RESEARCH REPORT

HEALTH EFFECTS INSTITUTE

Number 138
February 2009

PRESS VERSION

Health Effects of Real-World Exposure to Diesel Exhaust in Persons with Asthma

Junfeng (Jim) Zhang, James E. McCreanor, Paul Cullinan, Kian Fan Chung, Pamela Ohman-Strickland, In-Kyu Han, Lars Järup, and Mark J. Nieuwenhuijsen

Includes a Commentary by the Institute's Health Review Committee

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with a Commentary by the HEI Health Review Committee

Research Report 138

Health Effects Institute

Boston, Massachusetts

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Publishing history: The Web version of this document was posted at *www.healtheffects.org* in February 2009 and then finalized for print.

Citation for whole report:

Zhang J, McCreanor JE, Cullinan P, Chung KF, Ohman-Strickland P, Han I-K, Järup L, Nieuwenhuijsen MJ. 2009. Health Effects of Real-World Exposure to Diesel Exhaust in Persons with Asthma. Research Report 138, Health Effects Institute, Boston, MA.

When specifying a section of this report, cite it as a chapter of this document.

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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 research and analyses to public and private decision makers.

HEI 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 certain research programs. 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.

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 138, *Health Effects of Real-World Exposure to Diesel Exhaust in Persons with Asthma,* presents a research project funded by the Health Effects Institute and conducted by Junfeng (Jim) Zhang of the University of Medicine and Dentistry of New Jersey–School of Public Health and his colleagues. This report is part of an HEI research program designed to study the effects of diesel exhaust and other particles on human health. See the Preface at the beginning of the report for more information about this program.

HEI Research Report 138 contains three main 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 Health Review Committee's comments on the study.

The Investigators' Report, prepared by Zhang et al., describes the scientific background, aims, methods, results, and conclusions of the study.

The Commentary is prepared by members of the Health Review Committee with the assistance of HEI staff; it 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.

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. This draft report is first examined by outside technical reviewers and a biostatistician. The report and the reviewers' comments are then evaluated by members of the Health Review Committee, an independent panel of distinguished scientists who have no involvement in selecting or overseeing HEI studies. During the review process, the investigators have an opportunity to exchange comments with the Review Committee and, as necessary, to revise their report. The Commentary reflects the information provided in the final version of the report.

PREFACE

HEI's Research on Particles and the Exacerbation of Allergy and Asthma

Particles emitted from a number of sources (e.g. diesel and gasoline vehicles, electric power plants, and factories), and formed from gases also emitted from these sources, contribute to the exposure of urban populations to particulate matter (PM). Epidemiologic and experimental studies published in the 1990s suggested the possibility of adverse effects on the respiratory and immune systems of people exposed to diesel exhaust and other particles on a short-term basis. In particular, these effects could be more severe in persons with asthma and other allergic diseases. Some studies raised the question of whether various constituents of traffic-generated air pollution (including diesel exhaust particles) might contribute to an increase in symptoms of asthma or rhinitis or might enhance immune responses to allergens. However, the epidemiologic studies did not measure ambient concentrations of individual pollutants, and many of the experimental studies did not use relevant exposure routes and particle doses. In reviewing this literature, the HEI Health Research Committee noted that additional research with improved measures of exposure and outcomes was needed to elucidate dose–response relationships extending to relevant exposure concentrations and mechanisms of exposure in individuals at risk.

In 2000, HEI issued Request for Applications (RFA) 00-1, *Effects of Diesel Exhaust and Other Particles on the Exacerbation of Asthma and Other Allergic Diseases*. The RFA sought studies that would provide a better understanding of whether, and by what mechanism, particles from diesel engines and other sources may contribute to the frequency and severity of asthma attacks and to the exacerbation of allergic responses — especially in susceptible population groups such as children, older people, and people with chronic diseases. The Committee was interested in research proposals for epidemiologic studies, studies of controlled exposure in humans, and animal studies.

RFA 00-1 was part of a larger HEI effort to study the effects on human health of exposure to current and future diesel particulate emissions and to investigate how the toxicity of diesel particles compares with that of other ambient particles. HEI's earlier efforts included a comprehensive review of the health effects associated with diesel emissions (HEI Diesel Working Group 1995) and a project initiated in 1998 to examine more closely the epidemiologic studies used in quantitative risk assessment (HEI Diesel Epidemiology Expert Panel 1999).

Four studies have been funded under RFA 00-1; one is presented in this Research Report, one was published in 2008, one is in press, and one is continuing. All four are described briefly below. For additional information on this research program, see the HEI Program Summary *Research on Diesel Exhaust and Other Particles* (2003).

"Health Effects of Diesel Exhaust in Asthmatics: A Real-World Study in a London Street," Junfeng Zhang, University of Medicine and Dentistry of New Jersey–School of Public Health (Principal Investigator)

The study by Junfeng (Jim) Zhang and colleagues that is presented in this report (HEI Research Report 138, *Health Effects of Real-World Exposure to Diesel Exhaust in Persons with Asthma*) is the second to be completed and published (Zhang et al. 2009). The investigators used two locations in London, United Kingdom, to study the effects of a real-world exposure to urban diesel traffic in persons with mild or moderate asthma. The study tested the hypotheses that exposure to diesel exhaust leads to worsening of

asthma symptoms, with a reduction in lung function and evidence of increased oxidative stress and inflammation, and that such effects are dependent on the severity of asthma. Each subject participated in an experimental exposure session and a control exposure session by walking in each site for two hours while portable monitors determined concentrations of selected air pollutants. The exposure site was a busy street (Oxford Street) where motor-vehicle traffic was restricted to taxis and buses, the majority of which were powered by diesel engines; the control site was a nearby park with no motor-vehicle traffic (Hyde Park). The subjects' asthma symptoms, pulmonary function, and indicators of inflammation and oxidative stress were subsequently measured.

"The Relationship Between Pollutant Particles in Alveolar Macrophages from Normal Children and Proxy Markers of PM_{10} Exposure," Jonathan Grigg, University of Leicester, Leicester, United Kingdom (Principal Investigator)

The study by Grigg and colleagues examined whether the detection of PM (which was not specific to diesel) in macrophages obtained from sputum could be used as a biomarker of PM exposure in children. The investigators also explored the relationship between PM load in macrophages and markers of inflammation in children's lungs. This study was published as HEI Report 134, *Black-Pigmented Material in Airway Macrophages from Healthy Children: Association with Lung Function and Modeled PM_{10}* (Grigg et al. 2008).

"Fine Airborne Particles and Allergic Diseases," Jack Harkema, Michigan State University (Principal Investigator)

The study by Harkema and colleagues focuses on the effect of whole diesel exhaust and concentrated ambient particles on the induction (sensitization phase) and exacerbation (challenge phase) of antigen-induced epithelial remodeling and inflammation in Brown Norway rats. The underlying hypothesis is that inhalation of particles during either sensitization or challenge causes airway remodeling and exacerbates airway inflammation. This study has been completed and is expected to be published in 2009.

"Exacerbation of Allergic Inflammation in the Lower Respiratory Tract by Diesel Exhaust Particles," David Diaz-Sanchez, University of California–Los Angeles School of Medicine (original Principal Investigator); Richard Effros, Los Amigos Research and Education Institute (current Principal Investigator)

The goal of the ongoing study by Effros and colleagues is to test two hypotheses in allergic asthmatic subjects: first, that inhaled diesel exhaust particles alter lung function and some immunologic responses; and second, that inhaled diesel particles act synergistically with an allergen (cat dander) to exacerbate allergic immune responses. The study compares the effects of exposure to whole diesel exhaust and to NO_2, which is a major component of the gas phase. The research part of this study has been completed. The final report will be submitted to HEI in April 2009.

While the studies funded by this RFA were in progress, lower PM emission standards for diesel engines were introduced, which are expected to result in lower concentrations of several pollutants in the ambient air. As part of an effort to assess how emissions and health effects of diesel engines may be changing with the introduction of new technologies developed to meet the new standards, HEI initiated the Advanced Collaborative Emissions Study (ACES), in collaboration with the Coordinating Research Council. The study has two main components: the characterization of the emissions from four heavy-duty diesel engines meeting the 2007 PM standards (completed) and a chronic inhalation bioassay in rodents to evaluate both short- and long-term health effects of the emissions of one of the four engines. As the new diesel fleet slowly replaces older models, it will also be important to document the associated changes in air quality and health.

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HEI STATEMENT

Synopsis of Research Report 138

Health Effects of Real-World Exposure to Diesel Exhaust in Persons with Asthma

BACKGROUND

In the 1990s, results from several epidemiologic and controlled-exposure studies suggested an association between exposure to air pollution from traffic-derived particulate matter (PM) and increases in symptoms of airway diseases, including exacerbations of asthma. Some results also suggested that exhaust from diesel engines, used in a large fraction of vehicles worldwide and particularly in Europe, contributed to these effects. In response to HEI RFA 00-1, *Effects of Diesel Exhaust and Other Particles on the Exacerbation of Asthma and Other Allergic Diseases,* Dr. Junfeng (Jim) Zhang of the University of Medicine and Dentistry of New Jersey, proposed a study that would investigate how inhaling air with a high concentration of diesel exhaust from vehicular traffic while walking on a street in Central London, United Kingdom, might affect people who had either mild or moderate asthma. Dr. Zhang and his colleagues hypothesized that this exposure would exacerbate asthma symptoms, decrease lung function, and induce lung inflammation and oxidative stress responses. The HEI Research Committee recommended Dr. Zhang's proposal for funding.

APPROACH

The investigators recruited 60 nonsmoking participants of both sexes between 18 and 55 years old, with mild or moderate asthma. Each subject participated in one 2-hour exposure session by walking along Oxford Street, a busy Central London thoroughfare where vehicles are predominantly diesel-powered, and one 2-hour session walking at a nearby control site, Hyde Park, where there is no traffic.

Before, during, and after each session, the investigators evaluated pulmonary function parameters (forced expiratory volume in the first second [FEV_1], forced vital capacity [FVC], and forced expiratory flow during the middle half of the FVC [$FEF_{25–75}$]) and asthma symptoms. At some time points Zhang and colleagues also evaluated bronchial reactivity (PC_{20}) and markers of airway inflammation and oxidative stress. These markers included exhaled nitric oxide, the pH of exhaled breath condensate (EBC), blood thiobarbituric acid reactive substances (TBARS), sputum interleukin-8 and myeloperoxidase (MPO). Participants kept a record of asthma symptom scores, peak expiratory flow rate (PEFR), and asthma reliever medication use during the 7 days before and after each session.

Pollutant samplers, placed on a cart that accompanied the participants during sessions, measured concentrations of $PM_{2.5}$ ($PM \leq 2.5$ µm in aerodynamic diameter), ultrafine particles (UFP; PM < 100 nm in aerodynamic diameter), elemental carbon (EC), and nitrogen dioxide (NO_2).

STATISTICAL METHODS

Zhang and colleagues used two main statistical approaches: comparative analyses between the exposure and control sessions that took advantage of the paired design of the study to compare within-subject responses, and pollutant-specific exposure–response analyses that estimated associations between the concentration of an individual pollutant and a change in a health endpoint from its baseline value. In one set of pollutant-specific exposure–response analyses, each of the four pollutants was used as a single covariate; a second set of analyses used two of the four pollutants as covariates. They analyzed responses for all subjects, and also analyzed responses separately for participants with either mild or moderate asthma.

RESULTS

The investigators found that participants were exposed to higher average pollutant concentrations

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Junfeng Zhang, University of Medicine and Dentistry of New Jersey–School of Public Health. Research Report 138 contains both the detailed Investigators' Report and a Commentary on the study prepared by the Institute's Health Review Committee.

during the exposure session than during the control session: approximately 5-fold higher EC, 4-fold higher NO_2, 3.5-fold higher UFP, and 2-fold higher $PM_{2.5}$ mass.

FEV_1 and FVC were significantly lower after the exposure session compared with the control session (stratified analyses showed that these effects were dominated by responses in participants with moderate asthma). However, $FEF_{25–75}$ and PEFR did not differ significantly. Also not affected by exposure were asthma symptom scores, asthma medication use, PC_{20}, and blood TBARS; some of these responses showed nonsignificant trends. A significant relative decrease in EBC pH of 2.0% (correlating with an approximate half-log change in pH) was noted one hour after the exposure session; this effect was also dominated by observations in subjects with moderate asthma. Of the sputum parameters evaluated, only the neutrophil-associated enzyme MPO differed significantly with a 5-fold increase after the exposure session compared with after the control session.

In one-pollutant exposure–response analyses, UFP and NO_2 were associated with changes in the most endpoints, EC with fewer, and $PM_{2.5}$ with fewer still. In two-pollutant models, several associations between the measured pollutants and changes in endpoints lost significance after the investigators controlled for other pollutants. Some associations, however, were unaffected by the inclusion of a second pollutant. Associations of UFP with endpoints were not affected by adjusting for other pollutants, except NO_2. Adjusting for NO_2 generally appeared to reduce associations with the other pollutants.

SUMMARY AND CONCLUSIONS

The study by Zhang and colleagues, with an innovative approach, has provided interesting new findings. The effects with the most potential clinical significance were the relative decreases in FEV_1 (3.0%–4.1%) and FVC (3.1%–3.7%) during and several hours after the exposure ended. The magnitude of these decrements in lung function may be clinically relevant for patients with severe or uncontrolled asthma, whose lung capacity is severely diminished compared with healthy people.

Based on the statistically significant changes in one marker each of airway inflammation (MPO) and of airway acidification (EBC pH), and in conjunction with sputum findings of marginal statistical significance, results of this study suggest that the exposure session was associated with a mild increase in inflammatory response in the airways that was mediated by neutrophils.

Asthma symptoms and the use of asthma reliever medication increased only marginally after the exposure session. Thus, whereas exposure to a diesel-traffic–enriched environment may have produced changes in pulmonary function and inflammatory endpoints, the lack of significant changes in symptoms or the use of asthma reliever medication suggests that this single exposure did not affect the clinical status of asthmatic participants.

Analyses with stratification by the severity of asthma showed that changes in FEV_1, FVC, and EBC pH were significant only in subjects with moderate asthma. Because the majority of subjects with moderate asthma were taking corticosteroids, it is possible that corticosteroid use may have blunted responses in this group. Though the background severity of asthma may be an important factor affecting responses to diesel traffic exposure, further work is needed to confirm or disprove this hypothesis.

Exploratory one- and two-pollutant analyses to identify associations between specific components of the pollutant mix and changes in endpoints found that UFP and NO_2 were associated with the most endpoints, EC with fewer, and $PM_{2.5}$ with fewer still. All these pollutants are constituents of traffic emissions and EC is frequently used as a marker of diesel emissions, but none is absolutely specific to diesel. Thus, because the pollutants measured are not specific to diesel emissions, the results are only suggestive of the effects of DE on the endpoints measured.

Explanations for the effects observed, other than exposure to DE, also need to be borne in mind. One is that participants were almost certainly concurrently exposed to air pollutants not associated with diesel-powered engines, such as pollutants derived from tailpipe emissions of gasoline-powered cars on streets that cross Oxford Street, as well as particles not derived from tailpipe emissions — such as those generated by tire and brake wear and roadway dust produced by all vehicles. In addition, concentrations of several traffic-associated pollutants (including CO, organic carbon compounds, and particles in the coarse size range) were not measured in the current study and may be associated with the endpoints evaluated. Furthermore, the exposure

and control sites differed in other, unmeasured characteristics, particularly in noise levels and the amount of stress experienced by the subjects.

Although the findings of the current study indicate that lung function is slightly decreased and some markers of airway inflammation are increased in people with asthma who are exposed to ambient urban air in a roadside environment dominated by diesel vehicles, the study does not provide direct evidence that DE itself causes these effects. Additional studies would be needed to address that question, and to identify specific components of DE that might be responsible for any observed health effects. A final consideration is that since the study was completed, more stringent emissions and fuel standards have been implemented and new engine technologies introduced in both the United States and Europe. As older vehicles are replaced in the fleet, decreases in most traffic-related pollutant concentrations can be anticipated. The health impact of these changes will need to be assessed; this study may serve as a baseline analysis for future studies on the effects of such changes.

INVESTIGATORS' REPORT

Health Effects of Real-World Exposure to Diesel Exhaust in Persons with Asthma

Junfeng (Jim) Zhang, James E. McCreanor, Paul Cullinan, Kian Fan Chung, Pamela Ohman-Strickland, In-Kyu Han, Lars Järup, and Mark J. Nieuwenhuijsen

University of Medicine and Dentistry of New Jersey–School of Public Health (J.Z., P.O.-S., I.-K.H.); Environmental and Occupational Health Sciences Institute (J.Z.); and Imperial College London (J.E.M., P.C., K.F.C., L.J., M.J.N.).

ABSTRACT

Many people, including people with asthma, experience short-term exposure to diesel exhaust (DE*) during daily activities. The health effects of such exposures, however, remain poorly understood. The present study utilized a real-world setting to examine whether short-term DE exposure would (1) worsen asthma symptoms, (2) augment airway inflammation, or (3) increase oxidative stress burdens. The study also examined exposure–response relations for several DE components and the contribution of background asthma severity to individuals' respiratory responses to DE exposure. Sixty people participated in the study; 31 had mild asthma and 29 had moderate asthma. Each participant completed an exposure and a control session. During the exposure session, participants walked for 2 hours along a heavily trafficked city street where motor vehicle access was restricted to buses and official taxicabs. These vehicles were powered by diesel engines. During the control session, participants walked for the same duration and at the same speed in a public park where motor vehicle traffic was prohibited.

The concentrations of elemental carbon (EC), NO_2, ultrafine particles (UFP), and particulate matter less than or equal to 2.5 μm in aerodynamic diameter ($PM_{2.5}$) during exposure sessions were, on average, 4.8, 4.0, 3.4, and 2.0 times higher, respectively, than during control sessions. Increases in asthma symptom score and in the daily use of asthma reliever medication within the 7-day measurement period after exposure were not significant. Some effects on lung function were statistically significant. Compared with control sessions, forced expiratory volume in the first second (FEV_1) was reduced 3.0% to 4.1%, and forced vital capacity (FVC) was reduced 2.8% to 3.7% in the 5 hours immediately after the exposure sessions. Analyses of biomarkers showed that the exposure sessions led to a significant reduction in exhaled breath condensate (EBC) pH and to significant increases in induced sputum neutrophils and myeloperoxidase (MPO). The changes in lung function indices (FEV_1, FVC, and forced expiratory flow during the middle half of the FVC [$FEF_{25–75}$]) were most consistently associated with UFP and EC exposures, whereas the changes in EBC pH were most consistently associated with NO_2 exposure. In addition, NO_2 had a significant effect on bronchial reactivity and on the amount of interleukin-8 (IL-8) in induced sputum; it also modified the UFP effect on EBC pH and the EC effect on exhaled nitric oxide (eNO). However, our findings cannot be taken as demonstrating a causal association with any measured pollutant, because the measured pollutant concentrations may simply represent the entire roadside diesel-traffic exposure that comprises not only the pollutants measured in this study but also other pollutants in the complex DE mixture and resuspended coarse particles from road dust, engine debris, and tire debris.

The effects of exposure appeared to be larger in the more severe asthmatic group for most outcomes measured. In conclusion, short-term exposure to urban roadside diesel traffic led to consistent and significant reductions in lung function, accompanied by airway acidification and neutrophilic inflammation. Our findings help to explain the epidemiologic evidence on diesel traffic health effects in persons with asthma.

This Investigators' Report is one part of Health Effects Institute Research Report 138, which also includes a Commentary by the Health Review Committee and an HEI Statement about the research project. Correspondence concerning the Investigators' Report may be addressed to Dr. Junfeng Zhang, University of Medicine and Dentistry of New Jersey–School of Public Health, 683 Hoes Lane West, Piscataway, NJ 08854.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Award CR–83234701 to the Health Effects Institute, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency, and no official endorsement by it should be inferred. The contents of this document also have not been reviewed by private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views or policies of these parties, and no endorsement by them should be inferred.

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

INTRODUCTION

Diesel engines power trucks, buses, trains, ships, and machinery used in the mining, construction, and farming industries. Over the past two decades the proportion of diesel-powered vehicles to gasoline-powered vehicles has risen sharply and continues to do so. This is especially true in Europe, where more than 25% of the 2004 passenger car fleet had diesel engines. During this time, diesel engine performance improved and diesel fuel was promoted as an environmentally friendly alternative to gasoline. Emissions from diesel engines have lower CO_2 and CO concentrations than emissions from gasoline-powered vehicles. These gases are believed to contribute to global warming. The continuing increases in fuel prices may further accelerate the sales of fuel-efficient diesel engines in Europe and elsewhere.

However, exhaust from diesel-powered vehicles has long been recognized as a major contributor to urban air pollution (HEI 1995; WHO 1996). Diesel exhaust is a complex mixture comprising both particle-phase and gas-phase chemical species. Diesel engines have been shown to generate more particles by several orders of magnitude than gasoline-powered engines of comparable size (Alander et al. 2004). Diesel exhaust particles (DEP) are both ultrafine (from several nanometers to 0.1 μm in aerodynamic diameter) and fine ($\leq$ 2.5 μm in aerodynamic diameter), and thus are readily respirable and capable of being deposited in airways and alveoli (Parnia et al. 2002). UFPs may be able to pass through lung tissue and enter the blood stream (Oberdörster et al. 2005). The EC content in DEP is high (typically 60%–80% by mass), which leads to reduced atmospheric visibility (Zaebst et al. 1991). The large surface area of the DEP carbon core readily adsorbs chemical and biologic agents, some of which may be highly toxic. Identified chemical species of DEP include sulfate, nitrate, metals, and organic compounds such as polycyclic aromatic hydrocarbons (PAH) and nitro-PAHs. The gas-phase components of DE include oxides of nitrogen (NO_x = NO + NO_2), CO, CO_2, lighter-molecular-weight PAHs, and other hydrocarbons. The unique physicochemical properties of DE and DEP have important health consequences (Royal Society 2000).

Health concerns about DE exposure arose more than three decades ago and resulted in an extensive literature that includes publications from over 40 epidemiologic studies and numerous animal and in vitro toxicologic studies (Stayner 1999). The carcinogenic effect of long-term exposure to DE has been established, as documented in the literature (e.g., Pepelko and Chen 1993; HEI 1995; Cohen and Nikula 1999; U.S. Environmental Protection Agency [U.S. EPA] 1999; Garshick et al. 2004). Diesel exhaust, as a whole, is classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC) (Bunn et al. 2004). The effects of DE exposure on asthma and other respiratory illnesses have been examined in various types of studies, as briefly summarized later in this section.

Occupational exposures to DE can be high. For example, 8-hour average concentrations of DEP can be higher than 160 μg/m^3 as total carbon (defined as the final limit by the Mine Safety and Health Administration) or 20 μg/m^3 as EC (defined as the threshold limit value [TLV] limit by the American Conference of Governmental Industrial Hygienists) in underground nonmetal mines (Cohen et al. 2002). Substantially higher DEP concentrations, ranging from 100 to 1700 μg/m^3, were found in underground mines where diesel-powered vehicles and machineries operated within an enclosed space (HEI 1995). High occupational exposures to DE provide opportunities to study the potential health effects. Since 1970, about a dozen occupational studies have examined the effect of short-term (experienced during a work shift, typically 8 hours) or long-term (experienced throughout a work history, comprising years to tens of years) DE exposure on respiratory symptoms (particularly wheezing) and pulmonary function. The results from different studies are inconsistent and do not provide conclusive evidence linking occupational DE exposure and respiratory illness. The inconsistency may partly be due to the different occupational settings of the studies, which may have resulted in variations in exposure characteristics, including concentration of DE constituents and occupational copollutants (e.g., coal dust). In addition, most of the studies provide crude, nonquantitative exposure measures such as length of work shift, length of work history, or time spent driving diesel-powered farm tractors (Wade and Newman 1993; Hoppin et al. 2004).

Asthma is a chronic inflammatory disorder of the airways that causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough. The inflammation also causes an increase in the existing bronchial hyperresponsiveness to a variety of stimuli (Busse and Lemanske 2001).

Over the past two decades numerous epidemiologic studies relating traffic exposure to asthma prevalence or morbidity have been conducted, mostly on children. Overall, there appears to be a suggested association between the number of and subject proximity to diesel-powered vehicles and adverse respiratory outcomes such as wheezing and the use of asthma reliever medication in children with asthma (Lwebuga-Mukasa et al. 2004). This observation did not apply to adult subjects. The evidence linking DE exposure to changes in lung function is limited;

we found only one study with asthmatic children and a DE proxy in which deterioration in health was associated with increased DE exposure (Brunekreef et al. 1997). A few traffic studies have attempted to isolate DE from other ambient pollutants, but the results are not consistent, possibly because of methodologic biases. For example, a variety of methods were used to estimate subjects' exposures, including traffic counts, the proximity of the residence to roads, black carbon concentrations, and the self-reported truck and bus density. The differences in methods may affect the reported outcomes. In another example, preferential reporting bias may have been introduced when asthmatic children responded to questionnaire surveys in cross-sectional studies. Such children may be more aware of the potential adverse effects of exposure to motor vehicle exhaust than children without these symptoms (Brunekreef et al. 1997).

