston study; therefore in this section we use "source category" as a general term for the named source variables.)

Next, the team estimated the residual mass of SO₄²⁻, OC, and NO₃⁻ using a linear regression model. These unmeasured secondary aerosols, which form after pollutants are emitted into the atmosphere, are known to comprise the bulk of PM_{2.5} mass and may have their own associations with mortality. The investigators also constructed full-year all-U.S. models and seasonal and regional models to assess the sensitivity of the results to processes known to affect secondary aerosol formation.

Finally, the investigators performed an absolute principal component mass regression (Thurston and Spengler, 1985). They modeled PM_{2.5} mass data as a function of the APCA scores for the eight identified factors and the three

secondary aerosols (i.e., SO_4^{2-} , OC, NO_3^-) for each monitoring site and day. As a sensitivity test, they performed the APCA separately for the data sets with NO_2 data from the nearest-neighbor monitors located outside a city (about one-third of the NO_2 data and about one-half of the cities) and for data sets with component measurements only (no NO_2). The resulting concentrations of $\text{PM}_{2.5}$ mass apportioned to each of the eight source categories for each measurement day and monitor were then averaged over the entire measurement period in the same way that data for the individual components were averaged. The results were then used in the Cox model to assess the relationships of the mortality causes with $\text{PM}_{2.5}$, individual components, factors, and source-apportioned mass variables.

TRI Analysis

Thurston's team investigated multiple-pollutant exposure scenarios through the construction of a TRI. To formulate the TRI, they used the monitoring data for concentrations of $\text{PM}_{2.5}$ and the individual components as described above; they also used the factor scores, the source-apportioned mass variables, and the estimated concentrations of residual (i.e., not source-related) secondary aerosols (SO_4^{2-} , NO_3^- , and OC) from the absolute principal component mass regression. They included concentrations of the gaseous pollutants (NO_2 , SO_2 , and O_3), where available, as potential effect modifiers.

Statistical Analyses

Cohort Study In cohort survival analyses, a fixed number of participants are followed over time, mortality data are collected as members die, and no new members are recruited. Groups of participants are classified according to exposure level, and subgroups are compared with each other to understand how levels of exposure may affect the number of cohort members who die and the causes of death over the life of the cohort. Risk analyses are typically adjusted for age, smoking status, sex, and other variables known to affect longevity that could confound the relationship between pollutant exposure and death (in this case, the 44 individual-level covariates from the ACS enrollment questionnaire). The resulting comparisons yield a relative risk of mortality based on exposure to the pollutant of interest.

The standard Cox model, a common statistical analysis method for cohort studies, assumes that the mortality of individuals in the study is independent (after adjusting for covariates). However, it is possible that the health and pollution exposure of people in the same household or community will be more similar than those of people who live

in widely separated communities, even after controlling for all available personal risk factors. Therefore, for the more recent analyses of the cohort (Pope et al. 2002, 2004; Krewski et al. 2009), Dr. Krewski and colleagues used a random effects Cox model, as an extension to the standard Cox model, to control for the complex spatial patterns in the CPS-II data.

Thurston's team, which included members of Krewski's team, calculated relative risks for the various causes of death associated with the concentrations of $\text{PM}_{2.5}$, its components, and gaseous air pollutants to which cohort members were exposed. The investigators used the standard Cox model and the random effects Cox model, which included variables at the Zip Code area and city scales; each model was applied with and without the six ecologic covariates. Relative risks were expressed as the difference in risk across the IQR of concentrations for each pollutant index.

TRI Analysis To calculate the TRI, the investigators used the standard and random effects Cox models, described above, to simultaneously estimate the association between mortality and each set of pollutant variables. These models included the individual-level covariates (such as smoking status and dietary habits) with and without the ecologic covariates. The main difference between the conventional approach and the TRI analysis was that, instead of estimating a risk parameter for a single pollutant, they estimated a risk parameter for an entire group of pollutants taken together. The TRI represents the total relative risk for mortality associated with a mixture of pollutants, evaluated across their individual IQRs.

To construct each TRI, the investigators first specified two vectors: one vector was the parameter vector $\hat{\beta}$, which is estimated by the Cox model (and exponentiated to obtain the relative risk associated with the entire group of pollutants); the second vector took the place of the exposure variable (which would be used in an analysis with a single pollutant). The second vector (\hat{x}) was composed of the IQRs of the pollutants of interest. The TRI for the entire set of pollutant variables, taken together, was the exponentiation of the two vectors, or

$$\text{TRI} = \exp(\hat{\beta}'\hat{x}).$$

The TRI is thus analogous to the relative risk calculated for an individual pollutant, except that the parameter and variable are replaced by vectors.

The investigators also performed a number of analyses to evaluate to what extent gaseous pollutants would modify the associations between mortality and $\text{PM}_{2.5}$ mass

concentrations and how various TRI formulations would compare among those cities where data for the gaseous pollutants were available. For each TRI analysis, they included a single variable for gaseous pollutant concentrations in the standard Cox model and the random effects Cox model, both with and without the ecologic covariates. They compared the results of these models with TRI results that were estimated for the same sets of cities.

KEY RESULTS

Cohort Study

The analyses of the CPS-II cohort data were conducted for mortality from all causes, IHD, respiratory disease, and lung cancer using individual component concentrations, factor scores, and source-apportioned mass variables. The results from the various mortality analyses are presented in IR Figures 6 through 17, Study 4. In this Commentary, unless noted otherwise, we focus on the results from the most-controlled analyses; specifically, analyses using the random effects Cox model, which controlled for spatial correlations in the data for residents living near one another, with or without the ecologic covariates.

All-Cause Mortality The investigators reported the strongest associations between all-cause mortality and concentrations of As, Se, EC, S, and PM_{2.5} mass in the most highly adjusted models (random effects Cox model with ecologic covariates; see Commentary Figure 6). Of these, only the association of all-cause mortality with S was markedly stronger than the association with PM_{2.5} mass and was marginally increased with the addition of ecologic covariates. In contrast, inclusion of the ecologic covariates in the analyses of all-cause mortality with As and with Se reduced the magnitude of the associations. The association for EC, which was negative and not significant, was substantially affected by the inclusion of the ecologic covariates and became both positive and significant.

The Traffic and Coal Combustion factors were significantly associated with all-cause mortality in the most highly adjusted models. Although the association for the Traffic factor was initially negative and not significant in the less-adjusted model, it was substantially affected by the inclusion of the ecologic covariates and became both positive and significant (Commentary Figure 6). In contrast, the associations with the Coal Combustion factor were slightly attenuated when ecologic covariates were included in the model. The analysis did not find statistically significant associations with most of the other factors, such as Residual Oil Combustion and Biomass Combustion. The results for the analyses of source-apportioned mass variables were very similar to those for the factors.

IHD Mortality IHD mortality was associated with PM_{2.5} mass and the components As, Pb, Se, and S in the most highly adjusted models (Commentary Figure 6). Marginal associations (for which the lower bounds of the 95% CIs were 0.98–1.00) were noted for Cl, Fe, and EC. Most of these results were unaffected by the inclusion of the ecologic covariates, with the exception of S, which became significantly associated with IHD when the covariates were included. That association was also considerably stronger than the association between PM_{2.5} and IHD in the model with ecologic covariates.

The Coal Combustion and Metals factors were significantly associated with IHD mortality, but the Metals factor was significant only when ecologic covariates were included in the model (Commentary Figure 6). When source-apportioned mass variables were analyzed, only Coal Combustion was significantly associated with IHD (data not shown). Other source categories were not significantly associated with IHD mortality.

Respiratory Mortality PM_{2.5} and OC demonstrated significant associations with respiratory mortality, and Si and Ca had marginal associations (Commentary Figure 6). The PM_{2.5} and OC associations were of similar magnitude, and both increased when ecologic covariates were added to the model.

In the most highly adjusted models, the Traffic and Soil factors displayed the strongest associations with respiratory mortality. The inclusion of the ecologic covariates produced a small reduction in the association with the Soil factor, but had a strong, positive effect on the association with the Traffic factor (Commentary Figure 6). (The Residual Oil Combustion and Coal Combustion factors showed significant negative associations in the models.) Similar patterns of associations and responses to the inclusion of ecologic covariates were noted for the Traffic and Soil source-apportioned mass variables (data not shown). Other source categories were not significantly associated with respiratory mortality.

Lung Cancer S was strongly and significantly associated with lung cancer mortality in the most highly adjusted models, and PM_{2.5} and Se demonstrated positive, but marginally significant associations. Associations for PM_{2.5}, Se, and S were only slightly influenced by the inclusion of the ecologic covariates (Commentary Figure 6). For the associations of mortality with factors (as well as with source-apportioned mass variables), only Coal Combustion demonstrated a significant positive association with lung cancer mortality; inclusion of the ecologic covariates slightly reduced the magnitude of the association.