In laboratory animal studies, both restrictive and obstructive patterns of pulmonary dysfunction have been reported after chronic exposure to DE or DEP. Decreased lung volume and compliance have been observed, suggesting a restrictive disorder compatible with parenchymal fibrosis. Studies of animals chronically exposed to DE or DEP showed reduced expiratory flow rates, implying obstructive airflow (Vinegar et al. 1986; Heinrich et al. 1986; Lewis et al. 1986) and enhanced airway hyperresponsiveness, pulmonary inflammation, and cellular proliferation and fibrosis (Nikula et al. 1997). Despite these findings, a few cautionary considerations should be noted when extrapolating the results to humans. In most of the experiments, exceptionally high concentrations of DEP (350–12,000 μg/m^3) were delivered over prolonged periods of time (3–24 months). These concentrations are many times higher than those present in occupational settings with high DEP concentrations. Another consideration is that the response to DE exposure may vary across different animal and human models.

Only a few laboratory-based human exposure studies have been conducted to examine the health effects of DE exposure on healthy or asthmatic subjects. These studies had relatively small sample sizes. Typically the subject would spend two sessions in an exposure chamber: one session being exposed to DE and the other being exposed to clean air. The exposures typically used standardized concentrations of PM_{10} (e.g., 300 μg/m^3), NO_2 or NO_x. To increase the respiratory ventilation rate during exposures, subjects performed moderate exercise in most of the published studies (Nightingale et al. 2000; Chalupa et al. 2004). The duration of exposure varied between 1 and 2 hours. In general, there appears to be a methodologic consistency among different exposure chamber studies with human subjects. During DE exposure, most study subjects complained of eye and nose irritation and of an unpleasant odor.

Spirometric measurement of pulmonary function has consistently failed to show any changes after exposure to DE in published studies. However, this is in contrast to whole-body plethysmography, a more sensitive measure of pulmonary function that has repeatedly demonstrated increased airway resistance, particularly in asthmatic subjects (Rudell et al. 1996; Nordenhäll et al. 2001; Stenfors et al. 2004). Asthmatic subjects also exhibited greater airway responsiveness after DE exposure. Among a variety of pulmonary inflammatory markers, the neutrophil content of lung lavage fluid and of sputum has most consistently been shown to increase after DE exposure (Rudell et al. 1996, 1999; Salvi et al. 1999; Nightingale et al. 2000; Stenfors et al. 2004).

A variety of mechanisms have been proposed by which DE may interact with the respiratory and immunologic systems to cause a worsening of asthma symptoms. Many cells and cellular elements in the airways play a role in inducing airway inflammation. Mast cells, eosinophils, T lymphocytes, neutrophils, and epithelial cells are thought to be particularly important. Most experimental data are derived from in vitro studies using human bronchial epithelial cells and macrophages, because these represent a primary target for inhaled agents. Observed and expected effects of DE exposure, at a cellular level, include increased immunoglobulin E (IgE) production (Takenaka et al. 1995; Diaz-Sanchez et al. 1996; Tsien et al. 1997), altered cytokine expression (Fujimaki et al. 1994; Diaz-Sanchez et al. 1994, 1996; Takano et al. 1997, 1998; Bayram et al. 1998b; Miyabara et al. 1998; Ohtoshi et al. 1998; Abe et al. 2000; Salvi et al. 2000; Stenfors et al. 2004), increased cellular adhesion molecule expression (Bayram et al. 1998a,b; Devalia et al. 1999; Salvi et al. 1999), increased oxidative stress (Sagai et al. 1993; Lim et al. 1998; Hiura et al. 1999; Bonvallot et al. 2001; Li et al. 2002), and impaired host defense (Rudell et al. 1996, 1999; Bayram et al. 1998a,b).

Human nasal challenge studies have shown that DEP alone or coadministered with an allergen significantly enhanced local human IgE production (Takafuji et al. 1987; Diaz-Sanchez et al. 1994; Fujimaki et al. 1997). These results, however, were not reproduced in a study with mice; in that study IgE levels did not increase after exposure to DEP alone (Miyabara et al. 1998). A number of animal studies, on the other hand, suggest that DEP is an adjuvant for IgE production when coadministered with an allergen (Takafuji et al. 1987; Takano et al. 1997). The exact mechanism by which DEP enhances IgE production is unknown. One possible explanation comes from the

observed changes in local cytokine production after nasal DEP challenge.

Diesel exhaust can affect a diverse range of cytokines, although the precise underlying mechanisms are uncertain. Human in vivo and in vitro studies have demonstrated that a variety of proinflammatory cytokines and chemokines (e.g., IL-8) increase with increasing DEP concentrations (Bayram et al. 1998a,b; Ohtoshi et al. 1998; Devalia et al. 1999; Abe et al. 2000; Salvi et al. 2000; Stenfors et al. 2004). Other studies have also shown that the effects observed in those studies do not occur when the particles are removed from the DE or when the particles are replaced with inert particles (Boland et al. 1999; Abe et al. 2000). Moreover, DEPs scrubbed of their adsorbed organic components elicited a substantially diminished effect on cytokine production (Boland et al. 2000).

The intracellular adhesion molecule, ICAM-1, is important for the transit of neutrophils and other inflammatory cells from the blood to the airways, and plays a role in inflammatory diseases such as asthma. In vitro studies with human epithelial cells have demonstrated that exposure to DEP leads to the release of soluble ICAM-1 (Bayram et al. 1998a,b; Devalia et al. 1999). This effect was more marked in epithelial cells from people with asthma than in cells from people who did not have asthma (Devalia et al. 1999). Similarly, short-term DE exposure has been shown to increase ICAM-1 in healthy human subjects (Salvi et al. 1999). Increased ICAM-1 expression after DE exposure may explain the observed influx of neutrophils detected in the airways of DE-exposed subjects.

Oxidative stress may also be a key component in the inflammatory response (Rahman et al. 1997). Much of the literature suggests that the airways of asthmatic people have an increased oxidative burden (e.g., Nadeem et al. 2003). The cells involved in the chronic inflammation of asthma were all capable of generating reactive oxygen species (ROS) such as superoxide anion ($O_2\bullet-$) and the hydroxyl radical (•OH) (Henricks and NijKamp 2001). Increased ROS can result in direct oxidative damage to lung epithelial cells leading to cell injury, death, and shedding (Hulsmann et al. 1994). Neutrophils and mononuclear cells from asthmatic subjects generated proportionally more ROS than cells of matched healthy subjects; this activity correlated with airway hyperresponsiveness (Seltzer et al. 1986). Markers of oxidative stress in the exhaled breath, urine, plasma, and lung fluid of asthmatic subjects were also higher than those of healthy subjects. In vitro studies have demonstrated that human bronchial epithelial cells and macrophages generate ROS when exposed to DEP or DEP organic extracts (Sagai et al. 1993; Hiura et al. 1999; Bonvallot et al. 2001). Animal studies have also provided evidence supporting the role of oxidative stress in DEP-induced airway inflammation. Instillation of DEP into the trachea of mice led to inflammation and hyperresponsiveness of the airways, increased mucus, and production of nitric oxide (NO) and CO, both of which are markers of oxidative stress (Lim et al. 1998, Maines 2000). Human exposure studies have indicated an inflammatory response to DEP exposure (Holgate et al. 2003; Stenfors et al. 2004). Few of these studies, however, have directly linked the inflammatory response to oxidative stress by demonstrating that increased CO production in exhaled breath is paralleled by increased sputum neutrophil and MPO concentrations (Nightingale et al. 2000).

In vitro studies have shown that DEPs and their organic extracts induce dose-dependent apoptosis or apoptosis-necrosis in both epithelial cells and macrophages (Hiura et al. 1999, 2000). This combined apoptosis-necrosis event is seen at higher DEP extract concentrations (e.g., > 100 µg/ml in RAW 264.7 cells). Necrosis may augment the pulmonary effects of DEP because necrotic cell fragments induce further inflammation. The apoptosis of pulmonary macrophages and epithelial cells may have significant local effects. Macrophage apoptosis may well impede phagocytosis of harmful microorganisms, thus impairing host defense. Two in vivo studies of healthy human subjects in exposure chambers showed impaired macrophage phagocytosis after a 2-hour DE exposure (Rudell et al. 1996, 1999). DEP may further impair defense systems by hampering the action of airway cilia. Bayram and colleagues (1998a) observed a significant reduction in the ciliary beat frequency of human bronchial epithelial cells after a 24-hour exposure to DEP. Thus, in vivo DE exposure may reduce the mucociliary clearance of particles and microorganisms deposited in the airways.

Despite this relatively rich literature on the health effects of DE or DEP exposure in relation to asthma morbidity and mechanisms, few studies have directly examined the effects of short-term DE exposure in asthmatic patients (Svartengren et al. 2000; Kagawa 2002). However, many people, including those with asthma, experience short-term exposure to DE during their daily activities. In addition large discrepancies exist among the findings from different studies in the magnitude of the observed effects or in the relative importance of gaseous and particulate components. The evidence that healthy subjects experience an inflammatory response after inhaling particles suggests that exposure to DEP may increase the inflammatory responses already present in the airways of asthmatic patients, thus increasing asthmatic symptoms. This has not yet been confirmed. The contribution of asthma severity to respiratory response to air pollution effects remains unknown.

SPECIFIC AIMS

The overall goal of this study was to investigate the health effects of short-term exposure to urban diesel traffic in asthmatic patients. The study was designed to test the following hypotheses:

(A) Short-term exposure to urban diesel traffic leads to increased asthma symptoms and reduced lung function in asthmatic adult nonsmokers;

(B) The increase in asthma symptoms is accompanied by increased oxidative stress and inflammation of the lungs; and

(C) The increase in asthma symptoms depends on both the asthma severity and the exposure dose of DE.

The specific aims of the study included the following:

1. Examine the effect of diesel traffic and DE components (i.e., $PM_{2.5}$, EC, UFP, and NO_2) on asthma symptoms and pulmonary function in asthmatic adults, after a 2-hour exposure in a real-world setting.
2. Examine whether short-term diesel traffic exposure augments airway inflammation and increases oxidative stress burdens in adult asthmatic patients.
3. Examine the contribution of background asthma severity to individuals' respiratory response to diesel traffic exposure by using two asthma severity categories (moderate asthma vs. mild asthma).
4. Explore exposure–response relations for several DE components, using the natural day-to-day variation in DE concentrations in a real-world exposure setting.

METHODS AND STUDY DESIGN

ETHICS AND INSTITUTIONAL REVIEW BOARD APPROVAL

This study involved human subjects and was carried out at two collaborating institutions, the University of Medicine and Dentistry of New Jersey (UMDNJ), and the Imperial College of Science, Technology, and Medicine, in London. The study protocol was approved by both the Institutional Review Board of UMDNJ and the joint Ethics Committee of the National Heart and Lung Institute and Royal Brompton Hospital. Written informed consent was obtained from all potential subjects before screening for the study. Upon the completion of each experimental session, an honorarium was offered to each subject to compensate them for their time.

The questionnaire responses and data files containing subject identifiers were securely stored using either locked filing cabinets or computers with password protection. By securing the data and ensuring that only the investigators and designated study staff members had access to records, subjects' identities were completely protected in compliance with human subject guidelines.

SUBJECT SELECTION AND RECRUITMENT

The study protocol required the recruitment of 60 nonsmoking men and women, between 18 and 55 years, with current bronchial hyperresponsiveness: 30 with mild asthma and 30 with moderate asthma. The subjects were recruited from a variety of sources: (1) a patient database within the Royal Brompton Hospital asthma laboratory that contained information about known asthmatic patients who had previously taken part in research within the department; (2) respiratory outpatient clinics at Royal Brompton Hospital; and (3) an advertisement distributed throughout the Royal Brompton Hospital and other units of Imperial College London, a student newspaper, and an electronic student journal. A total of 81 individuals were screened, from whom 65 nonsmoking adult asthmatic patients were recruited. The other 16 were not eligible for the study because they did not demonstrate adequate bronchial hyperresponsiveness. Five of the 65 subjects withdrew from the study for nonmedical reasons before taking part in any experimental sessions.

Inclusion Criteria

Each subject's asthma diagnosis was based on a history of asthmatic symptoms, spirometric lung function testing, and evidence of either bronchial hyperresponsiveness or reversible airway obstruction. The baseline FEV_1 determined whether a subject would receive an assessment of bronchial hyperresponsiveness or of airway reversibility. Subjects with an FEV_1 of $\geq 70\%$ of the predicted value underwent a methacholine challenge to measure PC_{20} (the concentration of methacholine required to decrease FEV_1 by 20%). Subjects with an FEV_1 of $< 70\%$ of the predicted value had their airway reversibility measured. The inclusion criterion was either a $PC_{20} \leq 8$ mg/mL or an FEV_1 improvement of at least 12% after inhaling a short-acting β-agonist (200 μg albuterol, also known as salbutamol).

Asthma Severity Classification

During screening, potential subjects completed a baseline health questionnaire regarding their medical and asthma history (see Appendix A). This questionnaire was later used to help determine the asthma severity classification of each study subject. Asthma severity was defined by the Global Initiative for Asthma (GINA) criteria, either by

symptoms (Table 1) or by therapy (Table 2). Both definitions were used because potential study subjects were expected to be receiving treatment, but the treatment may not have been optimal for each subject. We used the highest step obtained from the two tables to define the severity level.

Each subject's asthma severity was classified as mild or moderate according to the GINA criteria (see Tables 1 and 2); 31 subjects had mild asthma (GINA steps 1 or 2) and 29 subjects had moderate asthma (GINA step 3 or 4). Skin prick testing was also performed on subjects using extracts of common allergens (house dust mite, grass pollen, *Aspergillus fumigatus*, and cat hair); the results were for descriptive purposes only.

Exclusion Criteria

Current cigarette smokers, former smokers of less than 1 year, pregnant women, and people with cardiovascular disease or other physical disabilities that would preclude exercise were not eligible for participation. We also excluded those whose occupations entailed unusually high exposures to DE and those who lived or worked close to Oxford Street (the exposure site); this was determined by means of a baseline exposure questionnaire during screening (see Appendix A). Subjects could not participate within 6 weeks of a respiratory tract infection or within 4 weeks of taking systemic corticosteroids.

Table 1. Global Initiative for Asthma (GINA) Asthma Severity by Symptoms[a]

	Symptoms	Day	Night	PEFR or FEV_1	PEFR Variability
Step 1—mild intermittent	Asymptomatic and normal PEFR between attacks	< 1/week	2/month	≥ 80%	< 20%
Step 2—mild persistent	Attacks may affect activity	> 1/week, but < 1/day	> 2/month	≥ 80%	20–30%
Step 3—moderate persistent	Attacks affect activity	Daily	> 1/week	60–80%	> 30%
Step 4—severe persistent	Limited physical activity	Continuous	Frequent	≤ 60%	> 30%

[a] Abbreviations and other terms in tables and figures may be found in a list at the end of the Investigators' Report.

Table 2. Global Initiative for Asthma (GINA) Asthma Severity by Therapy[a]

	Daily Preventive Medication	Other Treatment Options
Step 1—mild intermittent	None necessary	NA
Step 2—mild persistent	Low dose inhaled corticosteroid	Slow release theophylline, or Cromone, or Leukotriene modifier
Step 3—moderate persistent	Low to medium dose inhaled corticosteroid plus long acting β-agonist	Medium dose inhaled corticosteroid plus SR theophylline, or Medium dose inhaled corticosteroid plus long acting oral β-agonist, or Medium dose inhaled corticosteroid plus leukotriene modifier
Step 4—severe persistent	High dose inhaled corticosteroid plus long acting β-agonist, plus one or more of the following if needed: SR theophylline Leukotriene modifier Oral Corticosteroid	NA

[a] NA indicates not applicable.

EXPERIMENTAL DESIGN

The study consisted of one exposure session and one control session for each of the 60 subjects. Each session lasted 2 hours, with the session order selected at random. The subjects' usual asthma medications were continued except they were asked not to use bronchodilators 12 hours before and during a study day. Patients using a combination inhaler (e.g., a corticosteroid plus a long-acting β-agonist) were provided with a corticosteroid inhaler at the screening visit, enabling them to temporarily omit the bronchodilator component. The sessions were carried out during a period of asthma stability with no treatment changes in the 2 weeks before exposure. The exposure and control sessions were separated by a minimum of 3 weeks and took place between November and April, thus avoiding the pollen season. Subjects were required to walk within predesignated areas of the exposure and control sites at a steady walking pace (approximately 2 mph), resting 15 minutes every half hour. All sessions occurred during weekdays, starting at approximately 10:30 a.m. and finishing at approximately 12:30 p.m. To ensure the safety of subjects and their adherence to the designated routes, a physician and a field technician accompanied the subjects throughout each session.

The study subjects and field study staff obviously could tell whether a session was at the control or exposure site, so this part of the study could not be blinded. To reduce bias, the instructions given to the subjects during clinical measurements were the same for all subjects and for both sessions. In addition, subjects' clinical outcomes and exposure data were concealed from them. The stored clinical samples and all pollutant and environmental samples were labeled with unique identifying numbers and analyzed without knowledge of the sampling locations.

FIELD SITES FOR THE EXPERIMENTS

The experiments were carried out at a hospital, an exposure site, and a control site, all located in central London. Royal Brompton Hospital served as the hospital for clinical measurements, while Oxford Street and Hyde Park served as the exposure site and the control site, respectively. The selection of the exposure and control sites was achieved through a pilot study described in Appendix E.

Oxford Street is a busy and popular shopping district in London, situated approximately 2.5 miles from Royal Brompton Hospital. At the time of this study, only buses, official taxicabs, and pedestrians were permitted in the busiest half-mile section of this street (between Regent Street and Orchard Street) during the day. The buses and taxicabs were all powered with diesel engines. This section of the street, with its high density of idling and traveling buses and taxicabs, was selected as the exposure site for the present study. The pedestrian traffic along this section was also heavy, but the sidewalk was wide. Thus, we did not have difficulty ensuring that the subjects walked within designated paths at a steady walking pace.

The central part of Hyde Park served as the control site for this study for the following reasons: First, this area of the park was approximately 1 mile from Royal Brompton Hospital and 1 to 1.5 miles from Oxford Street. Second, the park was large (635 acres) and devoid of motor vehicle traffic. Motor vehicles were not permitted within the park boundaries; thus the impact of traffic pollution on the air quality of the central part of the park was relatively small. Finally, as with Oxford Street, the park provided an environment where subjects could walk naturally.

CLINICAL AND EXPOSURE MEASUREMENTS

Table 3 shows a timeline of health endpoints and exposure indices. These measurements were all subject-specific. On an experimental session day, participants came to Royal Brompton Hospital around 8:00 a.m. After the morning baseline measurements were taken, the subjects relaxed in a designated nonclinical day room within the hospital. They went to the same room throughout the day between clinical measurements. The participants were transported to the exposure site or the control site in a gasoline-powered taxi — a journey of less than 10 minutes. They were transported in the same manner to Royal Brompton Hospital for postsession measurements.

We also conducted an intensive field campaign during each of the two study winters to characterize air pollution related to DE in both exposure and control sites. That study was designed and carried out to provide additional information on ambient concentrations of a suite of pollutant species pertinent to diesel engine emissions. Results from this component of the study are not directly used in our exposure–response analysis, but details of this work are presented in Appendix F.

Pulmonary Function Measurement through Spirometry

Pulmonary function tests were conducted during the screening visit and throughout each session-day (see Table 3). We measured FEV_1 and FVC using a hand-held Vitalograph 2120 spirometer (Vitalograph, Buckingham, U.K.). The spirometer was linked to a laptop computer with Vitalograph Spirotrac software. Field measurements were stored in the handset and then downloaded to the laptop. The subjects' age, sex, race, and height were entered into the computer, for calculating absolute and percentage-predicted values for all measurements. The spirometer was calibrated daily, before any measurements were taken, using a precise 1-L syringe piston supplied by the manufacturer. A

Table 3. Timeline of Health Endpoint and Exposure Measurements

	Screening Visit	1 Week Pre-Session	Exposure or Control Session							1 Week Post-Session
			Day 1						Day 2	
Time of Day			08:00	~10:30 ~12:30	13:30	14:30	15:30	16:30	17:30	09:00	
Time point designation			hr −2	hr 0–2	hr 3	hr 4	hr 5	hr 6	hr 7	hr 22	
Spirometry	•		•	•	•		•		•	•	
Bronchial reactivity	•								•		
Baseline questionnaire	•										
Skin prick testing	•										
Asthma symptoms	•			•					•		
eNO			•		•	•	•	•	•	•	
EBC			•		•			•			
Venepuncture			•						•		
Induced sputum	•									•	
Symptom and PEFR		•									•
Baseline NO_2		•									
$PM_{2.5}$				•							
UFP				•							
EC				•							
NO_2				•							
Temperature and relative humidity				•							
Urine collection			•						•		

calibration figure of ± 3% was deemed acceptable. The ambient temperature was also entered into the spirometer; this was particularly relevant because lung function was measured both indoors and outdoors during the study. The subjects were trained how to breathe into the spirometer before the study began. All measurements were taken with the subjects standing up and not wearing a nose clip. The baseline value at each visit was taken after a minimum rest period of 15 minutes. The highest of three consecutive readings was recorded; this value was automatically selected by the Vitalograph software.

Bronchial Reactivity

Bronchial reactivity was measured as part of the screening process, and as an indicator of asthma severity after both sessions. If the subject's FEV_1 was ≥ 70% predicted, a methacholine challenge was undertaken; if FEV_1 was < 70% predicted, an assessment of airway reversibility was performed.

The methacholine challenge method used in the present study was derived from that previously described (Cockroft et al. 1977). Fresh solutions of methacholine (Stockport Pharmaceuticals, Manchester, U.K.) were prepared on each test day in 0.9% normal saline at concentrations of 0.1, 1.0, 2.0, 4.0 and 8.0 mg/mL. After a 15-minute rest, baseline lung function was measured. The subjects inhaled five breaths of 0.9% normal saline from a nebulizer that was attached to a breath-activated dosimeter (Mefar Dosimeter MB3, Brescia, Italy). The nebulizer delivers particles with an aerodynamic mass median diameter of 3.4–4 µm with an output of 9 µL per breath. The subjects inspired from functional residual capacity to vital capacity over a 3-second period, then held their breath for 6 seconds. The FEV_1 measurement was repeated two minutes later. If the value had fallen by ≥ 10% from baseline, the subject rested for 30 minutes, and the challenge was repeated. If the fall was < 10%, this value was recorded as the post-saline value. The subjects then inhaled the first dose of methacholine (0.1 mg/mL)

and FEV_1 was recorded as described earlier. Increasing doses of methacholine were administered until a ≥ 20% fall in FEV_1 from the post-saline value (PC_{20}) occurred or until the top dose (8 mg/mL) had been reached. PC_{20} was automatically calculated by the Spirotrac software, using the following equation:

$$\log(PC_{20}) = \log[A] \; + \; 0.301 \times \frac{FEV_1(A) - 0.8 \times FEV_1(\text{saline})}{FEV_1(A) - FEV_1(B)}$$

where [*A*] = the second highest methacholine concentration reached, FEV_1(saline) = FEV_1 after inhalation of saline, $FEV_1(A)$ = FEV_1 after inhalation of the second highest methacholine concentration, and $FEV_1(B)$ = FEV_1 after inhalation of the highest methacholine concentration.

On three occasions the computer failed and PC_{20} values were calculated manually.

Skin Prick Testing

Skin prick testing was performed on the volar aspect of the forearm. The forearm was cleaned with alcohol, and the test sites marked with a ball point pen. Each site was at least 2 cm apart to prevent the coalescence of positive reactions. Single drops of positive (histamine dihydrochloride 10 mg/mL) and negative (glycerinated saline) control solutions were placed at two separate marks. Each drop was placed on the skin with a 100-µL micropipette. The test solutions (grass pollen, cat hair, house dust mite, and *Aspergillus fumigatus*) were administered in the same manner at separate marks along the arm. A sterile lancet was pushed perpendicularly into the skin through each droplet and held for 5 seconds. A new disposable lancet was used for each solution. The droplet was then wiped off. After 15 minutes the test sites were observed for erythema and wheal formation. The wheal diameter was measured in millimeters using a transparent ruler. The longest diameter (d_1) and the diameter perpendicular to it (d_2) were measured and the average diameter was recorded — ($d_1 + d_2$)/2. A reaction of 3 mm or greater was considered positive. If a subject had a positive response to any of the test allergens described here, the subject was defined as being atopic.

Diary Card for Asthma Symptoms and Peak Expiratory Flow Rate

Subjects were given diary cards (see Appendix G) to record asthma symptoms, albuterol (bronchodilator) use, nocturnal awakenings, and measured peak expiratory flow rate (PEFR). They were asked to use the diary cards for the week before and the week after each exposure or control session. The symptom component of the diary included self-assessment of shortness of breath, chest tightness, wheezing, cough, and sputum production. Each was given a score ranging from 0 (none) to 3 (severe). The maximum possible score was 15. PEFR values were measured using a Mini-Wright peak flow meter. The subjects were trained to use the PEFR meter and asked to record the highest of three consecutive readings, four times daily.

Diaries for the week before an exposure session were collected the morning of the session day; diaries for the week after the session were mailed to the laboratory in a prepaid envelope. A diary was considered to be adequately completed when the subject recorded two or more PEFR measurements per day.

eNO

As a marker of airway inflammation, eNO was measured using an online Niox chemiluminescence analyzer (Aerocrine, Sweden) in accordance with the American Thoracic Society standardized procedure (American Thoracic Society 1999). The analyzer detection concentration was 1.5 ppb NO, with an accuracy of ± 2 ppb (for measured values ≤ 50 ppb) or ± 5% (for measured values > 50 ppb), with a response time of less than 1.5 seconds. The sampling flow through the reaction chamber was 50 mL/s for all measurements. The analyzer was calibrated every 13 days using a certified NO standard (200 ± 2 ppb) (AGA Linde Gases, Aerocrine, Sweden).

The subjects were trained how to breathe into the apparatus before beginning the study. All measurements were made with the subjects seated, after a period of rest and before spirometric testing. The subjects inspired filtered NO-free air by inhaling through the mouthpiece. They then performed a controlled expiration by exhaling through the mouthpiece for 10 seconds. A biofeedback display provided visual guidance to aid the subjects in maintaining a steady expiratory pressure and flow, thus improving reproducibility. Flows that did not meet the criteria were rejected by the analyzer. A resistive pressure against the flow (5–20 cm H_2O) was applied to ensure closure of the nasopharyngeal vellum to prevent eNO contamination from the nose and sinuses. The fractional eNO concentration value was the mean NO concentration during the NO plateau, between 5 and 8 seconds of exhalation (sampling frequency 20 Hz). The mean value of three consecutive measurements, taken at least 30 seconds apart, was the final recorded eNO (ppb). Reproducibility was usually within 5% among the three measurements.

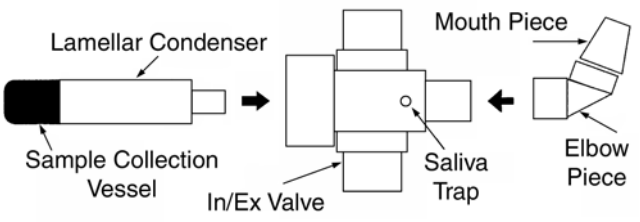

Figure 1. The sample collection vessel was lightly screwed onto the condenser to allow easy removal when condensate collection was complete. The lamellar condenser was pushed firmly into the In/Ex valve assembly to ensure an adequate seal. The mouth and elbow pieces were positioned so the saliva trap would be at the bottom of the device.

EBC

EBC Collection We attempted to measure biomarkers of oxidative stress and airway acidification and inflammation in collected EBC. These biomarkers included pH, 8-isoprostanes (8-ISP), nitrotyrosine, nitrite and nitrate, and free iron (redox-active chelatable iron). EBC was collected using a Jaeger ECOscreen (Erich Jaeger, Germany) condensing device. The machine was switched on at least 30 minutes before collection to allow the cooling cuff to reach operating temperature (−20°C). The sealing cap was applied to the cuff to insulate the internal cooling area and to avoid condensation of ambient moisture (which would freeze the lamellar condenser onto the cuff when it was inserted). The collection interface was assembled as shown in Figure 1. EBC was collected for 10 minutes, during which time subjects were seated. Each subject wore a nose clip and was instructed to breathe tidally. Approximately 1 mL of condensate was obtained per collection.

After collection the interface was removed from the cooling cuff, and the sealing cap was replaced. With the condenser held upright, the sample collection vessel was removed and its contents defrosted. Samples were deaerated with inert argon gas (350 mL/min for 10 minutes), and pH was measured (see next section). The samples were aliquoted into labeled cryotubes, and an antioxidant mixture (butylated hydroxytoluene, 2 mM in 99% ethanol, 10 µL per mL of sample) was added. The samples were immediately stored at −80°C for later analysis of the targeted markers. The reusable collection interface was sterilized according to the manufacturer's recommendation. All components were then thoroughly rinsed in double distilled and in deionized water to avoid sample contamination.

EBC pH EBC pH was measured using an electronic pH meter with a resolution of 0.01 and a working range of −2.00 to 16.00 (Jenway 350, Jenway, Essex, U.K.). The pH meter was calibrated each day before use with standard pH buffer solutions.

EBC 8-Isoprostanes The concentration of 8-ISP in EBC was measured by an enzyme-linked immunosorbent assay (ELISA) using a commercial assay kit (Cayman Chemicals, U.S.). Reagents were dissolved in the kit's phosphate buffer (pH 7.4) which contained sodium azide (0.1 g/L), sodium chloride (0.234 g/L), EDTA (0.37 g/L), and bovine serum albumin (1 g/L). 50-µL samples or an 8-ISP standard were added in duplicate to a 96-well plate precoated with murine anti-rabbit IgG antibody. 8-ISP-acetylcholinesterase conjugate (50 µL) and anti-8-ISP antibody (50 µL) were then added. The plate was incubated at room temperature for 18 hours, washed with phosphate buffer solution (PBS) (pH 7.4, containing 0.05% Tween-20), and developed with the Ellman reagent (200 µL per well) for 90 minutes. A microplate reader measured optical density. Unfortunately the 8-ISP data were not used in the current data analysis because of sample and data handling errors.

EBC Free Iron We used the bleomycin assay to determine redox-active chelatable iron (free iron) in EBC samples. This assay has been used to determine free iron concentrations in biologic samples in previous studies (Gutteridge et al. 1986, 1996). DNA (1 mg/mL), bleomycin sulfate (1.5 units/mL), Tris buffer at pH 7.4, magnesium chloride (50 mM), and EBC sample were mixed. To start the reaction, we added 7.5 mM of ascorbate and incubated the reactants at 37°C for 30 minutes. The iron chelated from EBC by bleomycin was reduced by ascorbate. In the presence of oxygen, the iron generated a ROS that degraded DNA and released malondialdehyde from its deoxyribose moiety. Malondialdehyde reacted with thiobarbituric acid. The product was extracted into butan-1-ol, and was then spectrophotometrically measured at 532 nm. Iron was quantitated using an iron calibrative curve made from iron standard solutions.

EBC Nitrite and Nitrate The concentrations of nitrite and nitrate in EBC samples were determined using the ISO-NOP Nitric Oxide Sensor (World Precision Instruments, Sarasota, FL), an amperometric sensor specific for NO (Machado et al. 2004). The sensor was immersed in 10 mL of 0.1-M sulfuric acid and 0.1-M potassium iodide, a solution in which NO is liberated from nitrite. Nitrite concentration is determined using a calibration curve made from nitrite standard solutions. Total nitrite and nitrate concentration was determined in replicate samples (15 µL) by reducing nitrate to nitrite with a cadmium-copper pellet (Nitralyzer II; World Precision Instruments, Sarasota, FL). The conversion efficiency using this protocol with authentic nitrate was determined to be 100%. After reduction, the nitrite was measured by the ISO-NOP. Nitrate concentration was determined by subtracting

nitrite concentration from total nitrite and nitrate concentration. However, because the volume of most samples was insufficient for the assay, we were able to measure nitrite and nitrate in only eight condensate samples (for 4 out of 60 subjects). We decided to exclude nitrite and nitrate from the data analysis. Nitrotyrosine assays also were not done because of low sample volumes.

Blood TBARS

Blood was sampled before and after exposure, using standard medical procedures, to analyze a product of oxidative stress: thiobarbituric acid reactive substances (TBARS). Blood (10 mL) was withdrawn, placed into a serum sample (yellow top) blood bottle, and left to clot (in the dark) for 20 minutes before being centrifuged for 10 minutes at 3500 rpm. The serum supernatant was aliquoted into labeled cryotubes and stored at −80°C. If the venipuncture failed and the subject agreed, one additional attempt was made at a different site.

The concentrations of TBARS were measured using the OXItek TBARS assay kit (Gentaur Molecular, Belgium). An aliquot of 100-µL serum supernatant or standard was added to the appropriately-labeled glass test tube followed by 100 µL of SDS solution. A thiobarbituric acid buffer reagent (2.5 mL) was carefully poured down the inside wall of the tilted test tube. The sample tubes were covered and incubated in a water bath (95°C) for 1 hour, and then cooled to room temperature in an ice bath for 10 minutes. The samples were centrifuged at 3000 rpm for 15 minutes and the supernatant was removed for fluorescence analysis (excitation wavelength 530 nm, emission wavelength 550 nm).

Sputum Induction and Processing

A sputum sample was obtained from each subject at the screening visit and after each exposure or control session. Although the informed consent process described the procedure and purpose of sputum induction, including the importance of collecting sputum rather than saliva, this was again explained in detail to each subject before each session. To protect the subject's airways, we administered 200 µg of albuterol 10 minutes before the procedure (via metered dose inhaler with spacer device). The sputum induction proceeded only if the post-albuterol FEV_1 was ≥ 60% of the predicted value. If that was not reached, a sample was not obtained. The induction process started with subjects inhaling 3.0% saline in three 4-minute cycles, via an ultrasonic nebulizer (DeVilbiss 2000, DeVilbiss Co., Middlesex, U.K.). The nebulizer was calibrated to deliver aerosol particles with a mass median aerodynamic diameter of 4.5 µm and an output of 4.5 mL/min. The aerosol was inhaled through a mouthpiece attached to a tube 110 cm long with an internal diameter of 22 mm. After 4 minutes, the subject attempted to produce sputum. To reduce contamination, subjects thoroughly rinsed their mouths and blew their noses before expectorating into a 50-mL polypropylene centrifuge tube. The subjects were asked to produce sputum at 4-, 8-, and 12-minute intervals, and whenever they needed to cough during induction. Pulmonary function was checked after each 4-minute cycle and whenever a subject experienced any symptom of bronchoconstriction. At the end of the procedure, the sputum sample was stored at 4°C and processed within 2 hours of collection. All reusable equipment was sterilized after each procedure.

The sputum samples were analyzed for their differential cell counts and soluble markers of inflammation. The whole sputum sample processing method was used throughout the study. The weight of the sample was gravimetrically determined. The sample was homogenized by adding 0.1% dithiothreitol (DTT, 4 mL per gram of sample; Sigma Chemicals, Poole, U.K.). This reduced the disulfide bonds of mucous glycoproteins so cells and proteins within the mucus would be released into suspension. To aid liquefaction the sample was gently mixed with a 3-mL pipette and left on a roller for 15 minutes at room temperature. A volume of PBS, equal to the DTT volume, was added to the sample. To remove any debris, the liquefied sputum was filtered through 48-µm nylon gauze into a fresh tube. The filtrate was then centrifuged at 400*g*, at 4°C for 10 minutes. Six 500-µL samples of the resultant supernatant were pipetted into labeled cryotubes and stored at −80°C for further analysis. The cell pellet was then resuspended in 1 mL of PBS. Total cell counts and viability were determined on an Improved Neubauer haemocytometer slide, using Kimura stain. The cell suspension was then diluted with PBS to a cell count of 3.5×10^5/mL. The cell suspension (100 µL) was added to six cytospin blocks (Shandon Cytospin, Runcorn, U.K.) and centrifuged at 450 rpm for 3 minutes. Slides were left to dry for 30 minutes and were then stained.

Sputum Cell Counts

Cytospin slides were coded before the differential cell counts so researchers were blind to whether samples were collected after an exposure or a control session. They counted 400 nonsquamous cells (neutrophils, macrophages, eosinophils, and epithelial cells) on each of two separate slides for each sputum sample. The differential cell counts were expressed as a percentage of nonsquamous cells. (An adequate sample was defined as having < 50% of squamous epithelial cells.)

Sputum Mediator Assays

IL-8 IL-8 concentrations were measured at room temperature using an amplified sandwich-type ELISA. The samples were diluted to 1 part supernatant in 5 parts reagent diluent (PBS containing 0.1% bovine albumin and 0.05% Tween 20). The dilution was selected from a series of trial dilutions, ranging from undiluted to 1 in 50; the most appropriate sample dilution generated values within the range of the standard curve.

We used 96-well microplates (IL-8 DuoSet, R&D systems, Abingdon, U.K.) that were precoated with 100 µL capture antibody (dilution ratio = 1:180), sealed, and incubated overnight. They were then washed with buffer (PBS containing 0.05% Tween 20), immediately treated with block buffer (PBS containing 1% bovine serum albumin, 5% sucrose, and 0.05% sodium azide), and left to stand for 1 hour. After an additional washing, IL-8 standards and appropriately diluted samples (100 µL) were added in duplicate to the plates, and were then covered and incubated for 2 hours. Samples were diluted to 1 part supernatant in 5 parts reagent diluent (PBS containing 0.1% bovine serum albumin and 0.05% Tween 20). The dilution was selected from a series of trial dilutions, ranging from undiluted to 1 in 50; the most appropriate sample dilution generated values within the range of the standard curve.

The plates were then washed with buffer and incubated for 2 hours with the detection antibody. After a final wash, the plates were incubated with streptavidin conjugated to horseradish-peroxidase for 20 minutes away from direct light. A stop solution (2N sulfuric acid) was added to the plates, and the optical density of the wells was determined with a microplate reader. IL-8 concentrations were reported in nanograms per milliliter.

MPO The sputum MPO concentrations were measured at room temperature using ELISA in 96-well microplates (TiterZyme human MPO, Ann Arbor, MI) that were precoated with a monoclonal antibody specific for human MPO. Standard and diluted samples (100 µL) were added in duplicate to the plates, which were then sealed and incubated for 1 hour on a plate shaker. The samples were diluted to 1 part supernatant in 30 parts standard diluent (assay buffer 13 plus Protein Cocktail Inhibitor: Sigma, 0.5 µL/mL, and PMSF 1 mM [phenylmethanesulphonyl fluoride]). This dilution was selected after a series of trial dilutions; the most appropriate sample dilution generated values within the range of the standard curve. The plates were then washed with wash buffer and 100-µL rabbit polyclonal anti-MPO antibody was added to each well. After incubating for an hour, the plates were washed again. Horseradish peroxidase conjugated to anti-rabbit IgG (100 µL) was added to the plates, which were incubated for another 30 minutes. After a final wash, 100 µL of the substrate solution was added to the wells. A stop solution (2N sulfuric acid) was added to the plates, and a microplate reader was used to determine the optical density of the wells at a wavelength of 450 nm. At the working concentration of 0.05%, there was no difference in standards diluted with standard diluent alone or standard diluent plus DTT; therefore all MPO analyses were done without adding DDT to the ELISA process. The sputum MPO concentrations were reported in nanograms per milliliter.

Eosinophil Cationic Protein Sputum eosinophil cationic protein (ECP) was measured by fluoroenzyme immunoassay (FEI) using an automated UNIcap100 device (Pharmacia & Upjohn, Sweden). The ECP-FEI was an in vitro test system for quantitative ECP measurement. With this assay, fluorescence intensity is directly proportional to the concentration of ECP in a sample. The method detection limit was 2 ng/mL.

In this assay anti-ECP, covalently coupled to Immunocap, reacted with ECP in the sputum supernatant sample. Immunocap is a part of the UniCap 100 instrument. Immunocap are flexible hydrophilic polymer carriers encased in a capsule. The carrier consists of an active cellulose derivative.

The sample was washed, and an enzyme labeled with antibodies against ECP was added, forming a complex. The samples were incubated, and unbound enzyme-anti-ECP was washed away, leaving the bound complex to incubate with a developing agent. The reaction was stopped, and the fluorescence of the reaction was measured.

Baseline NO_2 Exposure

To control for the potential effects of relevant baseline exposure, we measured personal NO_2 concentrations over the week before each exposure session. We did this for two reasons: (1) NO_2, along with particulate matter (PM), is released from the combustion of fossil fuels, including diesel; and (2) integrated NO_2 measurements over the course of a week are inexpensive and do not unduly burden subjects. Our hope was that this 1-week NO_2 measurement could add objective information about the subjects' baseline combustion pollution exposure to the subjective questionnaire data. Subjects wore a Palmes badge (a passive sampler for NO_2) (Gradko International, Winchester, U.K.) continuously for 7 days before each exposure. The subjects were instructed to wear the badges as close as possible to their breathing zones (e.g., on shirt pockets or neckties). The exposed badges were sent to the badge supplier for NO_2 analysis. NO_2 concentrations in the

air were calculated from the mass collected on the badges, the sampling rate, and the sampling duration.

During-Session Air Pollutant and Environmental Measurements

We measured air pollutants during the exposure and control sessions using real-time data-logging instruments that collected $PM_{2.5}$, UFP, and CO (as well as temperature and relative humidity). The values were logged every minute. In addition, the integrated samples of NO_2, $PM_{2.5}$ mass, and EC were collected throughout each 2-hour exposure or control session. To monitor personal breathing-zone concentrations, the sample inlets were placed at the average subject's breathing zone height (1.3–1.5 meters). The samplers were all placed on a pushcart. While the subjects walked on the designated path of the control site or the exposure site, a field technician walked behind them with the pushcart.

$PM_{2.5}$ $PM_{2.5}$ mass concentrations were measured using two independent methods. One used a DustTrak aerosol monitor (Model 8520, TSI Inc. St. Paul, MN). The DustTrak is a portable battery-operated laser photometer with real-time mass concentration readouts and data-logging capability (measurement range, 0.001–100 mg/m^3). $PM_{2.5}$ concentrations were measured at a 1-minute resolution. Throughout the study all guidelines provided by the manufacturer were strictly followed. Before each sampling session, the instrument was set to zero, and its internal clock was synchronized with the other instruments.

The second method was gravimetrically based. $PM_{2.5}$ mass was collected with a high-flow personal monitor using a method developed by Adams and colleagues (2001a,b). The monitor used a Vortex Ultraflow pump (Casella Ltd, U.K.) calibrated to a sampling flow rate of 16 L/min. The filters (37-mm diameter Teflon with a polymethylpentene support ring, Pall Life Sciences, U.S.) were weighed both before and after sampling using a Mettler MT5 microbalance (Mettler-Toledo Ltd., Greifensee, Switzerland) that was accurate to 1 µg. To eliminate any interference from electrostatic charge, we used a StatAttack Ionizing Blower (AEA Technology plc, Amersham, U.K.). The average of three consecutive filter weight measurements was recorded as the final weight; if the consecutive measurements were not all within 1 µg, the filter was reweighed.

All filters were equilibrated before weighing for at least 24 hours before and after sampling. The barometric pressure, temperature, and relative humidity were measured during the weighing sessions. These values were used to correct for air density changes between pre- and post-sampling weighing of filters (Koistenen et al. 1999; Mettler-Toledo 1998).

In the current analysis, we primarily used the $PM_{2.5}$ concentration data derived from the gravimetric method. $PM_{2.5}$ concentrations measured using the DustTrak were calibrated with the concurrently measured gravimetric data. The DustTrak data show $PM_{2.5}$ concentrations in 1-minute intervals during exposure and control sessions. These data may be used in future exposure–response analyses to examine the peak exposure effects.

EC We used another high-flow personal monitor to collect $PM_{2.5}$ on heat-treated quartz fiber filters (37 mm) which were subsequently analyzed for EC. (Organic carbon was also measured, but the results are not reported in this study.) The quartz fiber filters were analyzed either in-house at Rutgers University or at the Sunset Laboratory Inc., Forest Grove, Oregon. The method, designated as the U.S. National Institute for Occupational Safety and Health method 5040, was based on a thermal-optical technique described in detail by Birch and Cary (1996). Samples were analyzed for EC at two laboratories. Interlaboratory comparison was performed and proper adjustments, based on the interlaboratory comparison, were made (see Appendix D).

UFP The UFP concentrations were measured with a real-time condensation particle counter (model 3007 CPC, TSI Inc., St. Paul, MN). The instrument used a ribbon-laser light-scattering optical system to count particles. The particles passed through a saturator tube, where isopropanol vapor was added to the sample stream. The sample became saturated with isopropanol vapor, and then passed into a cooled condenser tube where the alcohol vapor supersaturated and condensed onto the particles that had a diameter greater than 10 nm. The resulting droplets exited the condenser and passed through a thin ribbon of laser light, causing the light to scatter. The scattered light was focused on a photodetector which converted the light intensity to an electric signal proportional to the number of particles. The alcohol source was an isopropanol-saturated wick, which provided enough isopropanol for 6 hours of operation at 35°C. This wick was recharged every day by soaking it in isopropanol. The measurement range of the instrument was 0 to 100,000 particles/cm^3. The instrument could measure up to 200,000 particles/cm^3 when concentrations exceeded 100,000, but with less accuracy. The accuracy was within ± 20% for concentrations within the measurement range. The particle counter had been factory-calibrated within a year of these measurements.

NO_2

Using a SKC personal-sampling pump (SKC Inc., Eighty Four, PA), we collected NO_2 onto a C_{18} Sep-Pak cartridge, coated with a solution of potassium hydroxide and triethanolamine, at a nominal flow rate of 1 L/min. When sampled air passed through the sampling media, NO_2 reacted with potassium hydroxide to form nitrite and nitrate. The cartridges were extracted with 2 mL of a solution containing high performance liquid chromatography (HPLC)-grade water and methanol (6:1 v/v) and analyzed for nitrite and nitrate using an ion chromatography technique. The sum of nitrate and nitrite molar concentrations was the molar concentration of NO_2. Final concentrations were then blank-corrected as described later in the Quality Control and Data Management section. More detailed descriptions of this method of analyzing NO_2 concentration can be found in published literature (Nishikawa and Taguchi 1987; Hisham and Grosjean 1990).

CO

To measure CO concentration, we used a real-time CO monitor — the Langan model T15v (Langan Products Inc., San Francisco, CA) with a resolution of 5 ppb. The monitor was equipped with an electrochemical cell suitable for monitoring CO. It stored and evaluated measured CO and temperature values with a built-in data logger. This information was downloaded from the data logger to a PC. Because of the temperature-dependent nature of the sensor, a temperature correction was applied to the collected CO data (Langan 1998, 2003). The electrochemical monitor was factory-calibrated but not field-calibrated. On close examination of the collected CO data, we were concerned about their quality (see Appendix F). Therefore, we did not report the CO measurements.

Temperature and Relative Humidity

The temperature and relative humidity measurements were electronically logged by a HOBO-Pro device (Onset Computer Corporation, MA) with a 1-second resolution. The device contained a two-channel logger with internal temperature and relative humidity sensors. The temperature measurement range was −30°C to 50°C (accuracy ± 0.2°C); the relative humidity measurement range was 0% to 100% (accuracy ± 3%). The instrument was reset before each sampling session, and its internal clock was synchronized with the other instruments.

Urine Collection and Analysis

Urine samples were collected for analysis of 1-hydroxypyrene (1-OHP), a metabolite of pyrene. Pyrene is one of the most abundant PAHs found in the atmosphere and is released during fuel combustion, including combustion of diesel fuel and tobacco. Urinary 1-OHP has been suggested as a potentially useful biomarker of DE exposure in urban residents (Northridge et al. 1999). Each subject contributed a preexposure and a postexposure urine sample for each exposure session. The preexposure sample was collected on arrival at the hospital, and the postexposure sample was collected before leaving the hospital. Up to 30 mL of urine void was collected in an opaque plastic container, stored at −20°C in the hospital, and then shipped on dry ice to the Principal Investigator's laboratory in New Jersey for 1-OHP analysis. The samples were slowly thawed and shaken. A 10-mL aliquot was used for 1-OHP analysis, and another 1-mL aliquot was sent to a commercial lab for creatinine analysis (Pro-Vet Diagnostics, Pittstown, PA). Because the proportion of 1-OHP to water in urine output is highly variable, 1-OHP concentrations were reported as micromoles 1-OHP per mole of creatinine.