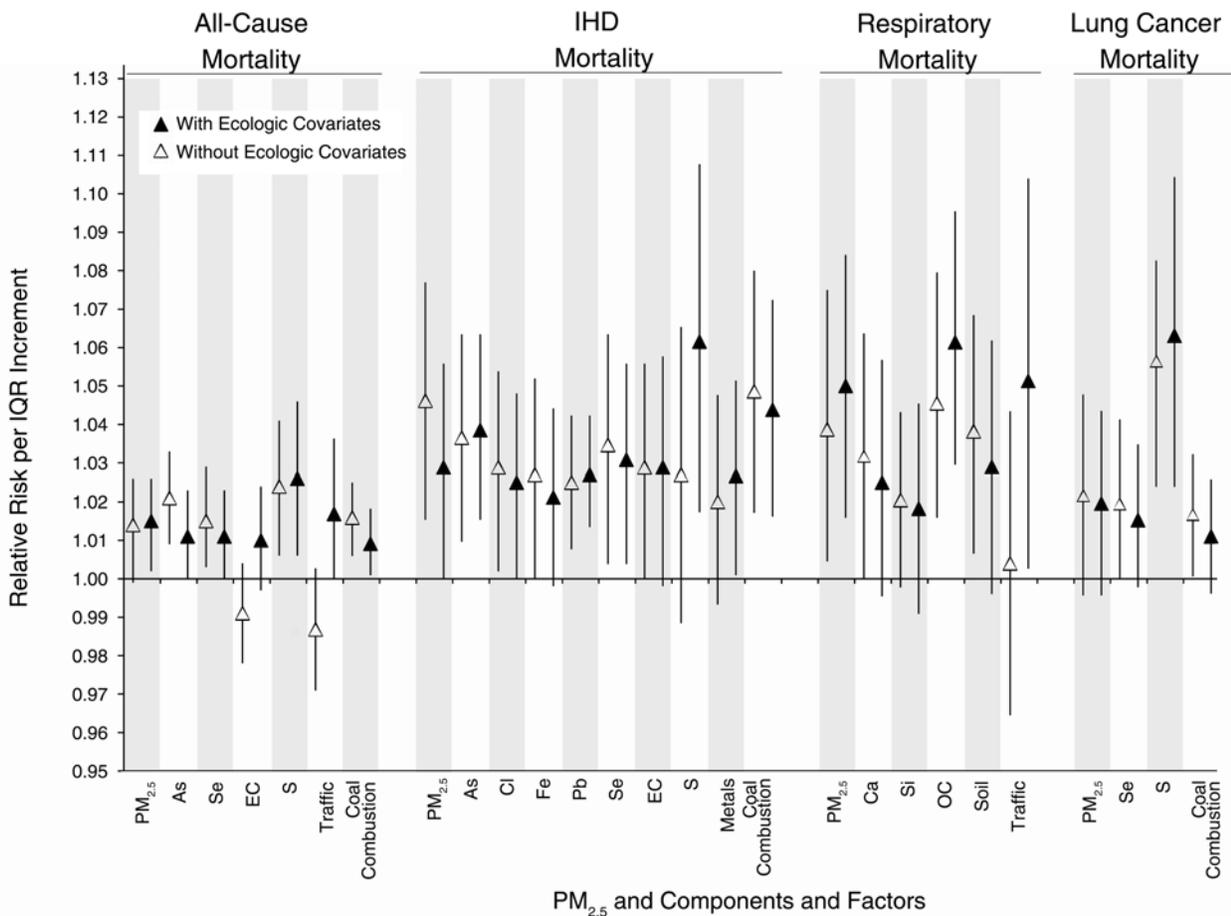
TRI Analysis

Thurston’s team presented numerous results from their TRI analyses for all-cause mortality, cardiopulmonary mortality, CVD mortality, IHD mortality, and lung cancer mortality; results are given for models with and without separate variables for exposure to pollutant gases. For consistency with the main cohort analyses, we have included here the key results for all-cause, IHD, and lung cancer mortality from the most highly adjusted model (random effects Cox model with ecologic covariates). Complete results are presented in IR Study 4, Appendix I, which is available on the HEI Web site.

Commentary Table 5 gives the effect estimates for PM_{2.5} mass and various TRI formulations (to include multiple-pollutant variables) for three primary causes of death. In

the table, each term refers to the entire set of factors, source-apportioned mass variables, components, or secondary aerosols. Each TRI formulation contained one or more of these sets. The relative strength of association for a particular TRI can be compared with PM_{2.5} or other TRI formulations for each mortality cause by comparing their respective effect estimates.

The all-cause mortality association was strongest for the TRI with the factors plus secondary aerosols, but all TRI formulations shown were significantly and positively associated. For IHD mortality, all TRI formulations were positive and significant (with PM_{2.5} only marginally so). The TRI with factors plus secondary aerosols was most strongly associated with IHD, followed by the TRI for factors alone.



Commentary Figure 6. Relative risks for all-cause and specific-cause mortality for the CPS-II cohort that were significantly or marginally associated with selected components and factors (Thurston Study 4). The data were analyzed using the random effects Cox model with (black symbols) and without (white symbols) ecologic covariates included in the model. Associations with other components and factors were not significant or were negative. Note that the IQR varied by pollutant and factor: IQRs for pollutants were 3.13 µg/m³ for PM_{2.5}, 0.6 ng/m³ for As, 0.8 ng/m³ for Se, 0.26 µg/m³ for EC, 0.53 µg/m³ for S, 33.5 ng/m³ for Cl, 47.8 ng/m³ for Fe, 2.6 ng/m³ for Pb, 43.6 ng/m³ for Ca, 42.9 ng/m³ for Si, and 0.98 µg/m³ for OC. IQRs for factors were 0.7 for Traffic, 0.3 for Coal Combustion, 0.2 for Metals, and 0.4 for Soil. (Based on data in IR Figures 6, 7, 9, 10, 12, 13, 15, and 16.)

EVALUATION OF THURSTON STUDY 4

Study 4 investigated whether exposures to source-related and specific components of ambient PM_{2.5} are more (or less) strongly associated with human mortality in the United States than is PM_{2.5} itself. The investigators concluded that long-term exposure to PM_{2.5} and the key tracers of the Coal Combustion source category explain most of the previously observed associations between PM_{2.5} mass and increased risk of all-cause, IHD, and lung cancer mortality.

Although the Review Panel noted that the study was designed and conducted appropriately and has yielded many important results, the Panel did not agree with the overall interpretation of this study as summarized in the above statement. The Panel came to this conclusion after evaluating several aspects of the study, as outlined in the following sections: strengths and limitations of the spatial design of the CPS-II cohort, choice of statistical model, other methodologic issues, and interpretation of the study findings.

Spatial Design of the CPS-II Cohort

A major characteristic of the CPS-II cohort, which made it particularly valuable to this study, is that it includes participants from 100 cities across the United States with monitors that collect detailed data on PM_{2.5} composition, which enabled a nationwide assessment of the effects of PM components on mortality. This design is well suited to investigating the effects of city-to-city differences among cities in concentrations of regional air pollutants (e.g., sulfates, which are transported over long distances). However, given that the CSN includes only one or two air pollution monitors per city that routinely collect data on

PM_{2.5} composition, it was not possible to assess the effects of within-city exposure variations.

One of the most important contributors to within-city differences in PM_{2.5} exposure is traffic, but any analysis of the CPS-II cohort will likely underestimate the effect of traffic-related air pollution, simply because it will not be able to capture the variation in traffic-related exposure due to the limited number of monitors. Although this is an intrinsic problem that cannot be solved with the existing air quality data set, the results of the study need to be interpreted in this context. The Traffic source category in this study is based mostly on between-city variation in average traffic-related PM_{2.5}, which represents only a very small part of the expected overall variation in traffic-related pollution, because *average* contributions of traffic to air pollution across North American cities are very similar. (As shown in IR Table 7, Study 4, the overall range in PM_{2.5} mass attributed to the Traffic source category is 3.6 to 4.0 µg/m³, indicating minimal contrast among cities.) The importance of the within-city differences, especially with regard to long-term PM_{2.5} exposure and CVD, has been shown before (e.g., Miller et al. 2007). In contrast, the Coal Combustion source category varies on a regional scale only (not within a city); therefore, it can be far better assessed by the methods used in this study. Thus, it is very difficult to directly compare the effects of the Traffic and Coal Combustion source categories in this study.

Choice of the Statistical Model

When attempting the difficult task of assessing whether one or more specific components are responsible for the overall observed effect of PM_{2.5} on mortality, the choice of the statistical model is crucial. Here, we discuss three

Commentary Table 5. TRI Results from the Random Effects Cox Model Including Ecologic Covariates Using Data from the CPS-II Cohort (in 100 Cities) in Thurston Study 4^a

TRI Formulation	All-Cause Mortality	IHD Mortality	Lung Cancer Mortality
PM _{2.5} mass	1.014 (1.002, 1.026)	1.028 (1.000, 1.056)	1.019 (0.995, 1.043)
Source mass variables	1.033 (1.002, 1.064)	1.112 (1.038, 1.192)	1.013 (0.949, 1.081)
Source mass variables + secondary aerosols	1.053 (1.009, 1.099)	1.121 (1.018, 1.233)	1.083 (1.007, 1.165)
Factors	1.040 (1.011, 1.070)	1.135 (1.067, 1.208)	0.963 (0.904, 1.026)
Factors + secondary aerosols	1.063 (1.027, 1.100)	1.173 (1.092, 1.260)	1.026 (0.948, 1.110)
Components	1.032 (1.004, 1.062)	1.069 (1.010, 1.130)	0.947 (0.882, 1.016)
Components + secondary aerosols	1.042 (1.007, 1.079)	1.097 (1.025, 1.174)	1.018 (0.932, 1.113)

^a Values show increase in risk per increase in exposure equal to the magnitude of the IQR (95% CI). **Bold** type indicates significant associations.

major methodologic issues regarding the choice of statistical model: controlling confounders, adjusting for PM_{2.5} mass and multiple-pollutant exposures, and using the same units to make comparisons across components.