The procedure for 1-OHP analysis is as follows: A 10-mL aliquot of a urine sample was adjusted to pH 5 with a buffer solution of 1-M hydrochloric acid and 0.1-M acetate, for a final volume of 30 mL. This mixture was incubated for 16 hours with 20 µL of glucuronidase-arylsulphatase at 37°C in an electronically controlled rotary shaking bath. After this enzymatic hydrolysis, the mixture was filtered through ashless filter paper (Whatman, Brentford, Middlesex, U.K.) and forced through a precleaned Sep-Pak C18 cartridge (Waters, Milford, MA) at a flow rate of 1 mL/min. (The cartridge was precleaned with 5 mL of methanol followed by 10 mL of deionized water.) This cartridge was first washed with 3 mL of deionized water and 3 mL of 50% methanol in water. (Both eluents were discarded.) Finally, the cartridge was eluted with 10 mL of HPLC-grade methanol. This final eluent was then concentrated by evaporating the methanol solution down to 1 mL. A 20-µL aliquot of the concentrated extract was injected into an HPLC system equipped with a 3.9 mm × 150 mm Nova-Pak C_{18} column and its guard column (Waters, Milford, MA).

In the mobile phase of 1-OHP analysis, we used Solution A (55% methanol in water) and Solution B (100% methanol) with a gradient program: 100% Solution A for 10 minutes and then a linear gradient from 100% Solution A to 100% Solution B in 45 minutes at a constant flow rate of 1.0 mL/min. The fluorescence detector of the HPLC system was set at an excitation wavelength of 265 nm and an emission wavelength of 430 nm. The 1-OHP was quantified using external calibration standards that were prepared by dissolving solid 1-OHP (98%, Aldrich Chem. Co., Milwaukee, WI) in HPLC-grade methanol.

STATISTICAL METHODS AND DATA ANALYSIS

Our statistical analysis plan comprised mainly three types of analysis: descriptive and correlation analysis, comparative analysis (exposure session vs. control session), and pollutant-specific exposure–response analysis.

DESCRIPTIVE AND CORRELATION ANALYSIS

We used histograms and Shapiro-Wilks normality tests to examine data distributions for each measured pollutant or health endpoint. These were done for all the subjects combined and separately for each of the two asthma severity groups. The results indicated that the data for FEV_1, FVC, $FEF_{25–75}$, PEFR, and eNO were close to normal distribution. Although log-transformed data for some of the biomarkers (sputum ECP, sputum IL-8, and sputum MPO) showed better normality than the raw data, we decided to use nontransformed data in all statistical analyses for all endpoints except PC_{20}, which was extremely right-skewed. This not only ensured consistency across different endpoints and biomarkers, but also allowed more straightforward interpretations of results. Significance levels did not substantially change when the data were transformed.

We also created time-series plots with a single line representing each individual at each session to assess the potential impact of outliers. Outliers, defined as values ≥ 3 SD above or below the mean, were excluded from further analysis of FEV_1, FVC, $FEF_{25–75}$, and eNO. (In some analyses outlier data from one or two subjects were excluded based on outlier testing results. Outlier tests were not conducted for other endpoints because the data were highly variable.)

Descriptive summaries of exposure and health outcomes at each time point included sample size, mean, SD or standard error (SE), median, minimum, and maximum. The results on health endpoints, in general, were summarized for the combined data across two asthma severity groups and separately by severity category.

We used the nonparametric Spearman rank correlation to examine correlations among pollutants and correlations among health endpoints. In addition, bivariate scatter plots were used, when necessary, to help explain the results from correlation analyses.

COMPARISONS BETWEEN EXPOSURE AND CONTROL SESSIONS

Data on central tendency and variability (arithmetic mean and SE) for each pollutant and each health endpoint or biomarker were compared to find any differences between exposure data and control data. In general, we use paired *t* tests to examine whether pollutants or health endpoints at baseline differed between the exposure and control sessions. The average changes in percentage from baseline for health endpoints were calculated and plotted for each during-session and postsession time point. Within the SE plots, SEs for each time point were calculated using the changes in percentage for each time point. The analyses of the plots provided initial insight for more sophisticated analyses. These analyses were used to evaluate changes in health endpoints over time and allowed us to test an overall effect of exposure for multiple time points. Subsequently, estimates and tests of the effect of exposure at various time points after exposure allowed us to examine which time points contributed to each significant result. These analyses are potentially powerful tests because they allow adjustments for known differences between subjects, such as age, but also for unknown differences through modeling of subject-specific random effects.

We used repeated-measures mixed-effects linear regression models to model the average values of the health outcomes for each session at each time point. These models included the health outcome (FEV_1, FEF, FVC, etc.) as the response (dependent variable). Exposure (exposure session vs. control session) and time were categorical fixed effects, and individual subject was a random effect. The models were created, parameters estimated, and statistical tests performed using PROC MIXED in SAS Version 9.1.3. Additional covariates, including ambient temperature and relative humidity, were entered for all the modeled health endpoints and biomarkers. In addition, age, sex, and ethnicity were entered as covariates for all endpoints and biomarkers except FEV_1, FVC, and $FEF_{25–75}$, all of which were already reported as percentage of predicted values using height, age, and sex. In addition to the random effect for individual subject, which was used to account for similarities of measures within an individual, we introduced a covariance structure to model correlations between repeated measurements taken across time on the same day for each individual subject. An additional random effect for date was added to account for correlations between subjects with concurrent exposure sessions.

The covariance structure that best fits the data was selected by examining the Akaike Information Criterion and the Schwarz Bayesian Criterion. After considering different first-order covariance structures (autoregressive, compound symmetry, unstructured, and spatial power), we selected the spatial power covariance structure as providing the best fit to the observed data. This covariance structure was appropriate for the unequally spaced repeated measurements. Because two subjects typically participated in each session, correlations between subjects within each session were tested using random-effects modeling; the correlations were not statistically significant.

The interaction between time and exposure (1 = exposure session and 0 = control session) was tested to examine the effect of exposure on changes from baseline (postsession minus presession) on health endpoints and biomarkers. If a significant effect of exposure was found and endpoints were measured at multiple time points during and after exposure, contrasts (linear combinations of regression coefficients that provided estimates of the exposure effect on changes in outcomes from baseline to each follow-up time) were used to identify the time points for which the changes from baseline were significantly different between the exposure and control sessions. The analyses were also stratified by asthma severity group (mild or moderate) to assess any differences between the two asthma severity groups for the effect of exposure on changes in health endpoints.

POLLUTANT-SPECIFIC EXPOSURE–RESPONSE ANALYSES

Because of day-to-day variation in pollutant concentrations, actual exposure varied across the subjects who participated in exposure or control sessions on different session dates. This allowed us to perform exposure–response analyses across subjects. To do this, we used a set of models based on the mixed-effects model structure described earlier. In these models, change in a health endpoint from baseline is the dependent variable. The fixed-effects variables include pollutant concentration and time of measurement. The covariates ambient temperature and relative humidity were described earlier as were age, sex, and ethnicity (covariates for all endpoints and biomarkers except FEV_1, FVC, and FEF_{25-75}). Random-effects variables and structure were the same as described in the earlier section on comparing exposure and control sessions — individual subject and date. One pollutant was modeled at a time. Each of the four pollutants, $PM_{2.5}$, EC, UFP, and NO_2, was examined in these analyses, because these pollutants were measured during the 2-hour exposure and control sessions of each subject. From these analyses, we report point estimates and 95% confidence intervals (CIs) of estimated regression coefficients (slopes) (i.e., change in health endpoint or biomarker from baseline, associated with a defined unit change of pollutant concentration). These analyses were performed using the combined exposure and control session data and also using data from the exposure session alone and the control session alone. To find potential correlations among the measured four pollutants, we also performed analyses using two-pollutant models in which two of the four pollutants were analyzed at a time. The two-pollutant model had the same structure as the single-pollutant model described earlier.

QUALITY CONTROL AND DATA MANAGEMENT

QUALITY CONTROL MEASURES AND DATA CORRECTION

A study protocol was established, including standard operating procedures and associated standard quality assurance procedures for every component of the project. The protocol was strictly followed throughout the study. The precision of the method and accuracy of each analysis and assay were verified at published or manufacturer-suggested levels. When possible, 10% to 20% of all measurements and analyses were dedicated to quality assurance, including analysis of instrument blanks, solvent blanks, duplicate samples, and split samples. All instruments used in the study were calibrated periodically per the suggested time intervals of the manufacturer or before each use.

A number of quality control measures were in place to assess measurement reproducibility and between-instrument consistency. The results are shown in Table 4. When applicable, field blanks were prepared identically to sample substrates, transported to the field, and returned and analyzed with the samples. Field blank distributions were used for determining blank corrections and method detection limits. Duplicate samples were collected from collocated samplers to determine method precision or reproducibility. When two units of a real-time monitor were used in the study, both units were placed side by side, and a comparison between the two units was established. When samples were analyzed at two different laboratories, a subset of the samples was analyzed at both laboratories to establish a correction factor.

Table 4. Method Detection Limits (MDLs) and Measurement Precision[a]

	MDLs[b]	Precision (%)[c]	N[c]
$PM_{2.5}$ (μg/m^3)	0.01	ND	
EC (μg/m^3)	0.08	2.20	11
NO_2 (μg/m^3)	2.18	17.0	5

[a] ND indicates not detectable.

[b] Detection limits were estimated as 3 × SD of the field or laboratory blanks.

[c] Values are expressed as the pooled CVs of pairs of collocated field measurements expressed as percentage. N = equals total number of pairs. ND (not determined) indicates that CVs were not determined because more than half the values were below detection.

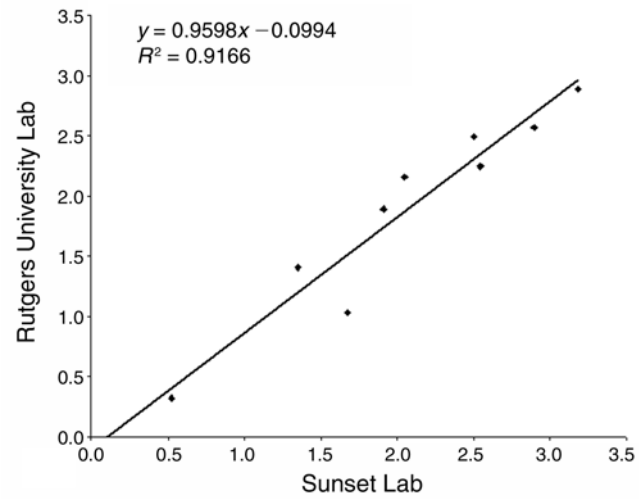

Figure 2. Linear regression comparing EC concentrations (μg/m^3) measured at the Sunset Lab and at the Rutgers University lab.

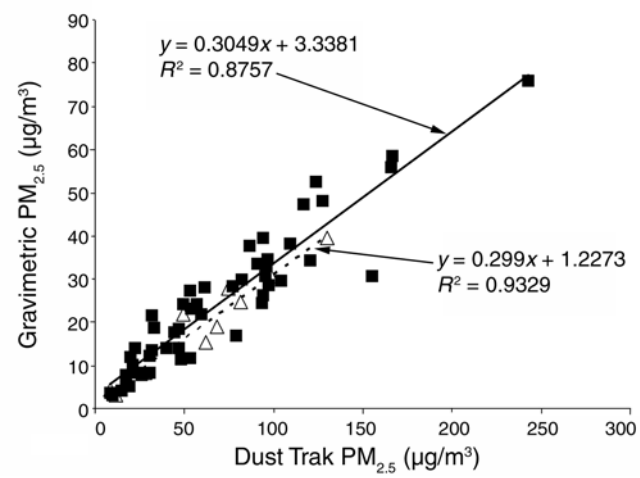

Figure 3. Regression coefficients of $PM_{2.5}$ mass concentrations (■ and solid line) and a subset of 9 samples with corrected $PM_{2.5}$ mass concentrations (△ and dotted line) were compared with DustTrak-derived $PM_{2.5}$ concentrations.

Interlaboratory Comparison of EC

In the first winter of the study (2003–2004), 55 samples, including 6 blanks, were analyzed for EC by the Sunset Lab. In the second winter of the study, 58 samples, including 2 blanks, were analyzed at an in-house Rutgers Lab (to reduce the cost of analysis). EC was not detected in 9 of the 58 samples analyzed at the Rutgers Lab. These 9 samples and another 9 samples (for which EC was detected) were sent to Sunset Lab for duplicate analysis. The EC concentrations in all 18 samples analyzed at the Sunset Lab were above the instrument detection limit. Comparing the 9 samples for which EC concentrations were detected at both laboratories, we found the results from the two laboratories agreed reasonably well (Figure 2). To be consistent with the 2003–2004 data, we adjusted the results for the 40 samples analyzed only at the Rutgers Lab using the regression results shown in Figure 2. Therefore, all the data were normalized to the Sunset results and blank-corrected.

$PM_{2.5}$ Concentration Validation

A site audit in the first study winter (January 2004) uncovered a leak in the Gilabrator flow rate meter (Gilian, Wayne, NJ) when it was used to measure sampling flow rates of the Vortex pumps. This leaking flow meter had affected nine $PM_{2.5}$ samples before it was replaced with a well-functioning Gilabrator flow meter. By comparing the flow rates measured by another well-functioning Gilabrator flow meter and the leaking flow meter, we derived a correction factor of 0.77, based on an average actual pump flow of 20.7 L/min when the pumps were set to operate at 16.0 L/min. To verify the appropriateness of the correction factor, we performed two sets of regression analysis on gravimetrically derived $PM_{2.5}$ concentrations versus DustTrak-derived $PM_{2.5}$ concentrations. The first analysis used data from the nine samples affected by the leaking flow meter and corrected the data using the correction factor. The second analysis used the data from all the other unaffected samples. The slopes from both regressions, shown in Figure 3, were nearly identical (0.299 for the affected data set and 0.305 for the unaffected data set). Therefore, we felt justified in using a correction factor of 0.77 to correct the $PM_{2.5}$ concentrations affected by the leaking flow meter.

Onsite Calibration of DustTrak Monitor

The DustTrak aerosol monitor (model 8520, TSI Inc., St. Paul, MN) measures particle concentrations based on light scattering; therefore, measurements are affected by particle composition. The manufacturer calibrated the monitor using standardized inert particles. To accurately measure particle mass concentrations, the monitor had to be calibrated with the ambient particles that would actually be measured. In the current study, our DustTrak monitor with a $PM_{2.5}$ inlet was collocated with a $PM_{2.5}$ sampler. The concurrent measurements of $PM_{2.5}$ using the DustTrak and gravimetric methods allowed us to calibrate the DustTrak using real-world particles from the exposure and control sites. The following equation was derived from these concurrent measurements of $PM_{2.5}$ mass concentrations:

$$[\text{DustTrak } PM_{2.5}] = -7.191 + 3.130\ [\text{gravimetric } PM_{2.5}]$$

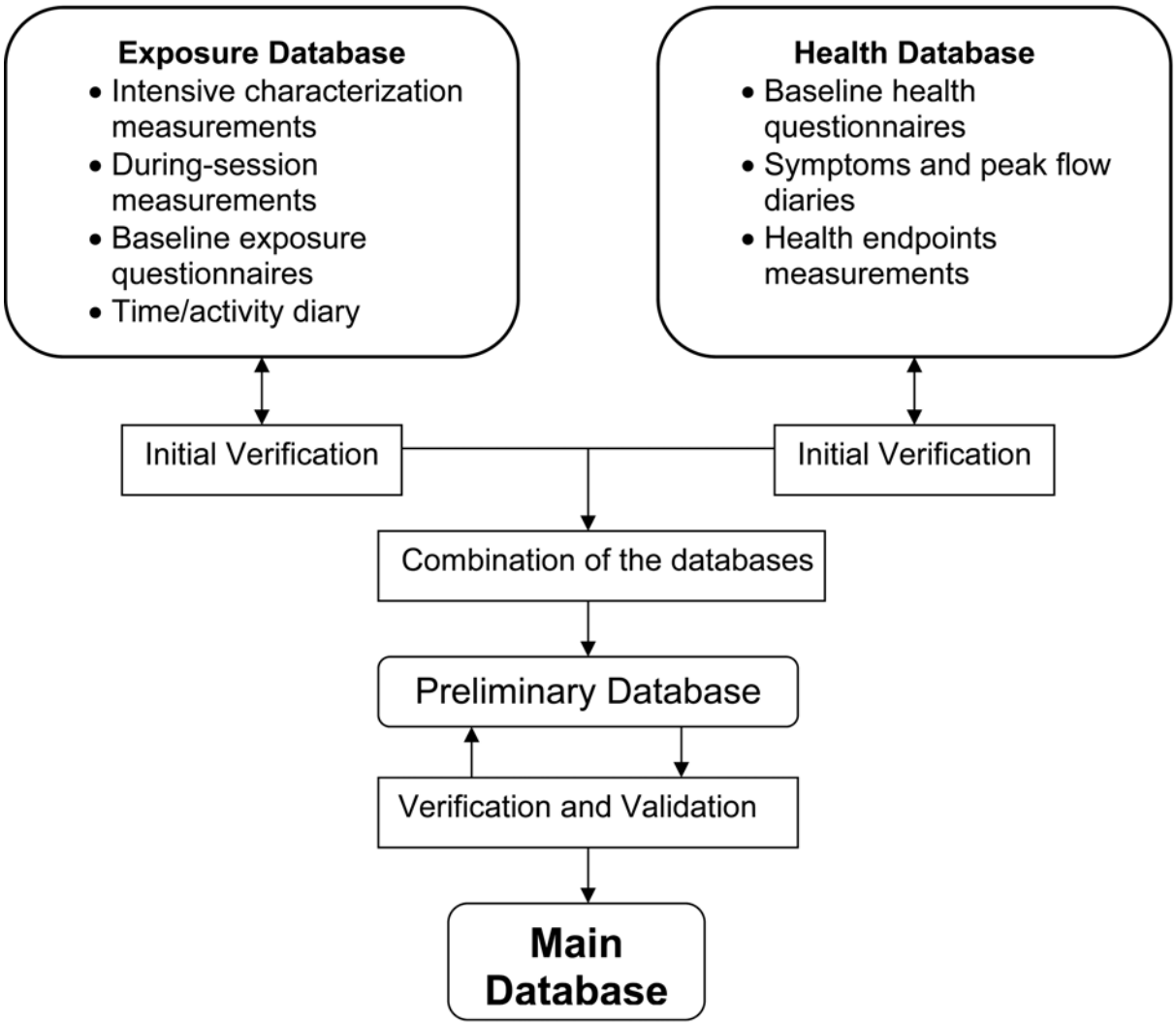

Figure 4. The data quality control and quality assurance processes.

Therefore, we adjusted the DustTrak raw data using the above regression results (r^2 = 0.908). The difference observed between the DustTrak readings and the gravimetric measurements was large but not uncommon, as discussed in Appendix E. This further supports the need for an onsite calibration of DustTrak when reporting absolute PM mass concentrations instead of just examining a trend and relative changes in PM.

DATA MANAGEMENT AND VALIDATION

Everything from the collection of samples and questionnaire information to the consolidation and entry of such items into a compiled database was done in an organized manner, as illustrated in Figure 4. All data were securely stored by locked filing cabinet or by computer file password. In compliance with human subject guidelines, we secured and restricted access to questionnaires and database records to protect subjects' identities and medical information. Data access was restricted to the designated field technicians and the investigators.

A chain of custody document (COC) was initiated for each batch of samplers and substrates. The COC accompanied that batch throughout the process. First, the laboratory technician who prepared the samplers and substrates signed and dated the COC before giving them to the field technician for a session. Likewise, the field technician signed and dated the COC to confirm receipt, recorded deployment and collection dates, and recorded the date when the samplers and substrates were returned to the

laboratory. In the same manner, the receiving laboratory's technician confirmed receipt. The laboratory technician then recorded when the samples were extracted from the samplers and substrates, when they were analyzed, and when they were stored.

As part of the field sampling and data collection process, sampling information sheets were completed by the field technician. These sheets contained the subject identification number, sample type, sample location, start and finish time, and sampling flow rates (when applicable). Once completed, the sampling information sheets were directly distributed to one or more of the investigators. To ensure that the analytical procedure was blind and consequently unbiased, the investigators stored these documents until all of the samples were analyzed; then they provided the sampling information sheets to the laboratory technician who keyed the information into an initial database. Some aspects of the sampling information sheets were used to determine the validity of the samples during the data validation process.

To review the initial data set, approximately 10% of the electronic data entries were randomly selected and cross-checked against the original printed hard copy. We also used scatter plots to identify unusual values. Original data records were retrieved to check these values. When sources of error were identified, the resulting values were either replaced with correct values or designated as invalid. When the reported values or the parameters that had been used to derive the values were physically impossible, the values were invalidated. When no source of error was found, the unusual values were retained in the database.

RESULTS

CHARACTERISTICS OF SUBJECTS

Sixty subjects participated in the study. Basic demographic information and some characteristics of the subjects are summarized by asthma severity category in Table 5. The mean age of the mild asthma group (31 subjects) was slightly younger than the mean age of the moderate asthma group (30.5 years vs. 34.4 years). Both asthma severity groups included more white subjects than subjects from other ethnic groups. Most of the subjects, regardless of their background asthma severity, were atopic as determined by skin prick tests. Ten subjects did not have the skin prick skin tests, so their atopy status was unknown. Within both asthma severity groups, more than 77% of the subjects were never smokers, and the rest were former smokers (quitting smoking at least 1 year before the start of the study). A similar fraction of the subjects in the mild asthma group (63%) and in the moderate asthma group (65%) reported that smoke affects their asthma. In contrast, a higher fraction of the subjects in the moderate (83%) than in the mild asthma group (65%) reported that exercise affects their asthma. All subjects reported using asthma reliever medication. More characteristics of the subjects are listed in Appendix H, Part 1, which reports

Table 5. Characteristics of Study Subjects[a]

	Mild Asthma					Moderate Asthma				
Variable	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum
Age (year)	31	30.5	9.1	20	49	29	34.4	11.6	19	55
Height (cm)	31	172.2	8.4	156	193	29	171.2	9.3	153	187
Sex										
Female	14	—	—	—	—	15	—	—	—	—
Male	17	—	—	—	—	14	—	—	—	—
Ethnicity										
White	26	—	—	—	—	21	—	—	—	—
Nonwhite	5	—	—	—	—	8	—	—	—	—
Atopy										
Unknown	4	—	—	—	—	6	—	—	—	—
Yes	24	—	—	—	—	18	—	—	—	—
No	3	—	—	—	—	5	—	—	—	—

[a] — indicates not applicable.

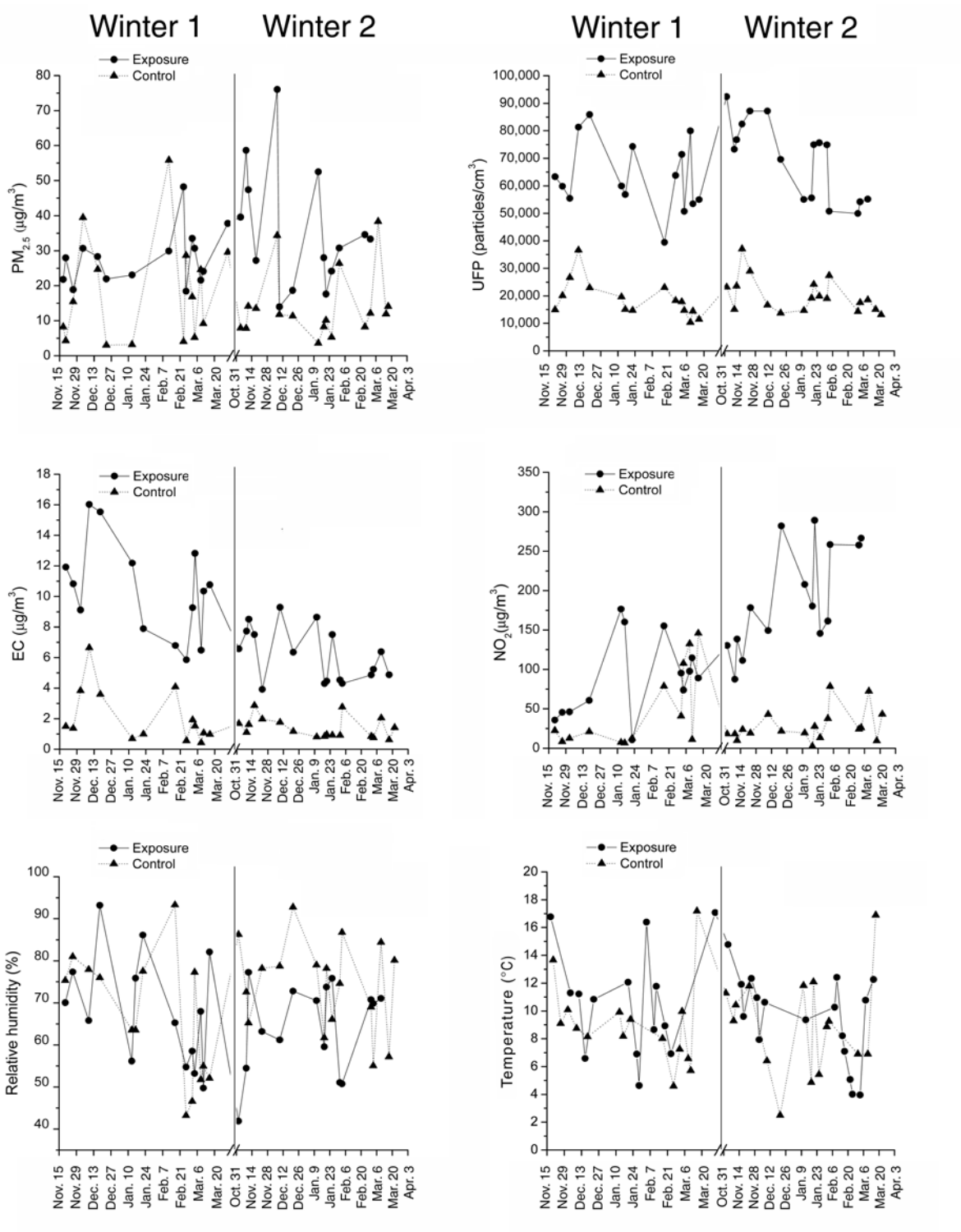

Figure 5. Time-series plots of air pollutants ($PM_{2.5}$, UFP, EC, and NO_2), relative humidity, and ambient temperature measured during exposure and control sessions. Winter 1 = November 15, 2003–March 20, 2004; Winter 2 = October 31, 2004–April 3, 2005.

responses to the Baseline Health Questionnaire. According to subjects' responses to the questionnaire (see Appendix H, Part 2), none of them used highly polluting indoor combustion devices such as wood- or coal-burning stoves, or portable kerosene or gas heaters. About 90% of the subjects within each severity group reported that they did not live with smokers, and 81% of the subjects reported that they worked away from home. The subjects' most common means of transportation to work were by bus, by train, and by foot.

SITE COMPARISON OF POLLUTANT EXPOSURES

Environmental measurements made during the sessions are summarized in Table 6 and plotted in Figure 5. $PM_{2.5}$, UFP, EC, NO_2, relative humidity, and temperature were all measured with the samplers and monitors that accompanied the subjects throughout each exposure or control session. Each subject's baseline NO_2 was taken from a passive sampler worn during the week immediately before each session and the 1-OHP was obtained from a urine void from each subject immediately before the start of each session.

Based on paired *t* tests, the subjects had significantly higher during-session exposures to all the measured pollutants (Table 7). As shown in Figure 5, the during-session concentrations of EC and UFP were always higher at the exposure site than at the control site for all the subjects. On some days $PM_{2.5}$ or NO_2 exposure was higher at the control site than the exposure site. This happened more often with $PM_{2.5}$ than with NO_2. (Note that the data shown in the plots were collected on different days at the two sites.) Exposures to the four pollutants were correlated with Spearman rank correlation coefficients ranging from 0.58 (for NO_2 with both UFP and EC) to 0.84 (for UFP with EC). The correlation coefficient for $PM_{2.5}$ and UFP was 0.62.

In contrast, the two markers of presession combustion exposure (baseline NO_2 and urinary 1-OHP) were not significantly different between the exposure and control sessions (Tables 6 and 7).

To further examine whether there were differences in presession exposure between the two sessions for each subject, we calculated mean within-subject differences for

Table 7. Within-Subject Differences in Baseline Exposure Measurements and Measurements Made During Sessions

Exposure Variable	*n*	Mean Difference (Exposure − Control)	*P*
$PM_{2.5}$ (μg/m^3)	53	13.7	< 0.001
UFP (#/cm^3) × 10^3	58	46.7	< 0.001
EC (μg/m^3)	56	5.9	< 0.001
NO_2 (μg/m^3)	53	109	< 0.001
Temperature (°C)	58	2.4	0.036
Relative humidity (%)	48	−5.5	0.026
Urinary 1-OHP[a]	40	−0.01	1.000
Baseline NO_2 (μg/m^3)	50	2.05	0.898

[a] Reported as μmol per mole of creatinine.

Table 6. Baseline Exposure Measurements and Measurements Made During Sessions[a]

Exposure Variable	Exposure Session						Control Session					
	n	Minimum	Maximum	Median	Mean	SD	*n*	Minimum	Maximum	Median	Mean	SD
$PM_{2.5}$ (μg/m^3)	29	13.9	76.1	28.3	31.7	13.8	32	3	55.9	11.9	16	12.8
UFP (#/cm^3) × 10^3	31	39.5	92.4	63.7	66.6	13.8	33	10.3	37.1	18.3	19.5	6.43
EC (μg/m^3)	31	3.9	16	7.5	8.1	3.2	32	0.4	6.7	1.3	1.7	1.3
NO_2 (μg/m^3)	28	10.7	289	142	143	77.7	31	2.4	146	21.7	36.1	37
Temperature (°C)	31	4	17.1	10.8	10.5	3.5	33	2.5	17.2	9.1	9.2	3.5
Relative humidity (%)	27	41.9	93.2	65.8	65.4	12	33	43.2	93.3	76	71.4	13
Urinary 1-OHP[b]	40	ND	0.72	0.03	0.06	0.11	59	ND	0.87	0.02	0.06	0.14
Baseline NO_2 (μg/m^3)	50	1.46	135	23.5	23.7	20.1	50	0.49	61.6	22.3	21.6	11.5

[a] ND indicates not detectable.

[b] Concentration reported as μmol per mole of urine creatinine; calculated based on assigning below-detection-values with halves of detection limit. Percent of samples below detection limit was 35% for the exposure session and 17% for the control session.

Table 8. Within-Subject Differences in Time Spent on Activities in the 48 Hours Before Each Session

Activity	*n*	Mean Difference in Time Spent (Exposure − Control) (Minutes)	*P*
Walking on foot	52	4.1	0.542
Motorbiking	52	0.3	1.000
Riding car	52	−6.3	0.986
Riding bus	52	2.0	0.933
Riding train	51	44	0.001
Staying home indoors	52	−81	0.304
Staying home outdoors	51	3.3	0.849
Working indoors	52	2.6	0.982
Working outdoors	50	−2.4	0.787
Staying indoors excluding home	52	89	0.281
Staying outdoors excluding home	52	65	0.242
Cooking	52	6.0	0.456
Sleeping	52	−12	0.448
Being close to a smoker	51	29	0.157

data describing the amount of time spent on the activities listed in Table 8 which took place over the 48-hour period before each session. The data were collected using a time-activity card. The differences were not statistically different from zero for all activities except *riding train*. Because the order of the exposure and control sessions for each subject was random, there is no reason to expect a difference in presession activity pattern between the two sessions. In view of that, we could not explain why there was a difference in time spent riding in a train.

COMPARISON OF HEALTH ENDPOINTS BETWEEN EXPOSURE AND CONTROL SESSIONS

Baseline Values of Health Endpoints

Because of the study protocol requirements, the presession samples or measurements were not necessarily taken at the same time (see Table 3). In reporting the change of a health endpoint from its baseline value, we defined the baseline time point as follows: For spirometric lung function (FVC, FEV_1, $FEF_{25–75}$), the baseline values were determined at the start of session (hour 0). For exhaled NO (eNO), EBC pH and free iron, and blood TBARS, the baseline values were measured at approximately 2 hours before the start of session (hour −2). For bronchial reactivity (PC_{20}) and biomarkers in induced sputum (number of epithelial cells, eosinophils, neutrophils, lymphocytes, and macrophages, and levels of ECP, MPO, and IL-8), session-specific baseline measurements were not possible because the procedures for sputum induction and PC_{20} measurements might affect the other health endpoints. Thus the measurements made at the screening visits were used as the baselines for PC_{20} and the sputum markers. For PEFR, symptom score, and asthma medication use, we used the presession 7-day means as the baseline values.

Each day of the week before a session, subjects recorded PEFR, asthma symptom score, and asthma reliever medication use. The results from the week-long diary records are summarized by asthma severity category in Table 9. As expected, there were no differences between the exposure and control sessions in PEFR mean daily values for any of the 7 days or for the 7-day mean PEFR for the week. Likewise, no differences in symptom score were observed for any of the 7 days or the 7-day mean for either asthma severity group. Within-subject difference results for asthma reliever medication use were similar except at 5 days before a session for the moderate asthma group.

The baseline values of the other endpoints and biomarkers, measured at the start (hour 0) or 2 hours before each session (hour −2), are summarized in Table 10 by asthma severity category. As expected, there were no significant within-subject differences in the baseline values of any endpoints or biomarkers between the exposure and control sessions. Therefore, we used the combined data set containing baseline values measured at both exposure and control sessions to compare baseline values between the two asthma severity groups. For PC_{20} and the sputum markers, only one baseline measurement was made for each subject at the screening visit.

Differences between baseline values of the mild and moderate asthma groups were based on 2-tailed *t* tests at $\alpha = 0.05$ (Table 11). The mild asthma group had significantly higher mean baseline values for measured lung function indices FEV_1, $FEF_{25–75}$, and PEFR, but not for FVC. The mild asthma group had significantly lower mean baseline values for asthma symptom score and frequency of asthma reliever medication use. When comparing EBC pH baseline values, we found a marginally significant difference between the two groups ($P = 0.060$). However, when H^+ concentrations in EBC were directly compared,

Table 9. Within-Subject Differences Between Exposure and Control Sessions in Self-Reported PEFR, Asthma Symptoms, and Asthma Reliever Medication Use Before Each Session

	Mild Asthma (n = 31)							Moderate Asthma (n = 29)						
	Exposure		Control		Exposure−Control			Exposure		Control		Exposure−Control		
	Mean	SE	Mean	SE	Mean	SE	P[a]	Mean	SE	Mean	SE	Mean	SE	P[a]
PEFR (L/min)														
Time before session (days)														
7	509	21	505	21	4	30	0.889	461	21	462	20	−1	29	0.968
6	505	21	508	20	−2	29	0.935	461	22	459	20	2	30	0.950
5	508	20	499	21	8	29	0.781	468	20	461	20	6	28	0.827
4	504	19	502	20	2	28	0.931	467	19	463	21	4	28	0.892
3	508	20	505	20	3	28	0.924	468	19	466	21	2	28	0.935
2	511	19	506	19	4	27	0.871	468	21	461	22	7	30	0.816
1	513	20	508	19	6	27	0.839	470	22	468	20	2	30	0.943
7-day mean	510	19	506	19	4	27	0.871	467	20	463	20	4	28	0.877
Asthma Symptom (Average Score per Day)														
Time before session (days)														
7	1.78	0.51	0.86	0.20	0.92	0.54	0.094	0.96	0.28	1.52	0.35	−0.56	0.45	0.220
6	1.33	0.41	0.97	0.24	0.37	0.47	0.435	1.36	0.28	1.40	0.34	−0.04	0.44	0.928
5	1.44	0.49	0.83	0.28	0.62	0.55	0.270	1.40	0.32	1.88	0.34	−0.48	0.47	0.309
4	1.19	0.40	1.52	0.31	−0.33	0.50	0.508	1.80	0.41	2.56	0.62	−0.76	0.74	0.311
3	1.30	0.40	0.90	0.23	0.40	0.46	0.384	1.88	0.47	1.64	0.36	0.24	0.59	0.686
2	0.96	0.30	0.72	0.22	0.24	0.37	0.522	1.80	0.45	1.44	0.37	0.36	0.59	0.542
1	0.82	0.31	0.72	0.20	0.10	0.37	0.792	1.72	0.44	1.24	0.32	0.48	0.55	0.385
7-day mean	1.22	0.32	0.93	0.18	0.29	0.37	0.426	1.58	0.32	1.67	0.29	−0.08	0.43	0.844
Asthma Reliever Medication Use (Number of Times Used per Day)														
Time before session (days)														
7	0.41	0.14	0.28	0.11	0.13	0.16	0.615	0.57	0.18	0.76	0.23	−0.19	0.23	0.688
6	0.48	0.20	0.31	0.11	0.17	0.19	0.516	0.60	0.22	0.52	0.15	0.08	0.17	0.828
5	0.59	0.22	0.38	0.13	0.21	0.15	0.270	0.52	0.18	0.92	0.21	−0.40	0.14	0.018
4	0.48	0.21	0.45	0.13	0.03	0.19	0.945	0.76	0.26	1.00	0.37	−0.24	0.27	0.523
3	0.44	0.14	0.34	0.12	0.10	0.18	0.842	0.92	0.21	0.88	0.34	0.04	0.39	0.633
2	0.39	0.15	0.38	0.13	0.01	0.13	1.000	0.72	0.20	0.84	0.34	−0.12	0.23	0.811
1	0.29	0.13	0.34	0.14	−0.05	0.11	0.766	0.52	0.18	0.68	0.41	−0.16	0.29	0.836
7-day mean	0.43	0.13	0.35	0.09	0.07	0.09	0.684	0.67	0.17	0.80	0.25	−0.13	0.14	0.554

[a] P from paired t tests of difference between exposure and control sessions.

Table 10. Within-Subject Differences Between Exposure and Control Sessions in Baseline Endpoints and Biomarkers

	Mild Asthma (n = 31)							Moderate Asthma (n = 29)						
	Exposure		Control		Exposure−Control			Exposure		Control		Exposure−Control		
Endpoint	Mean	SE	Mean	SE	Mean	SE	P[a]	Mean	SE	Mean	SE	Mean	SE	P[a]
FEV_1 (% predicted)	95.79	1.78	95.64	1.67	0.15	2.44	0.952	91.68	2.20	88.50	2.38	3.18	3.23	0.330
FVC (% predicted)	104.2	2.04	104.9	2.21	−0.61	3.00	0.840	102.8	2.54	100.7	2.16	2.09	3.34	0.534
$FEF_{25–75}$ (% predicted)	69.48	3.00	68.39	3.18	1.09	4.37	0.803	61.42	2.98	58.08	3.51	3.34	4.59	0.470
eNO (ppb)	46.88	7.12	50.28	7.76	−3.40	10.53	0.748	44.69	6.26	39.44	5.36	5.25	8.24	0.526
EBC														
pH	8.02	0.07	8.06	0.06	0.05	0.09	0.630	7.92	0.07	7.90	0.08	0.03	0.11	0.815
Iron (nmol/L)	68.0	32.3	135	35.3	−67.4	48.0	0.172	94.5	19.3	323	275	−228	276	0.418
Blood TBARS (µmol/L)	2.50	0.27	2.14	0.13	0.36	0.29	0.228	2.40	0.19	2.23	0.13	0.17	0.23	0.463

[a] P from paired t tests of difference between the exposure and control sessions.

Table 11. Differences Between Mild and Moderate Asthma Groups in Baseline Endpoints and Biomarkers

	Mild Asthma				Moderate Asthma				Difference (Mild−Moderate)		
Endpoint	*N*	Mean	SE	CV (%)	*N*	Mean	SE	CV (%)	Mean	SE	*P*[a]
FEV_1 (% predicted)	61	95.72	1.210	9.870	57	90.11	1.615	13.530	5.601	2.001	0.006
FVC (% predicted)	61	104.5	1.488	11.11	57	101.8	1.662	12.33	2.782	2.225	0.214
$FEF_{25–75}$ (% predicted)	61	68.95	2.169	24.57	57	59.78	2.285	28.86	9.163	3.149	0.004
PEFR (L/min)	56	507	12.8	19.0	51	465	14.6	22.4	42.1	19.4	0.032
eNO (ppb)	62	48.6	5.22	84.7	58	42.1	4.10	74.2	6.51	6.70	0.333
EBC											
Iron (nmol/L)	29	103	24.4	128	22	209	137	308	−106	122	0.391
pH	62	8.04	0.05	4.54	58	7.91	0.05	5.05	0.13	0.07	0.060
H^+ (nmol/L)	62	13.0	1.40	84.4	58	19.0	2.50	99.6	−6.00	2.80	0.034
Blood TBARS (µmol/L)	27	2.31	0.15	22.0	32	2.31	0.11	24.5	0.00	0.18	0.999
Symptom score	57	1.08	0.18	128	50	1.63	0.21	92.1	−0.55	0.28	0.050
Asthma reliever medication use (#/day)	57	0.39	0.08	154	50	0.74	0.15	144	−0.35	0.16	0.038
PC_{20} (mg/mL)	62	2.73	0.31	89.2	54	2.92	0.34	87.7	−0.19	0.46	0.684
Induced sputum											
IL-8 (ng/mL)	46	89.0	16.19	125	36	91.5	14.1	93.9	−2.44	22.2	0.913
MPO (ng/mL)	42	5.69	1.20	138	36	6.53	1.22	114	−0.84	1.72	0.629
ECP (ng/mL)	46	13.7	1.68	84.2	34	38.9	6.70	102	−25.3	6.07	< .0001
Eosinophils[b]	28	1.53	0.32	113	16	1.92	0.45	97.1	−0.39	0.54	0.478
Macrophages[b]	28	54.8	4.06	39.9	16	57.8	3.95	28.3	−2.97	6.16	0.632
Epithelial cells[b]	28	3.14	0.51	87.4	16	2.83	0.59	86.0	0.31	0.81	0.702
Lymphocytes[b]	28	0.62	0.11	94.9	16	1.03	0.26	103	−0.40	0.24	0.102
Neutrophils[b]	28	39.9	4.28	57.9	16	36.3	4.45	50.7	3.51	6.60	0.597

[a] Two-tailed *t* test was applied to test the mean difference between mild and moderate asthma group ($\alpha = 0.05$).

[b] % of total non-squamous cells.

the difference between the two groups was more significant ($P = 0.034$); H+ was lower for the mild asthma group. Among all the blood and induced sputum markers, only ECP was significantly different between the two asthma severity groups. (The mean for the mild asthma group was 65% of the mean for the moderate asthma group.)

Between-subject variability is reflected in the coefficient of variation (CV) values shown in Table 11. Within each asthma severity group, the following measured health endpoints and biomarkers had CV < 50%: FEV_1, FVC, FEF_{25-75}, PEFR, pH, TBARS, and macrophage count. Within-subject variability for all the other endpoints was > 50%.

Postsession Changes from Baseline

In this section, we descriptively contrast trends in postsession changes from baseline between the exposure and control sessions. In the next section, we will report between-session statistical significances determined from the repeated-measures mixed-effects linear regression models that account for potentially confounding covariates.

Figure 6 shows descriptive time-series plots of the mean percentages with changes from baseline values for each of the health endpoints measured at multiple time points during and after the exposure and control sessions. The time series were plotted separately for the mild asthma group and the moderate asthma group; they show group mean and SE at each time point of a measurement.

The spirometric tests began at hour −2 (~2 hours before the start of the session). Thus, the plots for FEV_1, FVC, and FEF_{25-75} show values at this time point (Figure 6). The group mean values of FEV_1 and FVC appear to be lower at hour −2 than those at hour 0 for both exposure and control sessions. Because the first reading at hour −2 was taken in the early morning, it would be reasonable to assume the low value was attributable to diurnal variation. Furthermore, this is likely to be exaggerated because subjects did not take their regular bronchodilator medication on the day of exposure. A spike at hour 0 was apparent at both the exposure and the control sites, with no differences between the two sites.

As shown in Figure 6, panel A, FEV_1 for the mild asthma group decreased from the baseline value at hour 1 of both the exposure and the control session. However, the decrease appeared to be larger during the exposure session than during the control session. At the end of each session (hour 2), the control session FEV_1 did not decrease from that taken at hour 1, but the exposure session FEV_1 dropped to its lowest point. After completing each 2-hour session, subjects returned to the base hospital for postexposure measurements starting at hour 3. FEV_1 values rose from hour 2 and remained relatively stable between hours 5 and 7. At all time points after the session, including hour 22, the FEV_1 concentrations did not return to the baseline values. However, they were closer to the hour −2 values than to those at hour 0. The difference between the two sessions decreased at hour 22 for the mild asthma group. The plots for the moderate asthma group showed a similar trend. However, the difference between the exposure and control sessions appeared to be larger than that for the mild asthma group.

Like FEV_1, FVC (Figure 6A) also decreased more during the exposure session than during the control session, especially between hours 1 and 2, and the largest reduction was at the end of the session (hour 2). Postsession measurements indicated that subjects in the mild asthma group experienced a gradual increase in FVC (recovery) between hours 3 and 22. However, recoveries for the moderate asthma group took longer. The difference in FVC change (from baseline) between the exposure and control sessions appeared to be much larger for the moderate asthma group.

Group mean values of FEF_{25-75} showed a pattern similar to those of FEV_1 and FVC (Figure 6, panel A). Exposure session values were lower at all time points of measurement during and after the session, compared with the corresponding control-session values. However, a noticeable reduction in FEF_{25-75} from baseline was found only at hours 1 and 2 during the exposure session for both asthma severity groups. In contrast, during and after the control session, an increase in FEF_{25-75} from baseline was observed for all time points in the mild asthma group and all but one time point in the moderate asthma group (the one exception was at hour 2).

The eNO measurements were made only at the hospital, and thus no during-session data were available. When viewing eNO changes from baseline after the exposure and control sessions (see Figure 6, panel B), we did not observe any clear trends for either asthma group.

The EBC samples were collected at hours −2 (baseline), 3, and 6. The plots for each of the two EBC markers (pH and iron) show changes from baseline at these two time points (Figure 6, panel B). For both asthma severity groups we observed a clear reduction in pH from baseline at both time points after the exposure session. The corresponding values after the control session were not reduced for either group. In contrast, we did not observe a consistent pattern for EBC iron across the two severity groups.