Controlling Confounders To the extent possible, all confounders should be controlled in an analysis. This is especially true for socioeconomic status, since it may be related to air pollution exposure (e.g., people with low socioeconomic status may be more likely to live near busy roads or coal combustion plants) and mortality (low socioeconomic status is strongly related to overall mortality; see Smith et al. 1990). The extent of such confounding may differ according to the outcome under study, but it would be unusual to find analyses of all-cause, IHD, respiratory, and lung cancer mortality in which socioeconomic status was not an important confounder.

In this study, socioeconomic status was based on individual addresses and Zip Code areas. Earlier analyses of the CPS-II cohort that specified such detailed models have found evidence of clustering of responses at the city level (Krewski et al. 2000a,b; Pope et al, 2002). In the current report, the investigators present findings for a variety of models because they did not believe that sole importance should be given to only the most complex model — with respect to either the number of covariates in the deterministic component of the model or the complexity of the stochastic component — but instead felt that all model results, and variation in estimated risk among models, should be considered:

“The models that included both the contextual ecologic covariates and the random effects assumption were generally the most conservative estimates (usually resulting in somewhat lower estimates with larger confidence intervals); but they are, conversely, the least parsimonious and therefore are most susceptible to possible over-specification. In our discussion, we have given the greatest weight to results that are least affected by choice of model (i.e., results that are most consistent across all four models).”

Particularly for analyses of the CPS-II cohort that attempt to assess variations in health status and air pollution across the entire United States, the Review Panel believed that careful adjustment for individual- and area-level variables is essential. In analyses of this type (in which an attempt is made to estimate the causal effects of one of more variables), the aim is to control for all important confounders while avoiding problems of multicollinearity. If adding contextual variables to the model results in large changes in the effect estimates, it is evidence of important confounding, which should be controlled for. If controlling for such confounding results in wider CIs, then those wider CIs correctly reflect

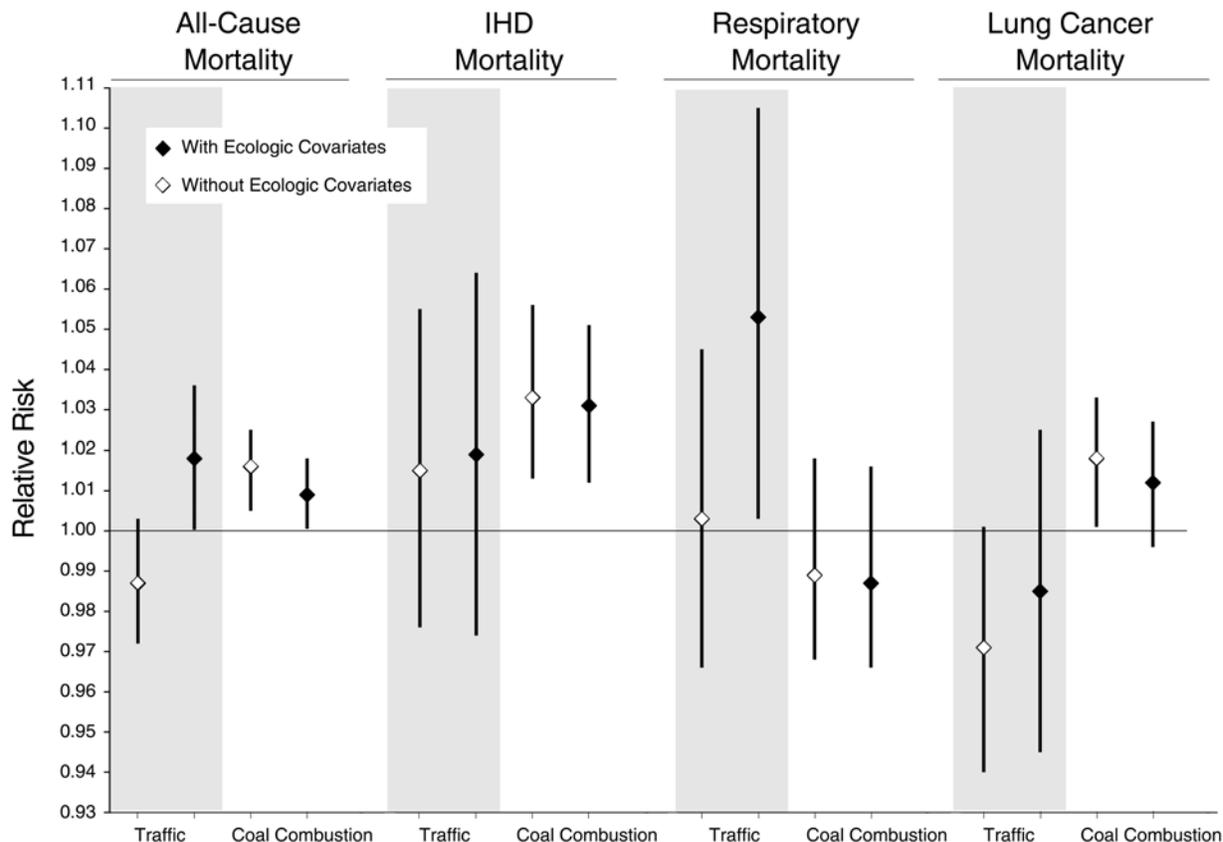
the difficulties of separating the effects of variables that are correlated.

For example, in Commentary Figure 7, the findings for the Traffic factor changed markedly for respiratory mortality and changed direction for all-cause mortality when adjusted for ecologic covariates, whereas the findings for the Coal Combustion factor changed to a smaller degree. The investigators therefore concluded that the findings for the Coal Combustion factor are more consistent. An alternative explanation, which the Review Panel favors, is that the results from the unadjusted models are strongly confounded and therefore should not be considered, whereas the results from the adjusted models indicate a stronger effect for the Traffic factor than for the Coal Combustion factor. Similar issues of interpretation occur throughout the reporting of results from this study. Because the CIs in the figures do not substantially change when the models are adjusted, and thus give no indication that there are substantial problems of multicollinearity, the Review Panel’s view is that the fully adjusted effect estimates are more reliable.

Adjusting for PM_{2.5} and Multiple-Pollutant

Exposures A second important issue related to choosing an analytic approach is whether to adjust for total PM_{2.5} mass when attempting to estimate the effects of particular components (see the sidebar What Different Statistical Models Can Tell Us About Associations Between Specific Components and Health Outcomes). The overall aim of the study was to assess whether associations between mortality and PM_{2.5} exposure were stronger with certain components or source categories of PM_{2.5} than with others. The problem with doing this one component at a time is that the specific exposures are correlated with each other and with total PM_{2.5} mass. Given that exposure to each component is associated with total PM_{2.5} exposure, one would expect each component to be associated with mortality. However, this does not mean that each component actually causes mortality. An analogy is that total consumption of tonic is likely to be associated with gin-and-tonic consumption, which in turn is associated with alcohol-related disease; but this does not mean that tonic consumption causes liver cirrhosis. What the study must do is examine the effect of each component while adjusting for total PM_{2.5} exposure (similar to assessing the effect of tonic consumption while adjusting for total gin-and-tonic consumption). The investigators point to this issue:

“Given the correlation between some pollutants, it is even difficult to confidently interpret the regression coefficient from a single-pollutant model because this single coefficient may reflect the incomplete but combined



Commentary Figure 7. Relative risks for all-cause and specific-cause mortality for the CPS-II cohort associated with the Traffic and Coal Combustion factors (Thurston Study 4). The data were analyzed with the random effects Cox model with (black symbols) and without (white symbols) ecologic covariates in the model. Note that the IQR varied by factor; i.e., 0.7 for Traffic and 0.3 for Coal Combustion. (Based on data in IR Figures 7, 10, 13, and 16.)

effects of multiple, correlated pollutants. These complex correlations among many pollutants make it even more difficult to interpret regression coefficients from multiple-pollutant models. Including several pollutants in a model can result in various outcomes: (1) coefficients that are mostly unaffected by others (suggesting independent effects); (2) coefficients that retain the same sign (positive or negative) but are each smaller in size (suggesting that they share the effect or are both imperfect indicators of a true risk estimate); or (3) coefficients that are highly unstable, some becoming inflated and others becoming null or changing signs.”

However, these general statements are not satisfactory unless results on correlations between components and $PM_{2.5}$ are provided. In the opinion of the Review Panel, the investigators missed an opportunity to include two-pollutant models in their study.

The investigators' development and use of TRIs was an interesting innovation that promises to add to the methods by which multiple-pollutant models, and particularly those that include multiple PM components, may be compared. Although promising, the Review Panel considered this to be a work-in-progress rather than a finished tool that can be relied on at this stage.

The investigators acknowledged that this approach has a scaling issue, which is not a trivial problem. The TRI for a group of pollutants represents the relative risk calculated by comparing mortality related to an atmosphere in which the concentration of every pollutant is in the upper quartile of its range, with mortality related to an atmosphere in which the concentration of every pollutant is in the lower quartile of its range. In reality, pollutants never rise and fall together in such an extremely correlated fashion. This extreme assumption — that all pollutants in the TRI would

be at either the high end or low end of their concentration ranges simultaneously — leads to higher relative risks for those analyses that involve a large number of pollutants that are positively correlated. For example, even if all $PM_{2.5}$ components had equal toxicity per unit mass, the TRI for all the components combined would be higher than that for total $PM_{2.5}$.