Figure 6, panel C also shows mean changes from baseline for the three health endpoints that were measured after each session every day for 7 days. For both asthma severity groups, daily mean PEFR values were lower after

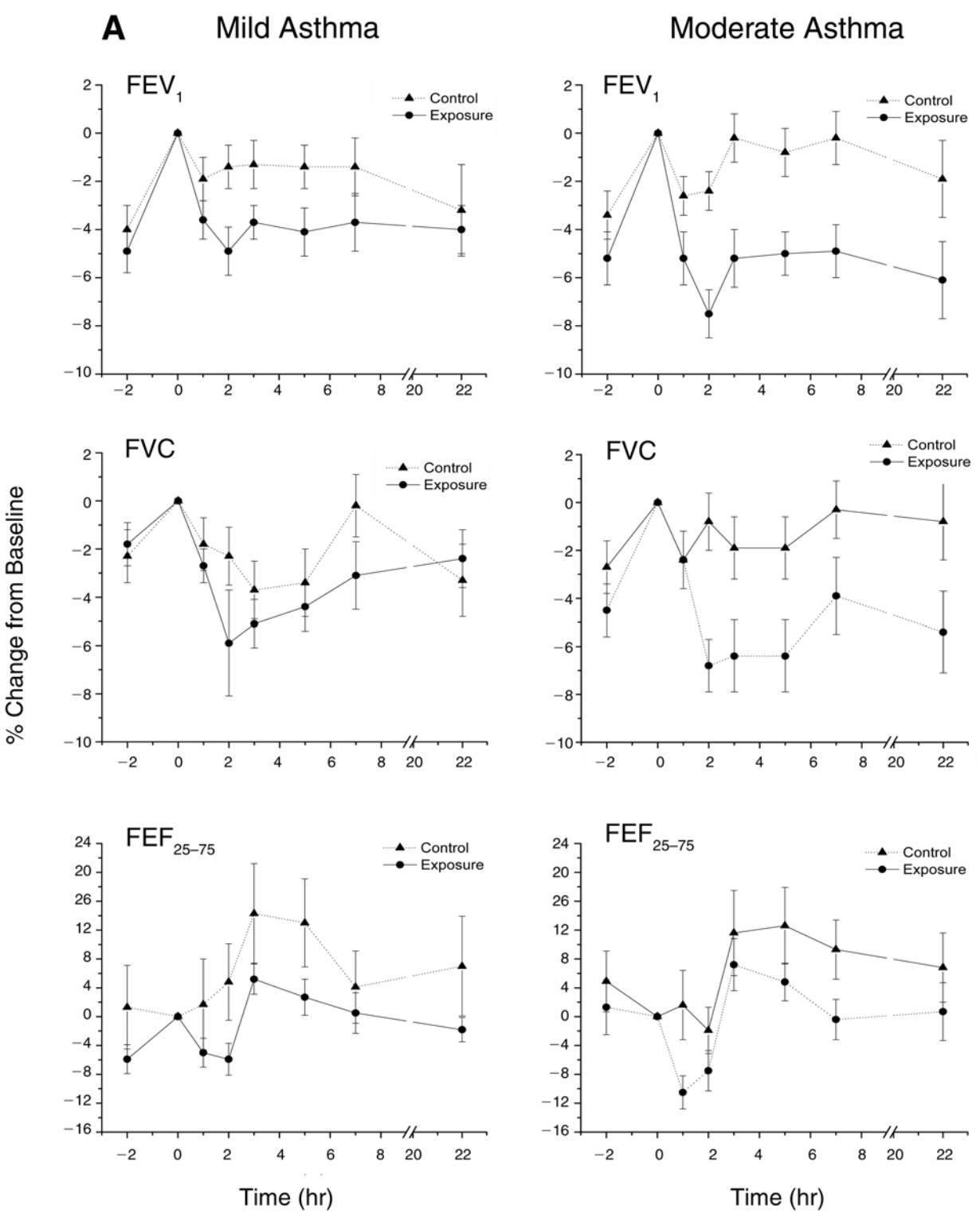

Figure 6. Mean percentage change from baseline in health endpoints and biomarkers for subjects with mild asthma and for subjects with moderate asthma. Baseline values were measured at the start of the session (Time = hr 0). Error bars represent SEs. A: FEV_1, FVC, and $FEF_{25–75}$. *Figure continues next 2 pages.*

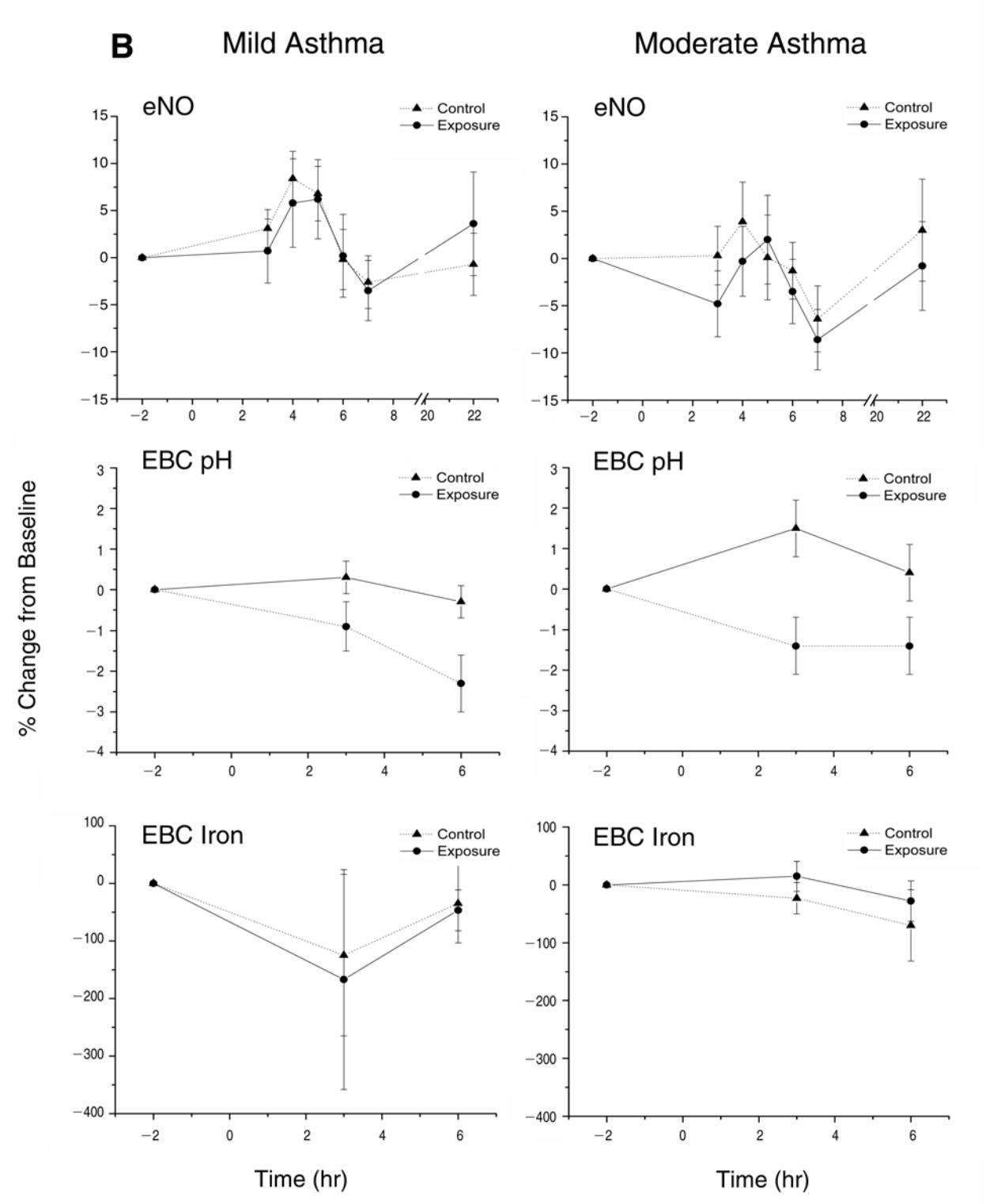

Figure 6 (*Continued*). B: eNO, EBC pH, and EBC iron.

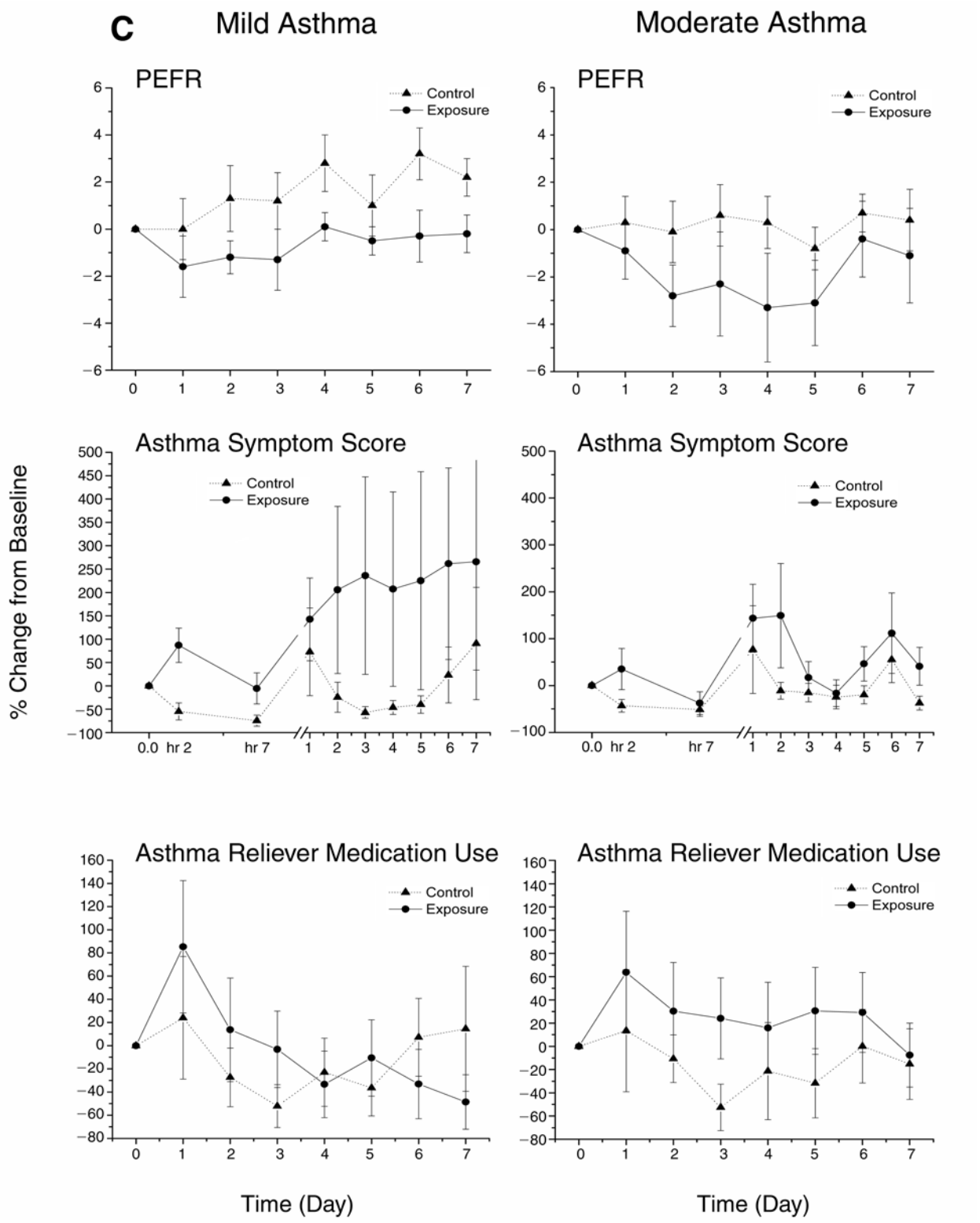

Figure 6 (*Continued*). C: PEFR, asthma symptom score, and asthma reliever medication use.

Table 12. Endpoint and Biomarker Changes from Baseline after Each Session, by Asthma Severity Category

	Mild Asthma (% Change)					Moderate Asthma (% Change)				
	Exposure		Control			Exposure		Control		
Endpoint	Mean	SE	Mean	SE	*P*	Mean	SE	Mean	SE	*P*
Blood TBARS	34	19	30	7	0.837	9	10	19	9	0.489
PC_{20}	122	40	189	57	0.339	87	37	157	52	0.273
Induced sputum										
IL-8	124	42	108	52	0.813	151	62	111	60	0.645
MPO	101	39	11	28	0.024	1552	1265	174	188	0.296
ECP	285	129	289	126	0.982	86	47	121	95	0.747
Eosinophils	184	93	47	38	0.196	19	25	68	75	0.557
Macrophages	−33	15	−13	16	0.353	−16	34	−29	12	0.728
Epithelial cells	18	23	53	31	0.358	72	72	85	37	0.882
Lymphocytes	284	131	181	54	0.481	183	108	294	159	0.574
Neutrophils	104	29	56	23	0.208	79	49	80	45	0.992

the exposure session than after the control session. The results were consistent throughout the week. In general, asthma symptom scores were consistently higher at hours 2 and 7 and in the days after the exposure session than the corresponding control session scores. The difference between the two sessions appeared to be greater for the mild asthma group than for the moderate asthma group. After the exposure session, the frequency of daily asthma reliever medication use appeared to be higher in the first 3 days for the mild asthma group and in the first 6 days for the moderate asthma group. (Among the 60 subjects, only 2 from the mild asthma group reported that they used asthma reliever medication during both the exposure and the control sessions.)

Blood TBARS, PC_{20}, and induced sputum markers were measured at only one time point after each session. The postsession changes from baseline for these markers are contrasted in Table 12. *P* values are given to compare the difference in the changes from baseline to postsession between the exposures. The mean TBARS concentrations (with large SE values) increased from the baseline values after each of the two sessions for both asthma severity groups, but there was no clear pattern indicating which session led to a higher increase across the two groups. The PC_{20} concentrations also increased after each of the two sessions, with smaller increases after the exposure session for both asthma groups. The moderate asthma group appeared to have smaller PC_{20} increases than the mild asthma group.

Among the sputum markers, IL-8 and MPO had similar patterns. After each exposure, the group mean values increased from the baseline values for both asthma groups. The increase appeared to be greater after the exposure session than after the control session, and the increase appeared to be greater for the moderate asthma group than for the mild asthma group. In general, a postsession increase in group mean from baseline was also observed for ECP, eosinophils, epithelial cells, lymphocytes, and neutrophils. However, a large SE was associated with the mean percentage change for each of these markers, and there appeared to be no clear pattern indicating which session led to a higher increase across the two groups. A postsession decrease in group mean (with large SE) was observed for macrophages, with no clear pattern indicating which session led to higher decrease across the two groups.

A similar presession and postsession comparison for 1-OHP was not conducted, because a substantial fraction of the samples had concentrations below the detection limit. (Among the presession samples, 17% and 35% were below the detection limits for the control and exposure sessions, respectively. Among the postsession samples, 36% and 32% were below detection limits for control and exposure sessions, respectively.)

Differences in Health Endpoints between Exposure and Control Sessions

Detailed results, both from the stratified analyses by asthma severity group and the nonstratified (combined) analyses, are summarized in Appendix H for every health endpoint and biomarker measured in the study. Differences in both absolute value and relative change from

Table 13. Nonstratified Analyses of Control-Subtracted Percent Changes from Baseline for Selected Health Endpoints During and After the Exposure and Control Sessions

Endpoint / Hour of Measurement	Mean (% Change)	SE (% Change)	P[a]
FEV_1			
1	−0.95	0.83	0.286
2	−3.23	1.04	0.004
3	−3.29	1.15	0.008
5	−4.11	1.25	0.002
7	−3.02	1.28	0.028
22	−2.01	1.30	0.151
Overall			0.043
FVC			
1	1.03	1.22	0.384
2	−3.06	1.45	0.029
3	−3.52	1.54	0.018
5	−3.68	1.59	0.018
7	−2.82	1.61	0.070
22	−1.90	1.61	0.226
Overall			0.012
EBC			
Iron			
3	4.26	64.8	0.923
6	96.4	85.9	0.099
Overall			0.103
pH			
3	−1.99	0.05	0.002
6	−1.33	0.05	0.052
Overall			0.008
Blood TBARS	6.38	0.26	0.573
PC_{20}	−19.6	1.09	0.612
Sputum			
IL-8	38.5	50.84	0.498
MPO	521	12.58	0.014
EOP	30.7	14.24	0.600
Eosinophils	25.0	0.78	0.591
Macrophages	−15.3	5.93	0.154
Epithelial Cells	−17.0	0.66	0.438
Lymphocytes	11.6	0.25	0.725
Neutrophils	23.3	6.04	0.142

[a] Statistical significance for percent change in individual time points of FEV_1 and FVC is $P \leq 0.01$. For eNO and EBC time points, $P \leq 0.008$ and 0.025 respectively. For blood TBARS, sputum, and PC_{20}, $P \leq 0.05$.

baseline are reported in Appendix B. For all endpoints, an overall P value represents whether there are significant changes in health endpoint at any time after baseline. Additionally, for FEV_1, FVC, $FEF_{25–75}$, a Bonferroni-corrected alpha level of 0.01 was required at each time point to maintain a family-wise error rate of 0.05 for all time points within each health endpoint. For eNO and EBC, individual time points were tested at 0.008 and 0.025 levels, respectively, using the Bonferroni correction.

The nonstratified analysis shows that FEV_1 reduction was significantly different (overall $P = 0.043$; see Table 13) after the exposure session from that after the control session. This reduction was significant at hours 2, 3, and 5. The largest was from baseline to hour 5, where the reduction was

Table 14. Stratified Analyses of Control-Subtracted Percent Changes from Baseline for Selected Health Endpoints During and After the Exposure and Control Sessions

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*
FEV_1 (From Table B.2)								
1	−0.61	−0.64	1.17	0.602	−1.19	−1.32	1.19	0.318
2	−2.54	−2.66	1.49	0.089	−3.48	−3.86	1.45	0.017
3	−1.84	−1.92	1.66	0.269	−4.38	−4.86	1.58	0.006
5	−2.93	−3.07	1.82	0.109	−4.81	−5.34	1.67	0.004
7	−1.88	−1.97	1.88	0.318	−3.88	−4.30	1.70	0.023
22	−0.51	−0.53	1.92	0.792	−3.35	−3.72	1.71	0.051
FVC (From Table B.4)								
1	0.20	0.19	1.52	0.895	1.97	1.94	1.93	0.309
2	−0.53	−0.50	1.82	0.772	−6.10	−6.00	2.27	0.008
3	−0.33	−0.32	1.94	0.864	−7.25	−7.12	2.38	0.003
5	−0.60	−0.58	2.01	0.765	−7.31	−7.18	2.44	0.003
EBC pH (From Table B.12)								
3	−0.10	−1.19	0.06	0.112	−0.23	−2.92	0.08	0.006
6	−0.09	−1.11	0.07	0.184	−0.12	−1.53	0.08	0.147

4.1% more for the exposure session than the control session. FEV_1 was reduced at all other time points as well. The results from the stratified analysis (Table 14) indicate that FEV_1 reduction was mainly driven by the moderate asthma group, with the largest reduction being 5.3% at hour 5 for the exposure session after subtracting out changes under the control session.

The results for FVC were similar (overall $P = 0.012$; Table 13), with reductions of more than 3% at hours 2, 3, and 5 which were not significant after a Bonferroni correction. The reductions in FVC for both asthma severity groups were mainly driven by reductions within the moderate asthma group at hours 2, 3, and 5 ($P < 0.01$; Table 14). For example, this group experienced a 7.2% reduction at hour 5.

The effect of exposure on changes in EBC pH was significant (overall $P = 0.008$; Table 13). EBC pH was significantly lower after the exposure than after the control session at hour 3 ($P = 0.002$). At hour 3, the pH reduction (group mean) was 2.0% more than that of the control session for all the subjects and 2.9% for the moderate asthma group ($P = 0.006$; Table 14). The between-session difference in EBC iron concentration, a 96% increase from baseline, reached marginal significance at hour 6 ($P = 0.099$). The eNO, PC_{20}, and blood TBARS measurements showed no significant differences between the exposure and control sessions. The only sputum biomarker that exhibited statistical significance was MPO. Compared with the control session, the exposure session led to a 521% increase in mean MPO for all subjects ($P = 0.014$; Table 13).

The $FEF_{25–75}$ values appeared to be lower after the exposure session than after the control session. The largest difference in change from baseline was 5.0% for all subjects and 6.9% for the mild asthma group (both at hour 5). However, these differences were not statistically significant (Tables 15 and 16). Similarly, no differences in PEFR, symptom score, and asthma reliever medication use were statistically significant at any of the time points (days 1–7). However, the trends from the nonstratified analysis were consistent with those shown in Figure 6. Compared with the control session, the exposure session led to a reduction in PEFR from baseline of up to 2.0% for all subjects and up to 3.4% for the mild asthma group (Tables 17 and 18), an increase in symptom score from baseline of up to 47% for all the subjects and up to 48% for the mild asthma group (Tables 19 and 20), and an increase in daily asthma reliever

Table 15. $FEF_{25–75}$ from Nonstratified Analysis[a]

Time (Hr)	Baseline (% Predicted)	β_3 (% Predicted)	β_3/Baseline (%)	SE	*P*
1	64.52	−2.30	−3.56	1.80	0.203
2	64.52	−2.23	−3.45	2.09	0.286
3	64.52	−0.98	−1.53	2.18	0.651
5	64.52	−3.21	−4.97	2.22	0.150
7	64.52	−2.54	−3.94	2.23	0.255
22	64.52	−2.54	−3.94	2.24	0.257
Overall		*df* = (6, 528)		*F* = 0.59	0.737

[a] This table was copied from Appendix Table B.5. See abbreviations list for *df*, *F* and β_3.

Table 16. $FEF_{25–75}$ from Stratified Analysis[a]

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*
1	68.95	−1.01	−1.46	2.26	0.656	59.78	−3.69	−6.17	2.82	0.193
2	68.95	−4.46	−6.47	2.77	0.109	59.78	0.12	0.19	3.03	0.969
3	68.95	−3.21	−4.65	3.02	0.289	59.78	1.42	2.38	3.05	0.641
5	68.95	−4.77	−6.92	3.20	0.137	59.78	−1.58	−2.64	3.06	0.606
7	68.95	−0.96	−1.39	3.25	0.768	59.78	−4.50	−7.52	3.06	0.143
22	68.95	−3.04	−4.40	3.29	0.357	59.78	−2.06	−3.45	3.06	0.501
Overall		*df* = (6, 271)		*F* = 0.84	0.538		*df* = (6, 243)		*F* = 1.03	0.407

[a] This table was copied from Appendix Table B.6.

Table 17. PEFR from Nonstratified Analysis[a]

Time (Hr)	Baseline (L/min)	β_3 (L/min)	β_3/Baseline (%)	SE	*P*
1	487	−0.24	−0.05	5.63	0.966
2	487	−5.81	−1.19	6.55	0.375
3	487	−3.69	−0.76	6.84	0.590
4	487	−9.58	−1.97	6.94	0.168
5	487	−5.92	−1.22	6.99	0.398
6	487	−8.68	−1.78	6.99	0.215
7	487	−3.13	−0.64	7.04	0.657
Overall		*df* = (7, 558)		*F* = 0.56	0.785

[a] This table was copied from Appendix Table B.13.

Table 18. PEFR from Stratified Analysis[a]

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline (L/min)	β_3 (L/min)	β_3/ Baseline (%)	SE	*P*	Baseline (L/min)	β_3 (L/min)	β_3/ Baseline (%)	SE	*P*
1	507	−1.14	−0.23	7.95	0.886	465	0.75	0.16	7.96	0.925
2	507	−10.70	−2.11	8.65	0.217	465	0.04	0.01	9.75	0.997
3	507	−3.19	−0.63	8.78	0.717	465	−4.08	−0.88	10.53	0.699
4	507	−11.25	−2.22	8.80	0.202	465	−7.28	−1.57	10.90	0.505
5	507	−15.51	−3.06	8.84	0.081	465	5.60	1.21	11.08	0.614
6	507	−17.35	−3.42	8.80	0.050	465	1.47	0.32	11.17	0.896
7	507	−9.99	−1.97	8.80	0.257	465	4.57	0.98	11.34	0.687
Overall		*df* = (7, 291)		*F* = 1.00	0.433		*df* = (7, 251)		*F* = 0.44	0.878

[a] This table was copied from Appendix Table B.14.

Table 19. Asthma Symptom Score from Nonstratified Analysis [a]

Time	Baseline	β_3	β_3/Baseline (%)	SE	*P*
Hr					
2	1.33	0.19	14.46	0.31	0.534
7	1.33	0.07	5.11	0.40	0.864
Day					
1	1.33	0.51	38.08	0.41	0.213
2	1.33	0.62	46.88	0.41	0.125
3	1.33	0.00	0.37	0.41	0.990
4	1.33	−0.19	−14.22	0.41	0.643
5	1.33	0.14	10.17	0.41	0.740
6	1.33	−0.27	−20.59	0.41	0.502
7	1.33	0.10	7.40	0.41	0.809
Overall		*df* = (9, 717)		*F* = 0.94	0.486

[a] This table was copied from Appendix Table B.15.

Table 20. Asthma Symptom Score from Stratified Analysis[a]

	Mild Asthma					Moderate Asthma				
Time	Baseline	β_3	β_3/ Baseline (%)	SE	*P*	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
Hr										
2	1.08	−0.01	−0.70	0.43	0.986	1.63	0.44	27.12	0.38	0.240
7	1.08	−0.27	−24.87	0.50	0.589	1.63	0.47	28.82	0.57	0.408
Day										
1	1.08	0.50	46.48	0.50	0.314	1.63	0.49	30.19	0.65	0.450
2	1.08	0.52	47.84	0.50	0.300	1.63	0.76	46.55	0.65	0.247
3	1.08	0.01	0.47	0.50	0.992	1.63	0.03	1.56	0.65	0.969
4	1.08	0.08	7.69	0.50	0.868	1.63	−0.48	−29.21	0.66	0.470
5	1.08	−0.02	−2.26	0.50	0.961	1.63	0.35	21.20	0.66	0.600
6	1.08	−0.02	−2.26	0.50	0.961	1.63	−0.60	−36.93	0.66	0.361
7	1.08	0.14	13.34	0.50	0.773	1.63	0.12	7.38	0.66	0.855
Overall		*df* = (9, 376)		*F* = 0.47	0.893		*df* = (9, 321)		*F* = 0.95	0.486

[a] This table was copied from Appendix Table B.16.

Table 21. Asthma Reliever Medication Use from Nonstratified Analysis[a]

Time (Hr)	Baseline	β_3	β_3/Baseline (%)	SE	*P*
1	0.55	0.07	13.28	0.17	0.676
2	0.55	0.01	1.15	0.20	0.975
3	0.55	0.12	21.36	0.21	0.570
4	0.55	0.04	8.14	0.21	0.831
5	0.55	0.07	13.29	0.21	0.728
6	0.55	−0.04	−7.16	0.21	0.851
Overall		*df* = (6, 525)		*F* = 0.19	0.981

[a] This table was copied from Appendix Table B.17.