Without resolving this issue, the Review Panel concluded that it is invalid to compare TRIs from different models because any differences in the effect estimates may simply reflect this scaling problem. Although the investigators attempted to address this in their report, the Panel noted some odd results, in particular in the CIs, when the investigators rescaled the TRIs. These difficulties suggest caution in interpreting substantive TRI results until the approach has been more thoroughly developed and investigated.

Using Comparable Units If we are to assess whether particular components of $PM_{2.5}$ are more important than others in determining health outcomes, or are more important than total $PM_{2.5}$, it is important to compare like with like, using the same units. In this study, the investigators presented the findings for Traffic, Coal Combustion, and other source categories in terms of IQRs; that is, the effect estimate expressed in terms of the concentration range for that particular component. This approach has merit because it compares the effect of the component based on its distribution in the cohort population. However, the approach does not estimate which component is more toxic on a same-unit basis, for example per $10 \mu\text{g}/\text{m}^3$. Even if all toxicities per unit were the same, the components with the greatest range in concentration across the population would have higher relative risks per IQR. To obtain a more direct comparison, therefore, it would be useful if results were also reported for a fixed exposure range and fixed units; that is, how does the effect of a $10\text{-}\mu\text{g}/\text{m}^3$ increase in total $PM_{2.5}$ compare with the effect of a $10\text{-}\mu\text{g}/\text{m}^3$ increase in traffic-related $PM_{2.5}$, coal combustion-related $PM_{2.5}$, and so on. Because this has not been done, the tables and figures do not compare like with like; thus the findings of which components have the strongest effects on mortality, based on absolute concentrations, are difficult to compare. The toxicity of different components would be easier to compare if the tables and figures had included findings for an absolute concentration contrast for each pollutant in addition to the IQR-based contrasts.

Other Methodologic Issues

When comparing the relative effects on mortality of different $PM_{2.5}$ source categories, another methodologic issue that needs to be taken into account is the differential

decrease over time in $PM_{2.5}$ emissions from specific sources due to regulatory actions and other factors (Pope et al. 2009). Whereas exposure to $PM_{2.5}$ from coal combustion in the United States has declined considerably during the last two decades, exposure to traffic-related $PM_{2.5}$ has declined less (U.S. EPA 2004b). Using today's exposure contrast (i.e., the difference in exposure between cities) — which is smaller for coal combustion-related $PM_{2.5}$ now than it was at the beginning of the CPS-II follow-up period — leads to a potential inflation of the effect estimates per unit mass concentration. A comparison of relative effects of different source categories and components needs to take these long-term changes in exposures into account, for instance by using a proper back-extrapolation algorithm. As an example, the analysis of the CPS-II cohort by Pope and colleagues (2002) showed that effect estimates per unit $PM_{2.5}$ mass were higher when they used the reduced 1999–2000 exposure contrast measured after the end of follow-up than when they used the 1979–1983 exposure contrast measured at the beginning of follow-up (1.06 versus 1.04 for all-cause mortality, respectively). With further analyses of the CPS-II cohort and continued reductions in emissions from coal-fired power plants, the potential for over-estimating effect estimates is problematic.

The investigators concluded that “controlling $PM_{2.5}$ emissions from coal-combustion and other sources that produce a similar mix of pollutants would influence the greatest mortality benefits”. The Panel believes this conclusion is overstated because of the methodologic problems identified above and issues related to the study's source-apportionment analyses. For example, in this study, the source category identified as Coal Combustion might also represent pollution transported over long distances from multiple combustion sources, not just from coal combustion.

Furthermore, this study did not include a health impact assessment of potential benefits: Without combining information on a population's exposure to emissions from specific sources and on the incidence of disease, it is not possible to predict to what extent specific preventive measures (e.g., reducing emissions from some sources but not others) will benefit health.

Interpretation of the Study Findings

Given the way the analyses were performed, the Review Panel concluded that the findings did not point to any particular component of $PM_{2.5}$ as consistently more strongly related to health outcomes than exposure to total $PM_{2.5}$. In particular, the evidence was relatively weak that air pollution from Coal Combustion was more strongly related to mortality than was air pollution from Traffic, especially

given the limited ability of the study design to detect traffic-related effects (because of the lack of within-city exposure information and between-city exposure contrast) and the marked influence of the ecologic covariates on the effects for the Traffic factor. This is illustrated in Commentary Figure 7, which compares the findings for the Traffic and Coal Combustion source categories for all-cause, IHD, respiratory, and lung cancer mortality, with and without adjusting for contextual variables.

Conclusions

Of the source categories identified, Coal Combustion and Traffic showed the strongest associations with mortality in the most highly adjusted models, as did the components that strongly drive their factor scores (S and EC). The Traffic and EC associations with mortality were, however, highly sensitive to use of the random effects Cox model and the inclusion of the contextual ecologic covariates.

Although the TRI analysis provided some interesting results that implied that exposure to combinations of components, secondary components, and gases in pollution atmospheres is more toxic than exposure to PM_{2.5} mass alone, the TRI approach does not specifically identify which of these components or source categories are potentially more toxic than others. In addition, because of the TRI scaling issues (as explained above), the Panel urges caution in interpreting these results.

The Review Panel found that the investigators had conducted a detailed and generally well-done analysis in this important population. It was not convinced, however, that the study has definitively demonstrated that long-term exposure to particular components of PM_{2.5} is more important than long-term exposure to other components or to total PM_{2.5} in causing adverse effects. In particular, the Review Panel thought these findings did not support the investigators' conclusions that exposure to air pollution from coal combustion emissions is more strongly associated with mortality than exposure to traffic-related air pollution. The results of the analyses based on factors showed marginally significant associations of the Metals factor with IHD mortality and of the Soil factor with respiratory mortality. Overall, the analyses did not consistently show associations for most other source categories, although a number of the uncertainties described above make it difficult to definitively determine whether or not these source categories are associated with health effects.

In the analyses based on PM components, PM_{2.5} mass concentrations exhibited associations with health outcomes that were as significant as those for any of the specific components. Because the source-apportionment analysis grouped components according to source categories, the potential

effects of correlation between components associated with the same source category were mitigated. Nevertheless, unresolved questions remain about the attribution of components to source categories given recent regulations for coal-fired power plant emissions, which have affected their source profiles (Morgenstern et al. 2012). In particular, emissions of sulfur oxides and NO_x have been reduced, but other components have likely been affected as well (e.g., As, which has been an important marker for the Coal Combustion source category). Although the results of the current study are suggestive of a link between mortality and the Coal Combustion and Traffic source categories, they are not definitive and need further exploration.

SUMMARY AND CONCLUSIONS FOR THE LIPPMANN STUDY

In their original proposal, Lippmann and colleagues stated that "Our overall objective is to conduct a coordinated program of toxicologic and epidemiologic research that will identify and characterize specific components of ambient air particulate matter (PM) that are responsible for excess mortality and morbidity in contemporary human populations." They further stated four hypotheses that guided their research:

1. Coarse, fine, and ultrafine PM are each capable of producing acute health effects of public health concern, but the effects may differ according to particle size and composition.
2. Long-term PM_{2.5} exposures are closely associated with chronic health effects.
3. The source-apportionment techniques that we have developed and refined in recent years provide a useful basis for identifying major categories of sources of PM in ambient air and specific chemical components that have the greatest impacts on a variety of acute and chronic health effects.
4. The health effects due to ambient PM exposures can best be seen in sensitive subgroups within overall human populations and in animal models of such populations.

The researchers conducted an extensive and detailed set of toxicologic and epidemiologic analyses to test these hypotheses. They addressed the first hypothesis in Study 2, in which they indeed found some differences in biological markers after exposure to different PM size fractions in vitro and in vivo. However, the overall results indicated that both coarse and ultrafine PM, in addition to fine PM, display important biological activity; it is difficult to assess the relative importance of each and it is unclear

how the findings of experiments in cell cultures and mice exposed to PM by aspiration can be translated to health effects and mortality in human populations. The second hypothesis, which has been supported by the results of numerous other studies of long-term PM_{2.5} exposure and mortality, was further verified in Study 1 — in which animals exposed to CAPs for several months showed signs of atherosclerotic plaque progression as well as some changes in HR measures — as well as in Study 4, in which long-term exposure to PM_{2.5} was associated with all-cause, IHD, and lung cancer mortality.

The authors and the Review Panel did not fully agree about the utility of the source-apportionment approaches (hypothesis 3). Although the authors did extensive work to identify source categories using well-developed methods, the source categories they identified were not the same across all of the different studies, even though the studies often described the same airsheds. In addition, the components contributing to those source categories varied among the studies, because the data sets of PM_{2.5} components were different for each study. The Panel further noted that concentrations of some components (e.g., As) used to apportion PM_{2.5} to a Coal Combustion source category are showing long-term decreasing trends and are no longer emitted in substantial concentrations by modern coal-burning facilities, given stringent controls on emissions after implementation of the 1990 Clean Air Act Amendments in 1995 and 1997 (Morgenstern et al. 2012). These substantial changes in emissions regulations were phased in during the latter part of the period during which the air quality data were collected (1988–1997) that were used to develop the source apportionment approach (Thurston et al. 2005); but the changes were completed during the time when the PM component data were collected for the current study (2000–2006). Although the investigators did find associations between health outcomes and Traffic, Coal Combustion, and Residual Oil Combustion source categories in animal and human studies, the Review Panel felt these findings should be interpreted with caution because of the uncertainties surrounding the attribution of selected PM_{2.5} component data to each source category. In other words, the investigators may have attributed a certain PM_{2.5} component profile to Coal Combustion, but, in reality, that category may represent combustion emissions from a number of sources, in addition to coal.

This report showed some interesting findings about sensitive populations (hypothesis 4). However, the overall design and execution of the studies did not typically lend itself to a more direct investigation of this hypothesis. One noteworthy finding, in Study 4, was how the associations of EC and the Traffic source category with major health outcomes changed when contextual ecologic covariates

were included in the analyses. The large change from a null to a positive association when socioeconomic information was introduced implies that the associations of EC and Traffic with IHD are confounded by socioeconomic status. However, the study did not attempt to specifically identify vulnerable populations. Likewise, the analytic models in Study 4 stratified for age and controlled for a large suite of individual-level covariates, but did not specifically identify and evaluate subpopulations. Although Study 3 analyzed data for CVD and respiratory hospitalizations in elderly populations, it made no comparisons with nonelderly populations, nor did it directly assess age-related patterns within elderly populations. Study 1 used mice that are susceptible to the formation of atherosclerotic plaques, showing that such animals do indeed respond to PM exposures, although it remains unknown whether they were more susceptible to the effects of PM than normal mice would have been.

Lippmann and his investigative team conducted a wide-ranging and carefully implemented program of both toxicologic and epidemiologic research aimed at determining whether PM_{2.5} components or source categories were more strongly associated with selected animal and human health outcomes than PM_{2.5} itself. Specific findings from each of the studies, as described in detail in the investigators' reports and the study-specific sections of this commentary, have provided some insights into the toxicity of components and source categories that may guide future research efforts (e.g., the possible implication of the Coal and Residual Oil Combustion, Traffic, and Metals source categories). However, these findings were not consistent across the Lippmann studies. The Review Panel concluded that this research has not shown conclusively that a single component, multiple components, or pollutants in a single source category were more strongly associated with the health outcomes than others. It remains possible that some components and source categories may be less likely to be associated with human or animal health outcomes on a national scale only because their concentrations in ambient air are low or below the limit of detection at the central monitors included in the CSN.

Overall, this comprehensive and ambitious research program has shown that research on the toxicity of PM components is not likely to easily identify a single culprit component or source category. More work remains to be done to accomplish the following:

- Refine statistical methods for simultaneously modeling multiple pollutants, particularly when pollutant concentrations may be correlated as a result of common source emissions, weather patterns, and other phenomena.

- Improve the representation of spatial contrasts in component concentrations, particularly for components with high spatial variability, to capture within-city contrasts in exposure.
- Improve source identification and attribution, including making use of local source inventories and sophisticated modeling techniques for evaluating the formation of secondary pollutants.
- Connect particle components with physiologic mechanisms, through further toxicologic assessments using *in vivo* and *in vitro* methods.
- Study further the toxicity of particles versus gases, and study atmospheric aging of complex mixtures so that toxicologic studies better reflect real-world conditions.

We need the results of such research before we can definitely determine which components of PM_{2.5}, if any, are responsible in whole or in part for the observed health effects associated with PM_{2.5}.

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Research Report 178, *National Particle Component Toxicity (NPACT) Initiative Report on Cardiovascular Effects*, Sverre Vedal, Matthew J. Campen, Jacob D. McDonald, Joel D. Kaufman, Timothy V. Larson, Paul D. Sampson, Lianne Sheppard, Christopher D. Simpson, and Adam A. Szpiro

INTRODUCTION

As outlined in the Preface, HEI funded the National Particle Component Toxicity (NPACT*) Initiative to provide more insight into which components of the particulate matter (PM) mixture may be responsible for its toxicity and human health effects. The initiative consisted of coordinated epidemiologic and toxicologic studies conducted in multiple cities to evaluate the toxicity of different chemical and physical properties of PM and their associated health effects, while taking into account the contribution of gaseous copollutants. The NPACT Initiative has spanned nearly a decade from its initial conception and the development of request for applications (RFA) 05-1, through the issuing of the RFA and study selection, to the conduct of research, submission of the final reports, and evaluation by the HEI NPACT Review Panel. It is important to take a broad look at the results of all the separate epidemiologic and toxicologic studies that were part of the two major research efforts and to consider them in the context of current scientific understanding of how particle components may affect health, and to what sources those components can be attributed.

This Synthesis looks broadly at the approaches and the results of the reports by Dr. Morton Lippmann at New York University (hereafter referred to as the Lippmann team, study, or report) and Dr. Sverre Vedal at the University of Washington (hereafter referred to as the Vedal team, study, or report). In this Synthesis, the HEI NPACT Review Panel

considers whether there is coherence and consistency in the epidemiologic and toxicologic results and discusses the larger scientific significance of the overall findings and their implications for future research into the health effects of particle components.

INITIAL OBJECTIVES OF THE NPACT INITIATIVE

The overall purpose of RFA 05-1 was “to develop a comprehensive research program to systematically address questions about the health effects related to different components” of the ambient PM mixture, and it specified several features of studies that would be considered for funding:

- Consideration of how gaseous pollutants may affect the toxicity of the PM components;
- A preference for studies that combined epidemiologic and toxicologic approaches; and
- A project plan that demonstrated a systematic comparative study design for the evaluation of PM characteristics that may be associated with toxicity.

At the time, several hypotheses regarding particle characteristics and toxicity were of interest, such as the possibility that some transition metals, sulfates, or certain organic compounds have stronger associations with adverse health effects than other PM components. In the interest of soliciting targeted research, RFA 05-1 specified that proposals “have a clear and defensible prior hypothesis to be tested, rather than involving large numbers of exploratory analyses.” The RFA also stated that investigators might use source apportionment in their investigations, but cautioned that “identifying sources responsible for toxic effects should be considered primarily as a step

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* A list of abbreviations and other terms appears at the end of this Synthesis.

toward identifying the components and characteristics of the emissions from those sources that have toxic effects.”

Both NPACT studies funded under this RFA[†] included a toxicology component, with in vivo exposures to laboratory-generated pollution mixtures in the Vedal report (the Campen study) and to concentrated ambient particles in the Lippmann report (the Chen study), and in vivo and in vitro exposures to particle extracts in the Lippmann report (the Gordon study). Both reports also included an epidemiology component, comprising a time-series study (the Ito study) and a cohort study (the Thurston study) in the Lippmann report and two cohort studies in the Vedal report (the Vedal epidemiologic study), investigating associations between particle composition and a variety of health outcomes in short- and long-term settings. Synthesis Table 1 summarizes the various studies that were conducted by the two teams of investigators.

DATA AND STUDY DESIGN

In addition to its detailed reviews of each study, the NPACT Review Panel considered, and discusses here, some of the strengths and limitations encountered by both teams in the design of the studies, the availability of data, exposure assessment and exposure atmosphere generation, and possible approaches to linking PM components to specific sources.

PM COMPOSITION DATA

Both the Lippmann and the Vedal epidemiologic studies relied on PM composition data available from the Chemical Speciation Network (CSN), operated by the U.S. EPA, which to date is the most comprehensive effort in the world to systematically collect such data nationwide. In addition, the Vedal team augmented the CSN data with their own monitoring data. Although these studies could not have been undertaken without the availability of the CSN data, the Panel noted that they also highlight some of the limitations of that network. First, the network is relatively sparse, comprising only about 200 locations nationally, such that the finer-scale spatial gradients in chemical components within cities are not captured. Second, although taking samples more often than many other efforts to collect PM component data, most CSN locations

collect samples only once every three or six days. This infrequency limits researchers' ability to evaluate associations of PM components with daily health outcomes in short-term study designs and (to a lesser extent) reduces the information available for long-term averaging in the longer cohort studies. Third, concentrations of many of the components measured in the CSN network, especially metals, are below their minimum detection limits (MDLs) on a large number of sampling days, limiting analyses to only those components that can be detected repeatedly and reliably. Fourth, the accuracy of measured concentrations of elemental carbon (EC) and organic carbon (OC) depends on the methods used to measure these components. Because the measurements are defined operationally (EC and OC are complementary fractions of total carbon, and their respective concentrations depend on the methods used for sampling and measuring carbonaceous material), there is considerable uncertainty associated with them, and comparing them across studies is difficult. These issues affect some of the chemical components most important to the NPACT studies.

The Vedal team addressed the sparseness of the monitoring network and non-continuous sampling by adding extra monitors in additional locations to measure EC, OC, and the other PM components measured by the CSN and by calculating average concentrations over longer (2-week) time periods. However, the Panel noted that they did not use the same measurement approach in their additional monitoring as was employed by the CSN, and their results did not agree well with measurements from collocated CSN monitors. Thus, although the increased spatial information provided by the additional monitoring might have reduced exposure measurement error, the different approach and sampling time used by the additional monitoring campaign might have actually enhanced such error.

In particular, the Panel considered the uncertainties in EC and OC measurements important because these components are used to help identify traffic as a source of PM. The Vedal team focused on these components in accordance with their hypothesis that traffic-related air pollutants drive the effects of PM on health. Source apportionment analyses conducted by the Lippmann team were also sensitive to these two components, because they were used in the estimation of traffic-related source categories. In addition to being operationally defined (see above), EC and OC are known to be subject to strong spatial and temporal gradients, making it likely that the small number of observations made at central monitoring stations do not adequately represent the highly variable concentrations observed across an entire urban area. Nonetheless, EC and OC continue to be important components to characterize in studies that evaluate the health impacts of PM components, particularly when there is an interest in traffic-related effects.