Table 22. Asthma Reliever Medication Use from Stratified Analysis[a]

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline	β_3	β_3/ Baseline (%)	SE	*P*	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
1	0.39	−0.07	−17.58	0.19	0.718	0.74	0.25	33.78	0.32	0.435
2	0.39	−0.03	−7.33	0.21	0.893	0.74	0.05	6.76	0.36	0.891
3	0.39	0.01	2.93	0.22	0.958	0.74	0.25	33.78	0.38	0.508
4	0.39	−0.11	−27.85	0.22	0.620	0.74	0.24	32.23	0.38	0.534
5	0.39	−0.11	−27.85	0.22	0.621	0.74	0.30	41.12	0.38	0.429
6	0.39	−0.11	−27.85	0.22	0.621	0.74	0.05	6.10	0.38	0.907
Overall		*df* = (6, 288)		*F* = 0.12	0.994		*df* = (6, 225)		*F* = 0.29	0.940

[a] This table was copied from Appendix Table B.18.

medication use of up to 21% for all subjects and up to 41% for the moderate asthma group (Tables 21 and 22).

Results from this mixed repeated-measures analysis were similar to those based on paired *t* test results with multiple testing corrections.

RELATIONS AMONG HEALTH ENDPOINTS

By examining changes in biomarkers, at individual subject levels, which were associated with FEV_1 changes from their baseline values, we obtained the results shown in Table 23. The Spearman rank correlation analysis indicates that FEV_1 changes at hour 2 were marginally correlated with changes in EBC pH measured at hour 6, PC_{20} measured at hour 7, and IL-8 measured at hour 22. Changes in FEV_1 measured at hour 5 were correlated only with changes in EBC iron measured at hour 6. Changes in FEV_1 measured at hour 7 were correlated with changes in EBC pH at hour 6, PC_{20} at hour 7, and eNO at hour 7. Changes in FEV_1 at hour 22 were correlated only with changes in sputum IL-8 measured at hour 22.

We also examined correlations in changes from baseline among all the health endpoints and biomarkers measured at the same time point. Significant correlations, however, were found only between some of the sputum biomarkers, (Table 24). The results indicate that an increase in MPO was associated with an increase in IL-8, an increase in neutrophils, and a decrease in macrophages, across the subjects. An increase in neutrophils was associated with an increase in IL-8, a decrease in macrophages, and a decrease in epithelial cells.

Table 23. Correlations Between FEV_1 and Each of the Health Endpoints that Show Statistical Significance ($P < 0.05$) or Marginal Significance ($P < 0.10$) [a]

Endpoints / FEV_1 Hour of Measurement	*r*	*P*	*n*
pH (hr 6)			
2	0.17	0.0679	117
7	0.18	0.0483	117
PC_{20} (hr 7)			
2	0.18	0.0563	110
7	0.21	0.027	110
Fe (hr 6)			
5	0.41	0.01	38
eNO (hr 7)			
7	0.23	0.0135	118
IL-8 (hr 22)			
2	0.19	0.0853	80
22	−0.28	0.0121	80

[a] *r* indicates Spearman correlation coefficient; *P* indicates probability of rejecting no correlation between the two variables; *n* indicates number of observations.

Table 24. Correlations Among the Sputum Markers that Show Statistical Significance ($P < 0.05$) or Marginal Significance ($P < 0.10$) [a]

Sputum Marker / Correlating Sputum Marker	*r*	*P*	*n*
MPO			
IL-8	0.58	< 0.0001	77
Macrophages			
IL-8	−0.46	0.0031	40
MPO	−0.58	0.0001	39
Epithelial cells			
IL-8	−0.47	0.0022	40
ECP	−0.28	0.0956	37
Macrophages	0.34	0.0250	44
Lymphocytes			
ECP	0.31	0.0658	37
Eosinophils	0.31	0.0509	40
Neutrophils			
IL-8	0.55	0.0003	40
MPO	0.55	0.0003	39
Macrophages	−0.91	< 0.0001	44
Epithelial cells	−0.40	0.0071	44

[a] *r* indicates Spearman correlation coefficient; *P* indicates probability of rejecting no correlation between the two variables; *n* indicates number of observations.

The raw data of neutrophils (the percentage of total nonsquamous cells) appeared to be normally distributed. Based on a paired *t* test of the raw data, group mean neutrophils significantly increased ($P = 0.049$) after the exposure sessions compared with group mean neutrophils after the control sessions (exposure: mean = 57.0%, SD = 22.8%; control: mean = 50.0%, SD = 23.6%). Using the mixed-effects model with adjusted covariates, we found a 23.3% increase from baseline in neutrophils with moderate statistical significance after exposure sessions, relative to control sessions ($P = 0.142$; Table 13). Across all of the subjects, MPO was significantly and positively correlated with neutrophils ($r = 0.55$, $P < 0.0003$; Table 24).

Because the distribution of IL-8 concentration data was skewed, it would be more appropriate to compare medians than means. There was a significant increase in median IL-8 concentration after the exposure sessions compared with the control sessions (exposure: median = 82.1 ng/mL, range = 6.9–1114 ng/mL; control: median = 66.8 ng/mL, range = 9.6–1076 ng/mL; $P = 0.045$ based on Wilcoxon Signed Rank test). In our analysis using the mixed-effects model with covariates adjusted, we also observed a 38.5% increase from baseline in sputum IL-8 after the exposure sessions compared with the control sessions, although this increase was not statistically significant ($P = 0.498$; see Table 13). Postsession changes from baseline were significantly correlated between IL-8 and neutrophils ($r = 0.55$, $P < 0.0003$) and between IL-8 and MPO ($r = 0.58$, $P < 0.0001$; Table 24). In addition, we found a significant and positive association between sputum IL-8 concentrations and during-session NO_2 exposure (Figure 7, panel D).

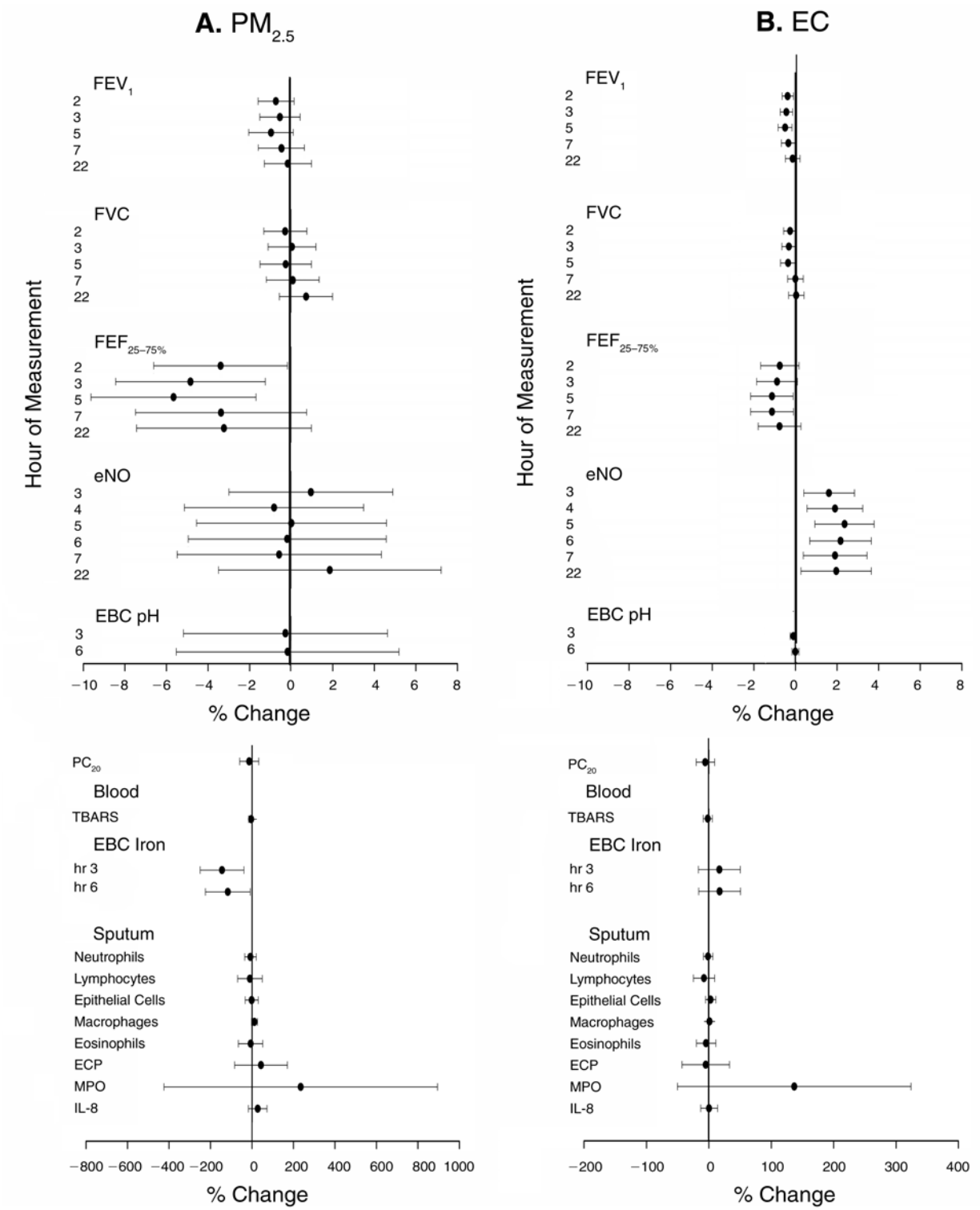

Figure 7. Point estimates and 95% CIs of the percentage change in health endpoints and biomarkers (see text for details of calculation) per A: 10-μg/m^3 incremental change in $PM_{2.5}$ concentrations averaged over the exposure and control sessions; **B:** 1-μg/m^3 incremental change in EC concentrations averaged over the exposure and control sessions. *Figure continues next page.*

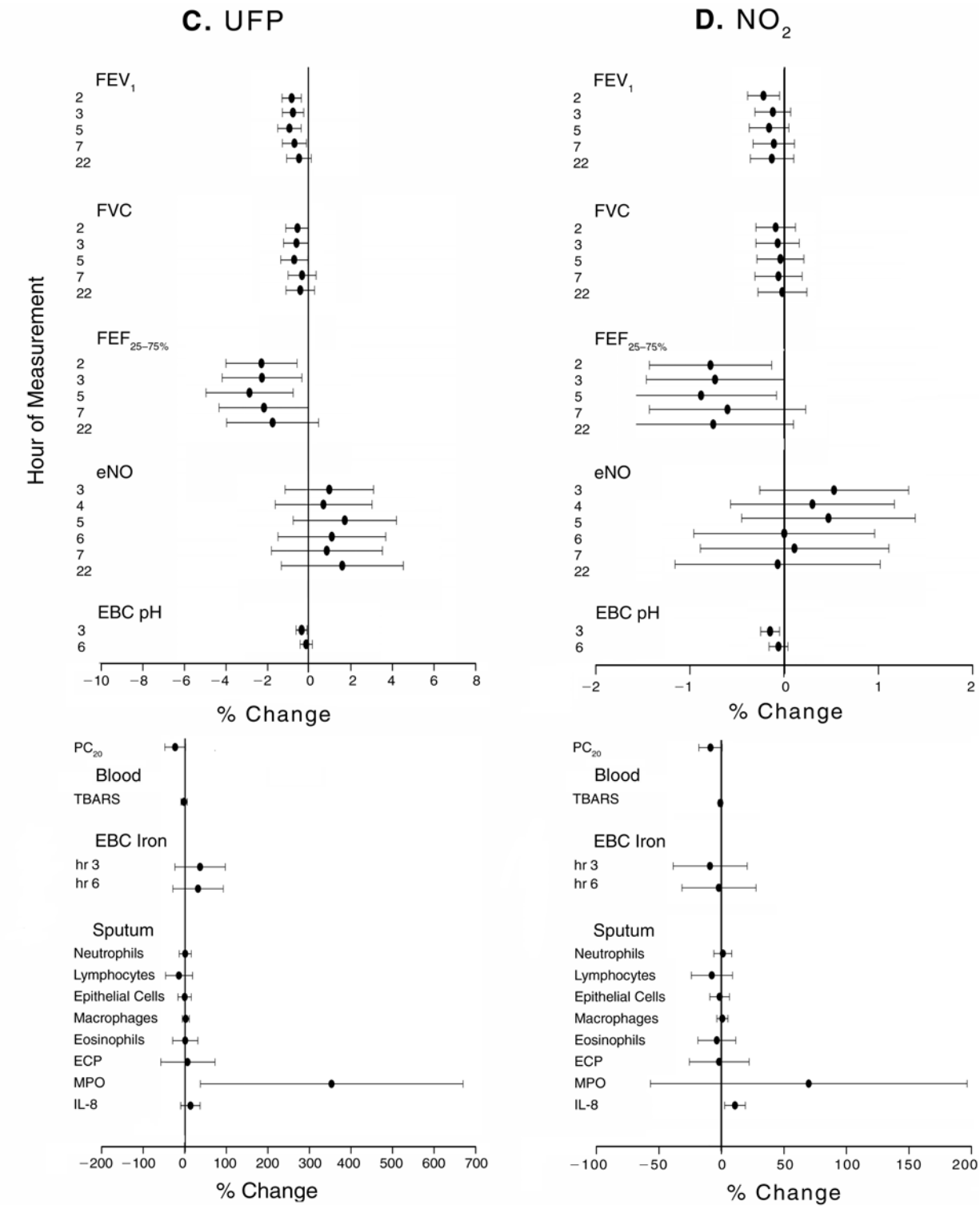

Figure 7 (*Continued*). C: 10,000-UFP/cm^3 incremental change in UFP concentrations; **D:** 10-µg/m^3 incremental change in NO_2 concentrations.

POLLUTANT-SPECIFIC EXPOSURE–RESPONSE RELATIONS

In this section, we report results from an exploratory analysis of pollutant-specific exposure–response relations. The relations, reported as point (mean) estimates and 95% CIs of percentage change in health endpoint or biomarker from the baseline, associated with a defined unit change of pollutant concentration, are summarized in Appendix C for each of the measured health endpoints. Unit changes of pollutant concentration were defined as 10 μg/m^3 for $PM_{2.5}$, 1 μg/m^3 for EC, 10,000 particles/cm^3 for UFP, and 10 μg/m^3 for NO_2. We used realistic scales of pollutant concentration change to obtain reasonable magnitudes of health endpoint change associated with a unit change in concentration for each of the four pollutants. The results are reported in Appendix C for nonstratified analyses (for all subjects) and for stratified analyses by asthma severity category.

Using the results from nonstratified analyses for all the health endpoints, we plotted the point estimates and 95% CIs of changes in endpoint per unit change in pollutant in Figure 7, panels A–D. For all the endpoints, an overall P value is used to determine whether there were significant changes in the health endpoint at any time after baseline. Additionally, for FEV_1, FVC, and $FEF_{25–75}$, a Bonferroni-corrected $\alpha = 0.01$ was required at each time point to maintain a family-wise error rate of 0.05 for all time points within each health endpoint. For eNO and EBC, individual time points were tested at $\alpha = 0.008$ and $\alpha = 0.025$, respectively, using the Bonferroni correction. In Figure 7, a few endpoints that had unusually large baseline variation are reported as showing more than a 100% reduction; this resulted from the way the estimates were calculated, first estimating the decrease and then dividing by the average baseline level.

As illustrated in Figure 7, panel A, $PM_{2.5}$ exposure was marginally associated with $FEF_{25–75}$ (overall $P = 0.100$; $P < 0.01$ at hrs 3 and 5). (See Table 25 for nonstratified data and Table 26 for stratified data; effect sizes at other time points followed a pattern similar to that for hrs 3 and 5, but with less significance.) Neither FEV_1 nor FVC were associated with $PM_{2.5}$. A 10-μg/m^3 increase in $PM_{2.5}$ during the 2-hour session led to an average reduction in $FEF_{25–75}$ of 4.8% at hour 3 and 5.6% at hour 5. An asthma-severity stratified analysis indicates that the slope significance of $FEF_{25–75}$ versus $PM_{2.5}$ was mainly driven by the mild asthma group (overall $P = 0.005$; $P < 0.01$ for all time points except hour 7). Within the mild asthma group, $PM_{2.5}$ was significantly associated with $FEF_{25–75}$ at all the time points, with the largest slope of −9.6% at hour 5. In contrast, a statistical significance was not found at any of the $FEF_{25–75}$ versus $PM_{2.5}$ time points for the moderate asthma group (overall $P = 0.793$).

Among the biomarkers in Figure 7, panel A, only EBC iron was significantly associated with $PM_{2.5}$ (overall $P = 0.020$; $P < 0.008$ for hour 3; Table 25). A 10-μg/m^3 increase in $PM_{2.5}$ during the 2-hour session led to an average reduction in the EBC free iron concentration of 144% at hour 3 and 116% at hour 6 for all the subjects combined.

The EBC free iron reduction was greater in the moderate asthma group (213% and 200%, hours 3 and 6 respectively; overall $P = 0.018$; Table 26). In contrast, no significant reduction was found for the mild asthma group.

As shown in Figure 7, panel B, health endpoints having a significant association with EC exposure included FEV_1 (overall $P = 0.043$; $P < 0.01$ at hours 2, 3, and 5, with other time points showing a similar trend), $FEF_{25–75}$ (marginally; overall $P = 0.315$; $P = 0.031$ and 0.034 at hours 5 and 7, respectively), and eNO (overall $P = 0.064$; $P < 0.009$ at hours 3, 4, 5, and 6). (See Table 27 for nonstratified data and Table 28 for stratified data.) A 1-μg/m^3 increase in EC during the 2-hour session led to an average reduction in FEV_1 of up to 0.49% (at hour 5) for all the subjects and of up to −0.63% (at hour 3) for the subjects with moderate asthma.

A 1-μg/m^3 increase in EC during the 2-hour session led to an average reduction in $FEF_{25–75}$ of 1.1% (at hours 5 and 7) for all the subjects and of 1.1% to 1.2% for the subjects with mild asthma. No statistically significant effect was found for the moderate asthma group, although the point estimates were all negative.

A 1-μg/m^3 increase in EC during the 2-hour session led to an average increase from baseline in eNO of up to 2.4% (at hour 5) for all the subjects and of up to 3.1% for the subjects with mild asthma. No significant effect was found for the subjects with moderate asthma, although the point estimates indicate an increase in eNO at each of the time points. No biomarkers were found to be significantly associated with EC (Table 27).

Figure 7, panel C shows that exposure to UFP was significantly associated with FEV_1 (overall $P = 0.015$; $P < 0.01$ at hours 2, 3, and 5), EBC pH (overall $P = 0.031$; $P < 0.018$ at hour 3), and sputum MPO ($P = 0.029$). (See Table 29 for nonstratified data and Table 30 for stratified data.) UFP exposure was marginally associated with $FEF_{25–75}$ at hours 2 and 5 ($P < 0.01$). An incremental change of 10,000 UFP/cm^3 during the 2-hour session led to an average reduction in FEV_1 of up to 0.90% (at hour 5) for all the subjects, of up to 0.89% for the subjects with mild asthma (at hour 5), and of up to 0.92% for the subjects with moderate asthma (at hour 3). The average reduction in $FEF_{25–75}$, associated with an increase of 10,000 UFP/cm^3, was largest

Table 25. Nonstratified Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in $PM_{2.5}$[a,b]

Endpoint / Hour of Measurement	Point Estimate	95% CI		*P*
		Lower	Upper	
FEV_1				
2	−0.65	−1.52	0.21	0.139
3	−0.46	−1.43	0.51	0.349
5	−0.89	−1.96	0.18	0.103
7	−0.39	−1.50	0.72	0.490
22	−0.08	−1.22	1.05	0.885
Overall		*df* = (5, 412)	*F* = 0.94	0.457
FVC				
2	−0.25	−1.30	0.79	0.635
3	0.07	−1.08	1.22	0.911
5	−0.23	−1.47	1.01	0.715
7	0.11	−1.16	1.38	0.865
22	0.75	−0.54	2.03	0.254
Overall		*df* = (5, 410)	*F* = 0.65	0.658
$FEF_{25–75}$				
2	−3.36	−6.58	−0.15	0.041
3	−4.81	−8.41	−1.21	0.009
5	−5.63	−9.59	−1.66	0.006
7	−3.34	−7.44	0.77	0.111
22	−3.20	−7.41	1.01	0.136
Overall		*df* = (5, 410)	*F* = 1.86	0.100
eNO				
3	0.98	−2.96	4.92	0.626
4	−0.79	−5.09	3.52	0.720
5	0.05	−4.53	4.62	0.985
6	−0.16	−4.92	4.61	0.949
7	−0.54	−5.45	4.37	0.828
22	1.89	−3.48	7.25	0.490
Overall		*df* = (6, 506)	*F* = 0.50	0.805
EBC				
Fe				
3	−144	−249	−38.2	0.008
6	−116	−223	−7.78	0.036
Overall		*df* = (2, 74)	*F* = 4.13	0.020
pH				
3	−0.24	−0.75	0.27	0.352
6	−0.17	−0.73	0.38	0.534
Overall		*df* = (2, 168)	*F* = 0.44	0.647
Blood TBARS (7)	−3.37	−16	9.21	0.586
PC_{20} (7)	−13.4	−59.1	32.4	0.562
Sputum (22)				
IL-8	28.3	−16.6	73.1	0.211
MPO	235	−424	894	0.477
ECP	43.6	−83.4	171	0.493
Eosinophils	−6.59	−65.5	52.4	0.820
Macrophages	12.6	−1.91	27.1	0.086
Epithelial cells	−1.17	−32.4	30.1	0.939
Lymphocytes	−9.42	−68.6	49.8	0.748
Neutrophils	−7	−34.8	20.8	0.611

[a] Change is averaged over the exposure and control sessions using single-pollutant models.

[b] This table was copied from Appendix Table C.1.

Table 26. Stratified Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in $PM_{2.5}$[a,b]

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	Point Estimate	95% CI		*P*	Point Estimate	95% CI		*P*
		Lower	Upper			Lower	Upper	
FEV_1								
2	−1.14	−2.52	0.24	0.104	−0.17	−1.30	0.95	0.761
3	−1.11	−2.65	0.44	0.159	0.17	−1.08	1.42	0.786
5	−1.36	−3.05	0.34	0.117	−0.46	−1.82	0.90	0.508
7	−0.62	−2.38	1.14	0.488	−0.18	−1.58	1.23	0.806
22	−0.74	−2.53	1.06	0.420	0.61	−0.81	2.04	0.398
Overall		*df* = (5, 209)	*F* = 0.77	0.575		*df* = (5, 191)	*F* = 0.64	0.670
FVC								
2	0.08	−1.33	1.48	0.915	−0.56	−2.15	1.04	0.492
3	0.13	−1.41	1.67	0.866	0.08	−1.63	1.79	0.927
5	0.34	−1.32	1.99	0.690	−0.68	−2.48	1.11	0.452
7	0.50	−1.19	2.19	0.559	−0.20	−2.01	1.61	0.826
22	0.90	−0.81	2.61	0.299	0.69	−1.13	2.51	0.454
Overall		*df* = (5, 208)	*F* = 0.26	0.936		*df* = (5, 190)	*F* = 0.70	0.625
$FEF_{25–75}$								
2	−7.35	−11.7	−2.99	0.001	0.25	−3.49	3.99	0.895
3	−8.98	−14.0	−4.00	0.001	−1.25	−6.36	3.86	0.631
5	−9.56	−15.2	−3.89	0.001	−2.40	−7.21	2.42	0.328
7	−5.92	−11.9	0.08	0.053	−1.26	−5.96	3.45	0.599
22	−8.34	−14.7	−1.99	0.010	1.19	−3.46	5.83	0.615
Overall		*df* = (5, 208)	*F* = 3.43	0.005		*df* = (5, 190)	*F* = 0.48	0.793
eNO								
3	6.75	1.28	12.2	0.016	−3.89	−9.6	1.82	0.181
4	5.17	−0.79	11.1	0.089	−5.67	−11.9	0.54	0.073
5	5.88	−0.44	12.2	0.068	−4.60	−11.1	1.95	0.168
6	6.81	0.24	13.4	0.042	−6.21	−13.0	0.59	0.073
7	6.55	−0.21	13.3	0.057	−6.34	−13.3	0.63	0.075
22	11.00	3.69	18.3	0.003	−6.18	−13.6	1.28	0.104
Overall		*df* = (6, 257)	*F* = 1.98	0.069		*df* = (6, 235)	*F* = 0.90	0.493
EBC								
Fe								
3	−70.9	−213	70.7	0.317	−213	−366	−59.9	0.008
6	14.3	−127	156	0.839	−200	−368	−32.1	0.021
Overall		*df* = (2, 38)	*F* = 0.77	0.470		*df* = (2, 31)	*F* = 4.59	0.018
pH								
3	−0.46	−1.1	0.17	0.152	−0.1	−0.93	0.72	0.805
6	−0.46	−1.18	0.25	0.201	0	−0.9	0.90	0.998
Overall		*df* = (2, 86)	*F* = 1.13	0.329		*df* = (2, 77)	*F* = 0.05	0.724
Blood TBARS (7)	−8.39	−35.8	19	0.506	−4.54	−18.3	9.19	0.473
PC_{20} (7)	−21.2	−94.8	52.4	0.563	−20.8	−86.4	44.7	0.522
Sputum (22)								
IL-8	46.3	1.56	90.9	0.043	46.8	−47.3	141	0.310
MPO	12.9	−25.9	51.7	0.498	611	−896	2117	0.406
ECP	38.5	−168	245	0.704	−23.5	−167	120	0.732
Eosinophils	−21.5	−150	107	0.722	−34.2	−117	48.4	0.361
Macrophages	0.98	−17.4	19.4	0.912	24.7	−6.83	56.2	0.106
Epithelial cells	7.07	−37.7	51.9	0.743	−41	−113	31	0.220
Lymphocytes	−72.4	−157	12.7	0.09	−27.3	−122	67	0.516
Neutrophils	3.73	−43.4	50.8	0.87	−5.26	−52.1	41.5	0.798

[a] Change is averaged over the exposure and control sessions. Analyses are stratified by asthma severity using single-pollutant models.