[†] A third study, *Assessment of the Health Impacts of Particulate Matter Characteristics*, by Dr. Michelle L. Bell of Yale University, was published as HEI Research Report 161 in January 2012. This study was funded through RFA 04-2, *Walter A. Rosenblith New Investigator Award*. Because the topic was very relevant to the NPACT Initiative, HEI decided to include this study under the umbrella of NPACT (although the study was reviewed separately and published earlier).

Synthesis Table 1. Broad Overview of NPACT Study Designs

Study Approach	Lippmann et al.	Vedal et al.
Exposure timescales	Short- and long-term	Long-term only
Health endpoints	Respiratory and cardiovascular	Cardiovascular only
Epidemiologic Studies		
Study design	Multicity time-series analysis and one cohort	Two cohorts
Health endpoints	Acute: respiratory and cardiovascular mortality and hospitalizations Chronic: mortality	Chronic: Subclinical markers of atherosclerosis; cardiovascular disease events (including mortality)
PM components and exposure assessment	EPA CSN monitors; MSA averages; sources	Cohort-specific and EPA CSN and IMPROVE monitors; individual-level exposure predictions; two exposure models; focus on OC, EC, silicon, and sulfur; included some evaluation of other pollutants and PM components
Source apportionment goal	Assessing exposure	Interpretation of exposure health effect estimates
Toxicologic Studies		
Study design	ApoE knockout mouse model (normal diet); 6-month exposures; FVB/N mice; 12-day and 100-day exposures	ApoE knockout mouse model (high-fat/high-cholesterol diet); 50-day exposure
Biologic endpoints	Cardiovascular effects and markers of oxidative stress and inflammation	Vascular effects and markers of oxidative stress and inflammation
Animal and cell culture exposures	Concentrated ambient particles (in vivo) and ambient particles collected on filters (in vitro and in vivo); five air sheds	Laboratory-generated complex mixtures: combinations of mixed vehicle emissions and non-vehicular primary particles (in vivo)

Abbreviations: ApoE indicates apolipoprotein E; CSN, Chemical Speciation Network; EC, elemental carbon; EPA, U.S. Environmental Protection Agency; IMPROVE, Interagency Monitoring of Protected Visual Environments; MSA, metropolitan statistical area; OC, organic carbon; PM, particulate matter.

On the other hand, sulfate (measured as elemental sulfur) is well captured by the CSN. Sulfur concentrations are typically well above detection limits, are measured with relatively high certainty, and have relatively low spatial variability. Therefore, exposure measurement error associated with sulfate is expected to be low. Selenium, arsenic, vanadium, and nickel, which are key components for identifying coal-burning and fuel-oil combustion, are often below the limit of detection in the CSN database. The low concentrations of those pollutants, which have been

decreasing over the past decades, hinder assessment of how they might be linked to health impacts. However, as reported by the Lippmann team in the current and prior studies, in some locations (notably New York City) concentrations of vanadium and nickel are sufficiently high that it has been possible to identify associations of these elements with health outcomes. However, new local regulations in New York City that address fuels used for residential heating are expected to reduce concentrations of nickel and vanadium in ambient air.

LINKING PM COMPONENTS AND SOURCES TO HEALTH OUTCOMES

For their epidemiologic analyses, the two NPACT teams adopted somewhat different philosophies on the use of source apportionment to link health outcomes to PM components. The Lippmann team relied heavily on a source apportionment approach that they had developed previously to link source categories directly to health outcomes in their epidemiologic analyses, whereas the Vedal team used source apportionment to assist in the interpretation of their health effects estimates and to support their focus on OC, EC, silicon, and sulfur as markers of specific sources in their analyses of health outcomes. An underlying question is which approach provides better information about which sources of PM components most affect health risks: Is it better to use source apportionment results, which may represent more accurately the combined effects of multipollutant atmospheres, but which require more effort and introduce additional uncertainties and assumptions, or is it better to simply use individual components that are typically linked to one or more specific sources? Each approach has its strengths, and there are strong reasons to use either method or both methods (as was done by the Lippmann team).

The Panel noted that all current source apportionment approaches (see the Source Apportionment sidebar in the Commentary) introduce uncertainty (Balachandran et al. 2012). Although some approaches may decrease uncertainty by reducing temporal variability, other approaches that produce source categories may increase temporal variability as compared with approaches using concentrations of individual components. For some approaches those potential errors can be quite large. In their analyses using an approach based on factor analysis methods that they had developed previously, the Lippmann team found differences among locations in terms of which components contributed to similar source categories, providing indications that source emissions vary spatially, that the factor analytic approaches are sensitive to measurement uncertainties, that there are temporal variations in the composition of the emissions, and that other factors may add uncertainty to this approach. Two of the limitations noted by the Panel were that the investigators did not account for how uncertainties in the component measurements affect the certainty of the source categories and that many of the concentrations were below the MDL. How their results might differ from those obtained using a different source apportionment technique and what the effect would have been of including measurement uncertainties and MDLs in the analyses remain unknown. Furthermore, it is not apparent which

chemical components drive the associations between source categories and key health outcomes in the Lippmann report (which is a different issue from determining which components are contained in the source categories that they identified). It was reassuring, however, that the Lippmann team came to consistent interpretations when they did include individual components in their analyses. We refer readers to the Commentary accompanying this report for a more detailed discussion of these issues.

The Vedal team applied positive matrix factorization (PMF), a widely used source apportionment approach, to support their focus on EC, OC, silicon, and sulfur as key components in their analyses of health outcomes. The Panel thought that their approach was defensible. The PMF factors they identified were reasonably consistent with what was expected in terms of sources and were also generally consistent with the source apportionment results of the Lippmann team. However, it would be of interest to compare the PMF results of the Vedal team directly with the source apportionment results of the Lippmann team in those cities that the two studies had in common.

The Panel thought that the question of how (or whether) to use source apportionment to identify which PM components have strong associations with adverse health outcomes is an important one. It is generally preferable to use both source categories and component concentrations directly in the health analyses, if the study design permits, with a focus on examining consistencies and differences between the two approaches. When source apportionment results are used for health analyses, researchers should recognize, discuss, and — if possible — address the uncertainties introduced by this method.

ESTIMATING EXPOSURE USING AIR QUALITY DATA

The Lippmann team approached the estimation of exposure from measured air pollutant concentrations in a straightforward fashion; they assumed that the monitored concentrations (or source apportionment results estimated for each city based on a single monitor or a few central monitors) can be used directly, with little additional spatial modeling to account for spatial gradients (e.g., variation due to different land uses and activities). The Vedal team, on the other hand, developed a more elaborate spatiotemporal exposure model, which estimated exposures at the individual level (i.e., the outdoor concentrations at participants' residences) for the Multi-Ethnic Study of Atherosclerosis (MESA) cohort. This approach was made possible by the intensive, dedicated monitoring conducted by the team in the six cities of the MESA study. The Vedal team also constructed a national spatial exposure model,

which also estimated component concentrations at participants' homes for their analyses of both the MESA cohort and the Women's Health Initiative Observational Study (WHI-OS) cohort.

The Panel thought that the initial formulation of the approach by the Vedal team was promising. However, the Panel noted that there were challenges associated with estimating EC and OC concentrations at the individual level. For instance, there were only small differences between EC concentrations measured at roadside locations and those at urban background locations, raising questions about the ability of the spatiotemporal model to accurately assign exposure at participant residences. The Panel identified additional concerns with the approach used by the Vedal team (as discussed in the Commentary accompanying the Vedal report, HEI Research Report 178), such as the varying R^2 values for the different components across the models (an indication of model accuracy in model validation) and the potential loss of volatile components over the longer sampling period of 2 weeks. At the same time, the Panel noted the more general challenge facing the primary alternative to such spatiotemporal modeling, which is the reliance on observations from just a few sites to characterize potential populationwide intra-urban exposures to pollutants such as EC, OC, and other primary pollutants (in much the same way the Lippmann team proceeded). Although using one or a few sites to characterize individual and populationwide exposures to certain secondary PM components, such as sulfate, may be sufficiently accurate, using this approach to estimate exposures to primary pollutants — such as metals — introduces larger uncertainties, potentially biasing the results.

SINGLE-POLLUTANT AND MULTIPOLLUTANT MODELS

When associations of $PM_{2.5}$ components and health outcomes are analyzed in single-pollutant models, potential interactions or high correlations between components could affect the analysis and lead to misidentification of which pollutants may be most strongly associated with the observed human and animal health effects. Furthermore, other constituents of inhaled atmospheres — such as gaseous pollutants — might complicate assessment of which associations may be causally related. The Lippmann team attempted to address these issues by employing source apportionment in all of their studies, two-pollutant models in time-series analyses in which they controlled for $PM_{2.5}$ mass, and a total-risk-impact approach in their cohort study. The Vedal team made simple comparisons between the results for individual components and those for

$PM_{2.5}$ mass in their epidemiologic study and carried out sensitivity analyses involving two-pollutant models. They performed a more sophisticated analysis (i.e., a multiple additive regression tree [MART] analysis) in their toxicologic study (the Campen study), in which they related the hundreds of compounds measured in their complex exposure atmospheres to biologic markers. Although the Panel appreciated the efforts of both NPACT teams, they concluded that any future research using PM component data needs to more directly address appropriate analyses for multipollutant atmospheres in the statistical design.

APPROACHES TO ANIMAL INHALATION EXPOSURES

The two NPACT teams exposed apolipoprotein E (ApoE) knockout mice to exposure atmospheres with pollutant concentrations that were by design higher than typical North American ambient concentrations, although such concentrations can be found in developing countries or occupational settings. The teams used different approaches to generate the pollutant mixtures, making it possible to compare responses to concentrated ambient PM and predetermined laboratory mixtures in a similar animal model. The Lippmann team (specifically the Chen study) used concentrators that pass ambient air through a cyclone that excludes particles larger than 2.5 μm , and then through a virtual impactor that concentrates particles between about 0.1 and 2.5 μm . The system does not exclude (or concentrate) gaseous pollutants or particles smaller than 0.1 μm (ultrafine PM). Thus, the resulting concentrated ambient particles (CAPs) exposure atmosphere is similar in pollutant composition to the ambient air, but the mixture is altered in terms of both particle concentration and relative composition. The Panel noted that this is an appropriate approach given the focus on PM components in the NPACT Initiative and the fact that much of the mass of ambient PM is within the size range ($PM_{2.5}$) that is being concentrated and of great interest regarding its health effects. The approach used by the Vedal team in their toxicologic study (conducted at the Lovelace Respiratory Research Institute (LRRI)) was to generate controlled atmospheres by mixing diluted and cooled exhaust from a gasoline and a diesel engine to provide a base pollutant mixture (i.e., mixed vehicular emissions, or MVE) and then removing PM from the mixture or adding different types of PM. This approach was driven by their general focus on PM components derived from traffic (vehicular) sources for both the epidemiologic and toxicologic studies. The Lippmann team measured about 30 components in the CAPs atmospheres, whereas LRRI measured close to 500 compounds (metals

and many organic compounds in the particle and gas phases) in their complex exposure atmospheres.

The inhalation exposures at LRRRI did not include secondary PM components that are formed by atmospheric processes (e.g., secondary organic aerosols). However, sulfate and nitrate ions, which are major PM components in ambient air, were added as primary particles, allowing the team to investigate the health effects of exposure to those components. In a typical city, secondary sulfate particles would form by oxidation of gaseous sulfur dioxide emissions from coal or oil burning, whereas secondary nitrate particles would be formed by oxidation of nitrogen oxides emitted by vehicles and other combustion sources. A unique feature of the Campen study was the addition of road dust particles in the fine fraction. In contrast, the animal exposure atmospheres used in the Chen study included secondary aerosols by design, although the extent to which this occurred likely varied by location (the West Coast of the United States versus the East Coast versus the Midwest). Exposure mixtures for both studies contained PM: at LRRRI, from engine emissions or added nitrate, sulfate, and road dust; for the Lippmann study, from general traffic sources. Gaseous pollutants in engine exhaust were included or excluded by design at LRRRI, and ambient gaseous pollutants were present by default (but not concentrated) in the CAPs exposures in the Chen study. In addition to the animal inhalation exposures in the two studies, the Lippmann team also used intratracheal aspiration of particles collected on filters (in the Gordon study), which allowed them to investigate the differences in biologic responses in mice exposed to different PM size ranges. This approach excluded gaseous components altogether. The investigators analyzed endotoxin content of the filter samples and elemental composition, but did not analyze OC, EC, or other organic compounds.

Because the Lippmann team did not use specific source mixtures for the exposures but conducted inhalation studies in five locations with different ambient air pollution mixtures, they conducted source apportionment to link their exposures back to source categories, such as emissions from mobile and stationary sources. Therefore, the animal exposure strategies of both teams had the potential to link biologic endpoints to similar types of sources, such as traffic, power generation, and dust, as well as to secondary aerosols (sulfates and nitrates). Furthermore, the parallel epidemiologic studies used similar markers for mobile-source emissions (EC and OC), although the source apportionment methods typically used in epidemiologic studies encounter difficulties in separating PM derived from gasoline engines from PM derived from diesel engines based on EC and OC concentrations.

The Panel thought that MVE was a reasonable representation of mobile source emissions for toxicologic studies that allowed a more direct comparison of the toxicologic results with epidemiologic results for non-source-specific estimates of traffic-related exposures. On the other hand, the sulfate added to the MVE exposures at LRRRI was a primary rather than secondary particle and did not include other components (e.g., selenium, arsenic, vanadium, or nickel) that are often found in emissions from sources that emit sulfur dioxide, and was thus less representative of real-world conditions.

COMPARING KEY FINDINGS ACROSS THE STUDIES

This section discusses the main findings in terms of what sources and PM components the teams found to play a role in the health outcomes they assessed, looking for consistency across the epidemiologic and toxicologic studies within and across the two main NPACT studies. Overviews of the main findings of the epidemiologic and toxicologic studies are presented in Synthesis Table 2 and Synthesis Table 3, respectively.

The Lippmann team's time-series study (the Ito study) identified a fairly large number of PM components associated with daily hospitalizations due to cardiovascular disease (CVD) and daily all-cause and CVD mortality. Source categories attributed to primary vehicle exhaust and secondary sulfate aerosols were found to be important in some of these short-term associations. The long-term American Cancer Society cohort study (the Thurston Study) also identified a number of PM components that could explain some of the mortality associations, including EC and sulfur. However, OC, silicon, and potassium (a marker for biomass combustion) were not associated with mortality in the cohort study. Source categories attributed to coal combustion and traffic pollution were found to be important in the associations with long-term effects, whereas little evidence was found for associations with source categories attributed to crustal sources or biomass combustion. There was minimal overlap between the PM_{2.5} components associated with short-term responses and those associated with long-term responses. Results for metals varied, but many effect estimates were highly uncertain (i.e., the confidence intervals were large), possibly due to the limited number of measurements above the limit of detection for metallic components in many cities.

The Vedal epidemiologic study focused primarily on EC and OC as markers of vehicle exhaust and other combustion emissions, on OC also as a marker of secondary organic

Synthesis Table 2. Approaches and Key Findings of the Epidemiologic Studies^{a,b}

	New York University / Ito	New York University / Thurston	University of Washington / Vedal ^c
Study design	Time series (short-term)	Cohort (long-term)	Cohort (long-term)
Population Cities Participants	U.S. MSAs 150 Cities Population >100 million	ACS CPS-II cohort 100 Cities ~450,000 People	MESA 6 Cities ~6,800 People
Health endpoints	Hospitalization Mortality	Mortality	Time to first event (MI, stroke, cardiac procedures, and CVD deaths)
PM components and source categories associated with health outcomes	Cold season: PM _{2.5} , NO ₂ , CO, EC, OC, and Cu Modified ^d by PM _{2.5} ; Cu, Ni, and V Sources: Vehicle exhaust	Warm season: PM _{2.5} , As, Se, sulfur, Cl, Fe, Pb, and EC Not associated: OC, silicon, and K Source Categories: Coal combustion and possibly traffic; little evidence for soil or biomass combustion	Best evidence for OC and sulfur; little evidence for EC or silicon; some evidence for Cu

^a Abbreviations: ACS CPS-II indicates American Cancer Society Cancer Prevention Study II; As, arsenic; CAC, coronary artery calcification; Cl, chlorine; CIMT, carotid intima-media thickness; CO, carbon monoxide; Cu, copper; CVD, cardiovascular disease; EC, elemental carbon; Fe, iron; K, potassium; MESA, Multi-Ethnic Study of Atherosclerosis; MI, myocardial infarction; MSA, metropolitan statistical area; Ni, nickel; NO₂, nitrogen dioxide; NO₃, nitrate; OC, organic carbon; Pb, lead; PM, particulate matter; PM_{2.5}, particulate matter ≤ 2.5 μm in aerodynamic diameter; Se, selenium; V, vanadium; WHI-OS, Women's Health Initiative Observational Study.

^b Epidemiologic studies at New York University were headed by George Thurston and Kazuhiko Ito and at the University of Washington by Sverre Vedal.

^c Source apportionment results supported the focus on four main components for evaluation in the Vedal study: EC and OC as markers of vehicle exhaust and other combustion emissions; OC also as a marker of secondary organic aerosols; silicon as a marker of crustal PM; and sulfur as a marker of secondary PM.

^d Risk estimates for these components were substantially modified when PM_{2.5} was included in a two-pollutant model.

Synthesis Table 3. Approaches and Key Findings of the Toxicologic Studies^{a,b}

	New York University / Chen	New York University / Gordon	University of Washington / Campen
Study design	Short-term (daily time-series)	Long-term (6 months)	Medium-term (50 days)
Exposures	Inhalation of CAPs at five locations (Manhattan, Tuxedo, East Lansing, Seattle, and Irvine)	Aspiration of PM collected on filters at five locations (Manhattan, Tuxedo, Ann Arbor, Seattle, and Los Angeles area); three size fractions	Inhalation of combinations of MVE or MVE gases with non-vehicular PM _{2.5} (sulfate, nitrate, or road dust)
PM concentrations	60–138 µg/m ³	In vitro: 50–100 µg/mL In vivo: 50 µg	100 or 300 µg/m ³
Model	ApoE knockout mice (normal diet)	FVB/N mice; epithelial cells, endothelial cells, cardiomyocytes	ApoE knockout mice (high-fat/high-cholesterol diet)
Biologic endpoints	Heart rate and heart rate variability	Mice: lung inflammation Cells: viability, ROS, inflammatory markers, beat frequency	Aorta: lipid peroxidation, vascular function and remodeling, plaque growth and inflammation
PM components and sources associated with biologic findings	Effects after exposure to Ni (residual oil combustion) > to Al, EC, and P (traffic) > to sulfur (coal combustion) > to PM _{2.5}	Complex interaction of particle size and composition (location and season); nothing ruled out	Effects after exposure to MVE > to MVE gases; fewer effects of nitrate and sulfate; no effects of road dust Effects after exposure to non-vehicular PM combined with MVE > non-vehicular PM without MVE

^a Abbreviations: Al indicates aluminum; CAPs, concentrated ambient particles; MVE, mixed vehicular engine emissions; Ni, nickel; P, phosphorus; PM, particulate matter; ROS, reactive oxygen species.

^b Toxicologic studies at New York University were headed by Lung-Chi Chen and by Terry Gordon and for the University of Washington study by Matthew Campen at the University of New Mexico and Jacob McDonald at the Lovelace Respiratory Research Institute.

aerosol, on silicon as a marker of crustal PM, and on sulfur as a marker of secondary PM. Results suggested that OC and sulfur were associated with several of the endpoints studied, but EC and silicon were not. The Panel agreed with the investigators that this suggests that traffic-related pollution and secondary PM could be playing a role in PM toxicity.

The Lippmann team's animal inhalation study (the Chen study) showed that a large number of components were positively or negatively associated with acute changes in heart rate and heart rate variability in mice. When the investigators tried to rank these components, they concluded that nickel, aluminum, EC, phosphorus, and sulfur had stronger associations with the cardiac endpoints than did PM_{2.5} mass. Effects of CAPs exposures on plaque progression in mice were primarily seen at Tuxedo, New York, Manhattan, New York, and East Lansing, Michigan, where the investigators deemed pollution mixtures to be more influenced by coal-fired power plant emissions than at Irvine, California, and Seattle, Washington. The Lippmann teams' *in vitro* and *in vivo* study of PM collected on filters (the Gordon study) found that PM size and composition (determined by location and season) played a complex role in PM toxicity. The Panel noted that no size classes or components could be ruled out.

The toxicologic study conducted at LRRI (the Campen Study) used laboratory-generated atmospheres based on MVE and MVE gases combined with non-vehicular PM. Several combinations of particles and gases were found to affect different biologic markers in aortic tissues. The whole MVE mixture produced the largest changes, with MVE gases producing smaller and fewer changes. Fewer effects were observed with primary nitrate and sulfate particles, and none with fine road dust particles. Combining non-vehicular PM with MVE gases increased the effects over non-vehicular PM alone, but generally did not exceed the effects of MVE by itself. Thus there was little evidence of a more-than-additive effect when exposure atmospheres were combined. The results support the role of both particulate and gaseous components in the induction of various cardiovascular outcomes, but whether there are important particle-gas interactions remains unclear and requires further research.

REFLECTIONS ON THE MAIN FINDINGS

Both the Lippmann and Vedal studies found that adverse health outcomes were consistently associated with sulfur and sulfate (markers primarily of coal and oil combustion) and with traffic-related pollutants, although the relative importance of the latter remains unclear because exposure to traffic-related pollutants varies within metropolitan areas and thus is more subject to uncertainty than exposure to

pollutants from other source categories. On the other hand, there were only small differences in EC concentrations measured at roadside locations compared with urban background locations, indicating either spatial homogeneity in concentrations or, as noted above, potentially high measurement error for EC due to the 2-week sampling protocol. The results for sulfur and sulfate may have been more consistent because their concentrations were more accurately estimated (due to their spatial homogeneity) than were concentrations of other pollutants.

Biomass combustion, crustal sources, and related components were not generally associated with short- or long-term epidemiologic findings in these studies, but there were only a few cities where these sources (and their attributed components) were likely to be measured consistently. The possibility remains that biomass combustion contributed to OC concentrations, and thus to the associations reported for OC and cardiovascular outcomes. There were few consistent associations with other components or sources, although the Panel cautioned that is not conclusive evidence that these components and sources do not have adverse health effects. Further analyses of some of these sources are warranted.

With regard to the association of health effects with EC compared with those associated with OC, the differences in findings between the Lippmann and Vedal studies are surprising. In typical urban environments, mobile sources are expected to be the major source of EC and important contributors to OC. It is noteworthy that these studies report such prominent differences between the results for EC and OC, given the strong correlation between the two in many cities. Again, these differences may be due to the stronger spatial gradients between cities for OC than for EC, the exposure models and study designs, or the difficulties involved in measuring OC and EC.

One limitation of the CSN is that it is by design focused on PM_{2.5}, while it is becoming increasingly clear that coarse PM remains of interest. For example, the Lippmann team's *in vitro* and *in vivo* toxicologic evaluations (in the Gordon study) found stronger associations per unit mass between coarse PM, which is often associated with dust, and certain biologic endpoints than for fine PM. However, associations of silicon, a marker for dust, with health effects or clinical markers in the epidemiologic studies were often fairly weak (with the exception of CIMT in the Vedal epidemiologic study), as would be expected.

Both studies highlight how important the CSN is to research on the health effects of components of air pollution and to air quality management. Neither study could have been performed without CSN data, although the studies highlighted some limitations that suggest that further efforts

would be helpful to characterize EC, OC, and metals (i.e., combustion- and traffic-related components); to lower the detection limits of some components; and to collect daily measurements. In summary, the Panel concluded that — except for the fairly consistent associations of many of the health outcomes with sulfur and sulfate, which may, in part, be due to better exposure assessment — associations with other components were mixed, and linkages to sources were not definitive.

How do these two major studies compare with the published literature? Quite a few investigators have performed smaller-scale studies and analyses to identify which PM components and sources are associated with a variety of adverse health outcomes. Not surprisingly, the results of those studies have been mixed, if only because of the differences in the selection of PM components and health outcomes of interest, study time frames (short- and long-term), and the imprecision of estimates because of the difficulties in obtaining truly large data sets on PM composition and sources.

In the third NPACT study (see footnote on page 293), Bell (2012) used daily Medicare hospitalization data to evaluate the effects of short-term exposures to various components of the PM_{2.5} mixture on daily morbidity. She focused on the average values of seven PM_{2.5} components (those accounting for $\geq 1\%$ of PM_{2.5} mass in the GSN) in 187 U.S. counties, using national, regional, and seasonal models. For her all-year analysis of the entire United States, Bell reported strong and statistically significant increases in the association between cardiovascular hospitalizations and an interquartile range increase in EC, nickel, and vanadium (Bell 2012).

It is beyond the scope of this summary to provide a detailed review of the literature on the health effects of PM components and sources. A recent systematic review of the findings of animal toxicology, human chamber, and field epidemiology studies (Stanek et al. 2011) presents results from five epidemiologic studies on total mortality (see Table 3 of that paper), which among them found that soil, sea salt, local sulfur dioxide, secondary sulfate, motor vehicle emissions, coal burning, wood smoke, biomass combustion, copper smelter emissions, residual oil combustion, and incinerator emissions were associated with health outcomes. This is just one illustration of the variety of results reported in the literature.

Together, the two studies discussed here, as well as the study by Bell, follow the conclusion of Stanek and colleagues (2011) that “apportionment methods have linked a variety of health effects to multiple groups of PM components and sources of PM, but the collective evidence has

not yet isolated factors or sources that would be closely and unequivocally related to specific health outcomes.”

Overall, this comprehensive and ambitious research program has shown that research on the toxicity of PM components is not likely to easily identify a single culprit PM component or source category or to identify a unique set of biomarkers that could be reliably used to monitor exposure. More work remains to be done to refine statistical methods for simultaneous modeling of multiple pollutants; to improve the representation of spatial contrasts in component concentrations, especially within cities; and to improve source identification and attribution. Further toxicologic studies are needed to connect particle components with physiologic mechanisms, to study the relative toxicity of particles and gaseous pollutants, to study atmospheric aging of complex mixtures to better reflect real-world conditions, and to provide more insight into the role of PM_{2.5} components in causing tissue injury and dysfunction.

The NPACT studies, which are to date the most systematic effort to combine epidemiologic and toxicologic analyses of these questions, found associations of secondary sulfate and, to a somewhat lesser extent, traffic sources with health effects. But the Panel concluded that the studies do not provide compelling evidence that any specific source, component, or size class of PM may be excluded as a possible contributor to PM toxicity. If greater success is to be achieved in isolating the effects of pollutants from mobile and other major sources, either as individual components or as a mixture, more advanced approaches and additional measurements will be needed so that exposure at the individual or population level can be assessed more accurately. Such enhanced understanding of exposure and health will be needed before it can be concluded that regulations targeting specific sources or components of PM_{2.5} will protect public health more effectively than continuing to follow the current practice of targeting PM_{2.5} mass as a whole.

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ABBREVIATIONS AND OTHER TERMS

ApoE	apolipoprotein E
CAPs	concentrated ambient particles
CIMT	carotid intima-media thickness
CSN	Chemical Speciation Network
CVD	cardiovascular disease
EC	elemental carbon
EPA	Environmental Protection Agency
LRR	Lovelace Respiratory Research Institute
MART	multiple additive regression tree
MDL	minimum detection limit
MESA	Multi-Ethnic Study of Atherosclerosis
MVE	mixed vehicular engine emissions
NPACT	National Particle Component Toxicity (Initiative)
OC	organic carbon
PM	particulate matter
PM _{2.5}	particulate matter ≤ 2.5 μm in aerodynamic diameter
PMF	positive matrix factorization
RFA	request for applications
WHI-OS	Women's Health Initiative Observational Study

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