[b] This table is copied from Appendix Table C.2.

Table 27. Nonstratified Analyses for Percentage of Change in Health Endpoints per 1-μg/m^3 Increase in EC[a,b]

Endpoint / Hour of Measurement	Point Estimate	95% CI		*P*
		Lower	Upper	
FEV_1				
2	−0.36	−0.63	−0.09	0.010
3	−0.42	−0.72	−0.12	0.007
5	−0.49	−0.81	−0.16	0.004
7	−0.33	−0.67	0	0.053
22	−0.12	−0.46	0.23	0.497
Overall		*df* = (5, 427)	*F* = 2.31	0.043
FVC				
2	−0.26	−0.57	0.06	0.111
3	−0.32	−0.66	0.02	0.066
5	−0.35	−0.72	0.01	0.059
7	0	−0.37	0.37	0.986
22	0.04	−0.33	0.41	0.841
Overall		*df* = (5, 425)	*F* = 1.83	0.105
$FEF_{25–75}$				
2	−0.74	−1.65	0.18	0.116
3	−0.87	−1.85	0.12	0.084
5	−1.12	−2.14	−0.10	0.031
7	−1.11	−2.14	−0.08	0.034
22	−0.75	−1.78	0.28	0.152
Overall		*df* = (5, 425)	*F* = 1.19	0.315
eNO				
3	1.63	0.41	2.85	0.009
4	1.91	0.57	3.25	0.005
5	2.37	0.95	3.79	0.001
6	2.18	0.70	3.67	0.004
7	1.92	0.39	3.46	0.014
22	1.97	0.28	3.65	0.022
Overall		*df* = (6, 523)	*F* = 2.00	0.064
EBC				
Fe				
3	16.9	−16.5	50.4	0.316
6	17.4	−16.3	51.1	0.307
Overall		*df* = (2, 74)	*F* = 0.72	0.490
pH				
3	−0.1	−0.26	0.06	0.210
6	< 0.01	−0.17	0.17	0.985
Overall		*df* = (2, 175)	*F* = 1.43	0.243
Blood TBARS (7)	−1.54	−8.86	5.79	0.670
PC_{20} (7)	−5.52	−20.3	9.22	0.458
Sputum (22)				
IL-8	0.59	−13	14.2	0.931
MPO	137	−50.3	324	0.148
ECP	−4.84	−42.8	33.1	0.799
Eosinophils	−4.27	−19.7	11.2	0.577
Macrophages	1.15	−3.4	5.69	0.611
Epithelial cells	3.01	−5.29	11.3	0.467
Lymphocytes	−7.65	−24.7	9.36	0.368
Neutrophils	−1.08	−8.68	6.52	0.774

[a] Change is averaged over the exposure and control sessions using single-pollutant models.

[b] This table is copied from Appendix Table C.3.

Table 28. Stratified Analyses for Percentage of Change in Health Endpoints per 1-μg/m^3 Increase in EC[a,b]

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	Point Estimate	95% CI		*P*	Point Estimate	95% CI		*P*
		Lower	Upper			Lower	Upper	
FEV_1								
2	−0.40	−0.75	−0.04	0.028	−0.35	−0.79	0.09	0.120
3	−0.33	−0.72	0.06	0.098	−0.63	−1.12	−0.14	0.012
5	−0.48	−0.91	−0.05	0.028	−0.53	−1.06	0	0.050
7	−0.31	−0.75	0.13	0.163	−0.40	−0.94	0.15	0.154
22	0.01	−0.44	0.46	0.962	−0.41	−0.97	0.14	0.141
Overall		*df* = (5, 219)	*F* = 1.81	0.113		*df* = (5, 196)	*F* = 1.41	0.224
FVC								
2	−0.08	−0.39	0.24	0.639	−0.63	−1.23	−0.02	0.043
3	−0.06	−0.47	0.34	0.758	−0.86	−1.51	−0.20	0.010
5	−0.14	−0.52	0.24	0.479	−0.79	−1.47	−0.11	0.023
7	0.07	−0.31	0.45	0.716	−0.14	−0.83	0.55	0.684
22	0.34	−0.04	0.71	0.078	−0.56	−1.25	0.14	0.115
Overall		*df* = (5, 218)	*F* = 1.81	0.113		*df* = (5, 195)	*F* = 2.13	0.064
$FEF_{25–75}$								
2	−1.10	−1.97	−0.22	0.014	−0.15	−1.61	1.30	0.834
3	−1.05	−2.05	−0.05	0.039	−0.48	−2.44	1.48	0.629
5	−1.24	−2.37	−0.12	0.031	−0.83	−2.68	1.02	0.377
7	−1.05	−2.24	0.13	0.082	−1.16	−2.97	0.65	0.208
22	−0.84	−2.09	0.41	0.185	−0.56	−2.35	1.23	0.536
Overall		*df* = (5, 218)	*F* = 1.44	0.210		*df* = (5, 195)	*F* = 0.39	0.855
eNO								
3	2.45	1.08	3.83	0.001	0.18	−2.09	2.45	0.875
4	2.86	1.37	4.36	0	0.17	−2.31	2.66	0.892
5	2.92	1.34	4.51	0	1.41	−1.23	4.05	0.295
6	2.92	1.28	4.57	0.001	0.96	−1.79	3.72	0.491
7	2.65	0.96	4.33	0.002	0.57	−2.26	3.41	0.690
22	3.10	1.28	4.91	0.001	0.15	−2.96	3.26	0.924
Overall		*df* = (6, 269)	*F* = 3.17	0.005		*df* = (6, 241)	*F* = 0.54	0.781
EBC								
Fe								
3	16.3	−18.0	50.5	0.342	21.8	−45.3	89.0	0.512
6	18.1	−16.1	52.4	0.290	20.6	−51.5	92.8	0.564
Overall		*df* = (2, 38)	*F* = 0.71	0.496		*df* = (2, 31)	*F* = 0.26	0.771
pH								
3	−0.02	−0.19	0.15	0.808	−0.24	−0.55	0.08	0.139
6	0.02	−0.17	0.21	0.817	−0.02	−0.36	0.33	0.925
Overall		*df* = (2, 88)	*F* = 0.19	0.831		*df* = (2, 79)	*F* = 1.84	0.166
Blood TBARS (7)	−2.61	−16.7	11.5	0.685	−0.93	−10	8.13	0.822
PC_{20} (7)	−15.2	−36.3	5.9	0.153	0.48	−22.7	23.7	0.966
Sputum (22)								
IL-8	1.11	−12.9	15.2	0.873	1.25	−28.6	31	0.931
MPO	4.86	−6.26	16	0.377	256	−177	689	0.231
ECP	−16.5	−77.1	44.1	0.581	3.37	−44.2	50.9	0.883
Eosinophils	−2.35	−27.6	22.9	0.846	−11.1	−35.6	13.3	0.325
Macrophages	−0.98	−6.44	4.48	0.712	6.55	−4.6	17.7	0.212
Epithelial cells	3.32	−6.44	13.1	0.486	6.16	−13.2	25.5	0.484
Lymphocytes	−18.1	−36.3	0	0.05	−12.1	−53.4	29.3	0.52
Neutrophils	−0.48	−10.9	9.94	0.925	−5.85	−19.7	7.98	0.358

[a] Change is averaged over the exposure and control sessions. Analyses are stratified by asthma severity using single-pollutant models.

[b] This table is copied from Appendix Table C.4.

Table 29. Nonstratified Analyses for Percentage of Change in Health Endpoints per 10,000-UFP/cm^3 Increase[a,b]

Endpoint / Hour of Measurement	Point Estimate	95% CI		*P*
		Lower	Upper	
FEV_1				
2	−0.79	−1.25	−0.33	0.001
3	−0.73	−1.24	−0.21	0.006
5	−0.90	−1.46	−0.34	0.002
7	−0.66	−1.24	−0.08	0.026
22	−0.44	−1.04	0.15	0.141
Overall		*df* = (5, 437)	*F* = 2.86	0.015
FVC				
2	−0.56	−1.11	−0.01	0.046
3	−0.61	−1.21	0	0.049
5	−0.71	−1.37	−0.06	0.032
7	−0.34	−1.01	0.33	0.317
22	−0.42	−1.10	0.26	0.223
Overall		*df* = (5, 435)	*F* = 1.28	0.272
$FEF_{25–75}$				
2	−2.24	−3.94	−0.54	0.010
3	−2.22	−4.12	−0.32	0.022
5	−2.81	−4.88	−0.73	0.008
7	−2.12	−4.27	0.02	0.053
22	−1.71	−3.91	0.48	0.125
Overall		*df* = (5, 435)	*F* = 1.82	0.107
eNO				
3	1.01	−1.11	3.12	0.350
4	0.73	−1.58	3.05	0.534
5	1.74	−0.72	4.19	0.166
6	1.13	−1.44	3.70	0.388
7	0.89	−1.76	3.54	0.510
22	1.62	−1.30	4.53	0.276
Overall		*df* = (6, 535)	*F* = 0.72	0.632
EBC				
Fe				
3	36.3	−24.2	96.7	0.236
6	31.7	−28.9	92.4	0.301
Overall		*df* = (2, 78)	*F* = 0.87	0.422
pH				
3	−0.32	−0.58	−0.05	0.018
6	−0.1	−0.38	0.19	0.515
Overall		*df* = (2, 179)	*F* = 3.55	0.031
Blood TBARS (7)	−2.24	−9.91	5.42	0.552
PC_{20} (7)	−23.4	−48.3	1.49	0.065
Sputum (22)				
IL-8	13.1	−10.2	36.4	0.266
MPO	353	37	669	0.029
ECP	6.29	−59.7	72.3	0.849
Eosinophils	0.68	−29.6	31	0.964
Macrophages	1.89	−6.78	10.6	0.661
Epithelial cells	−0.84	−16.9	15.3	0.916
Lymphocytes	−14.3	−46.7	18.1	0.377
Neutrophils	0.39	−14.1	14.9	0.957

[a] Change is averaged over the exposure and control sessions using single-pollutant models.

[b] This table is copied from Appendix Table C.5.

Table 30. Stratified Analyses for Percentage of Change in Health Endpoints per 10,000-UFP/cm^3 Increase[a,b]

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	Point Estimate	95% CI		*P*	Point Estimate	95% CI		*P*
		Lower	Upper			Lower	Upper	
FEV_1								
2	−0.84	−1.53	−0.15	0.017	−0.73	−1.34	−0.11	0.021
3	−0.51	−1.28	0.26	0.194	−0.92	−1.60	−0.24	0.008
5	−0.89	−1.75	−0.03	0.042	−0.90	−1.64	−0.16	0.017
7	−0.68	−1.57	0.22	0.137	−0.65	−1.40	0.11	0.092
22	−0.16	−1.08	0.76	0.728	−0.70	−1.46	0.07	0.075
Overall		*df* = (5, 224)	*F* = 1.84	0.107		*df* = (5, 201)	*F* = 1.70	0.137
FVC								
2	−0.20	−0.91	0.51	0.578	−0.91	−1.76	−0.06	0.037
3	−0.02	−0.79	0.76	0.965	−1.17	−2.08	−0.26	0.012
5	−0.16	−0.98	0.67	0.709	−1.25	−2.20	−0.29	0.011
7	−0.21	−1.05	0.63	0.620	−0.47	−1.43	0.5	0.343
22	0.31	−0.54	1.16	0.473	−1.12	−2.09	−0.15	0.024
Overall		*df* = (5, 223)	*F* = 0.45	0.815		*df* = (5, 200)	*F* = 2.16	0.060
$FEF_{25–75}$								
2	−3.45	−5.67	−1.23	0.003	−1.09	−3.11	0.94	0.292
3	−3.26	−5.80	−0.72	0.012	−1.19	−3.94	1.56	0.393
5	−3.81	−6.70	−0.92	0.010	−1.84	−4.43	0.75	0.163
7	−2.50	−5.56	0.56	0.109	−1.76	−4.30	0.77	0.171
22	−2.35	−5.59	0.89	0.155	−1.07	−3.58	1.43	0.399
Overall		*df* = (5, 223)	*F* = 2.22	0.053		*df* = (5, 200)	*F* = 0.53	0.755
eNO								
3	3.02	0.21	5.84	0.035	−0.93	−4.10	2.24	0.565
4	3.09	0.02	6.16	0.048	−1.49	−4.96	1.98	0.398
5	3.41	0.16	6.66	0.040	0.16	−3.53	3.84	0.932
6	3.24	−0.14	6.62	0.060	−0.92	−4.77	2.92	0.637
7	3.16	−0.32	6.64	0.075	−1.26	−5.22	2.7	0.532
22	3.85	0.08	7.61	0.045	−0.62	−4.96	3.73	0.780
Overall		*df* = (6, 275)	*F* = 1.08	0.373		*df* = (6, 247)	*F* = 0.66	0.680
EBC								
Fe								
3	35.4	−35.0	106	0.315	39.1	−64	142	0.446
6	29.7	−40.7	100	0.398	41.1	−69.2	151	0.454
Overall		*df* = (2, 40)	*F* = 0.62	0.546		*df* = (2, 33)	*F* = 0.39	0.680
pH								
3	−0.16	−0.49	0.16	0.323	−0.47	−0.91	−0.04	0.033
6	−0.09	−0.46	0.27	0.612	−0.10	−0.59	0.38	0.673
Overall		*df* = (2, 90)	*F* = 0.53	0.593		*df* = (2, 81)	*F* = 3.28	0.043
Blood TBARS (7)	−2.36	−19.2	14.5	0.759	−1.85	−9.69	6	0.607
PC_{20} (7)	−37.4	−77.4	2.62	0.066	−19.3	−52.7	14	0.247
Sputum (22)								
IL-8	8.49	−18.5	35.5	0.525	15.4	−26.3	57	0.451
MPO	5.18	−16.8	27.2	0.632	586	−23	1196	0.058
ECP	−16.4	−134	101	0.777	19.5	−43.2	82.1	0.521
Eosinophils	5.55	−47.8	58.9	0.828	−7.2	−48.4	34.1	0.702
Macrophages	−1.01	−12.5	10.5	0.857	6.72	−11.8	25.2	0.433
Epithelial cells	4.83	−15.8	25.4	0.630	−4.42	−40.7	31.8	0.789
Lymphocytes	−26.6	−66.6	13.5	0.182	−28.7	−93.1	35.7	0.340
Neutrophils	−1.45	−23.3	20.4	0.891	0.21	−24	24.5	0.985

[a] Change is averaged over the exposure and control sessions. Analyses are stratified by asthma severity using single-pollutant models.

[b] This table is copied from Appendix Table C.6.

Table 31. Nonstratified Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in NO_2[a,b]

Endpoint / Hour of Measurement	Point Estimate	95% CI		*P*
		Lower	Upper	
FEV_1				
2	−0.22	−0.40	−0.05	0.012
3	−0.12	−0.32	0.07	0.217
5	−0.16	−0.37	0.06	0.152
7	−0.11	−0.34	0.11	0.310
22	−0.13	−0.35	0.10	0.270
Overall		*df* = (5, 402)	*F* = 1.53	0.180
FVC				
2	−0.09	−0.30	0.11	0.376
3	−0.07	−0.30	0.16	0.549
5	−0.04	−0.29	0.21	0.761
7	−0.06	−0.31	0.20	0.666
22	−0.02	−0.28	0.23	0.863
Overall		*df* = (5, 400)	*F* = 0.18	0.971
$FEF_{25–75}$				
2	−0.78	−1.44	−0.13	0.019
3	−0.73	−1.46	0	0.051
5	−0.88	−1.69	−0.08	0.031
7	−0.60	−1.43	0.23	0.155
22	−0.75	−1.60	0.10	0.084
Overall		*df* = (5, 400)	*F* = 1.46	0.202
eNO				
3	0.53	−0.27	1.32	0.191
4	0.30	−0.57	1.17	0.498
5	0.47	−0.45	1.40	0.315
6	0	−0.97	0.96	0.994
7	0.11	−0.88	1.11	0.827
22	−0.07	−1.16	1.03	0.906
Overall		*df* = (6, 493)	*F* = 0.98	0.438
EBC				
Fe				
3	−9.01	−38.6	20.6	0.546
6	−1.92	−31.6	27.7	0.897
Overall		*df* = (2, 69)	*F* = 0.20	0.823
pH				
3	−0.15	−0.25	−0.06	0.002
6	−0.06	−0.16	0.04	0.246
Overall		*df* = (2, 165)	*F* = 5.58	0.005
Blood TBARS (7)	−0.84	−2.45	0.77	0.294
PC_{20} (7)	−8.68	−17.9	0.52	0.064
Sputum (22)				
IL-8	10.9	2.63	19.3	0.011
MPO	69.9	−56.7	197	0.272
ECP	−1.72	−25.7	22.3	0.886
Eosinophils	−3.62	−18.8	11.5	0.629
Macrophages	0.78	−3.63	5.19	0.722
Epithelial cells	−1.46	−9.37	6.45	0.709
Lymphocytes	−7.58	−24	8.85	0.354
Neutrophils	1.14	−5.99	8.27	0.746

[a] Change is averaged over the exposure and control sessions using single-pollutant models.

[b] This table is copied from Appendix Table C.7.

at hour 5 for all subjects combined (2.81%) and for the subjects with mild asthma (−3.81%). No significance was found for the subjects with moderate asthma, although the point estimates were all negative at all the time points of measurement. A 0.32% reduction in EBC pH at 3 hours, associated with a 10,000-UFP/cm^3 increase, was observed for all subjects, mainly driven by pH reduction in the subjects with moderate asthma (0.47%). A large increase (353%) in sputum MPO was associated with a 10,000 UFP/cm^3 increase, also driven by the change in the subjects with moderate asthma.

As shown in Figure 7, panel D, 2-hour exposure to NO_2 was significantly associated with EBC pH (P = 0.005; $P < 0.025$ at hour 3) and sputum IL-8 (P = 0.011). (See Table 31 for nonstratified data and Table 32 for stratified data.) Marginal significance was seen for effects in FEV_1 at hour 2, $FEF_{25–75}$ at all the time points except hour 7, and PC_{20} (see Figure 7, panel D). A 10-μg/m^3 (~ 5.3-ppb) increase in NO_2 concentration during the 2-hour session led to an average reduction in FEV_1 of 0.22% at hour 2 for all the subjects. The same incremental change in NO_2 concentration led to an average reduction in $FEF_{25–75}$ of up to 0.88% (at hour 5) for all subjects and of up to 0.93% for the subjects with mild asthma (at hour 2). No significant effect of NO_2 on $FEF_{25–75}$ was found for the subjects with mild asthma, although the point estimates of the coefficients were negative at all time points. A 10-μg/m^3 increase in the 2-hour NO_2 exposure was associated at hour 3 with a 0.15% average reduction in EBC pH for all subjects, a 0.12% reduction for subjects in the mild asthma group (P = 0.072), and a 0.19% reduction for subjects in the moderate asthma group (P = 0.01). The same amount of increase in NO_2 exposure led to an average 10.9% increase in sputum IL-8 percentage for all the subjects, mainly driven by the 18.1% increase in the subjects with moderate asthma. Interestingly, unlike the other three pollutants, NO_2 was significantly associated with bronchial reactivity. A 10-μg/m^3 NO_2 increase was associated with an 8.68% reduction in PC_{20} for all of the subjects (P = 0.064) or an 18.1% reduction for the subjects with mild asthma (P = 0.029).

The exposure–response relations, described above and in Appendix C, can be used to predict differences in health endpoints between exposure and control sessions. To do so, we simply scaled the slopes derived from the unit changes in pollutant concentration with the actual differences in pollutant concentration between the exposure and control sessions. The predicted results are summarized in Table 33, showing only the largest changes when values were available at multiple time points of measurements.

Table 32. Stratified Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in NO_2[a,b]

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	Point Estimate	95% CI		*P*	Point Estimate	95% CI		*P*
		Lower	Upper			Lower	Upper	
FEV_1								
2	−0.22	−0.53	0.09	0.167	−0.20	−0.41	0.01	0.064
3	−0.20	−0.56	0.15	0.253	−0.05	−0.29	0.18	0.668
5	−0.23	−0.62	0.16	0.242	−0.12	−0.38	0.13	0.348
7	−0.25	−0.65	0.15	0.220	−0.05	−0.31	0.21	0.714
22	−0.15	−0.57	0.26	0.462	−0.09	−0.36	0.18	0.496
Overall		*df* = (5, 190)	*F* = 0.49	0.785		*df* = (5, 201)	*F* = 1.20	0.310
FVC								
2	−0.14	−0.45	0.18	0.394	−0.08	−0.38	0.21	0.581
3	−0.23	−0.57	0.10	0.174	0.02	−0.30	0.34	0.885
5	−0.12	−0.47	0.24	0.520	−0.01	−0.35	0.32	0.938
7	−0.34	−0.70	0.01	0.060	0.08	−0.26	0.42	0.627
22	−0.15	−0.52	0.21	0.400	0.05	−0.30	0.39	0.793
Overall		*df* = (5, 189)	*F* = 0.95	0.449		*df* = (5, 200)	*F* = 0.29	0.916
$FEF_{25–75}$								
2	−0.93	−1.97	0.11	0.078	−0.59	−1.28	0.10	0.091
3	−0.81	−1.99	0.38	0.183	−0.60	−1.54	0.34	0.210
5	−0.88	−2.24	0.48	0.203	−0.89	−1.78	−0.01	0.048
7	−0.49	−1.94	0.96	0.508	−0.68	−1.55	0.19	0.123
22	−0.65	−2.21	0.91	0.413	−0.78	−1.63	0.08	0.075
Overall		*df* = (5, 189)	*F* = 0.73	0.601		*df* = (5, 200)	*F* = 1.14	0.339
eNO								
3	−0.09	−1.33	1.14	0.885	0.80	−0.29	1.88	0.149
4	−0.86	−2.20	0.48	0.208	0.94	−0.25	2.13	0.120
5	−0.74	−2.15	0.67	0.303	1.13	−0.13	2.39	0.080
6	−0.83	−2.30	0.63	0.264	0.29	−1.03	1.61	0.665
7	−0.72	−2.23	0.78	0.343	0.52	−0.84	1.88	0.451
22	−0.84	−2.45	0.76	0.301	0.04	−1.45	1.54	0.950
Overall		*df* = (6, 234)	*F* = 0.72	0.631		*df* = (6, 247)	*F* = 1.36	0.232
EBC								
Fe								
3	−16	−55.7	23.7	0.418	−9.58	−57.9	38.8	0.690
6	2.01	−37.7	41.7	0.919	−2.26	−53.4	48.9	0.929
Overall		*df* = (2, 32)	*F* = 0.48	0.622		*df* = (2, 33)	*F* = 0.09	0.915
pH								
3	−0.12	−0.25	0.01	0.072	−0.19	−0.34	−0.05	0.010
6	−0.11	−0.24	0.03	0.119	−0.05	−0.21	0.11	0.541
Overall		*df* = (2, 78)	*F* = 1.82	0.169		*df* = (2, 81)	*F* = 4.61	0.013
Blood TBARS (7 hr)	−1.4	−4.63	1.83	0.346	−0.4	−3.41	2.61	0.772
PC_{20} (7 hr)	−18.1	−34.3	−2.01	0.029	−6.12	−18	5.8	0.303
Sputum (22 hr)								
IL-8	6.65	−4.47	17.8	0.228	18.1	4.85	31.4	0.010
MPO	−0.51	−9.31	8.29	0.905	149	−90.1	387	0.209
ECP	3.22	−47.6	54	0.897	−7.89	−29.8	14	0.458
Eosinophils	−8.24	−36.4	19.9	0.538	12.6	−12.6	37.7	0.288
Macrophages	0.12	−5.68	5.93	0.965	2.61	−9.65	14.9	0.642
Epithelial cells	−1.33	−11.5	8.84	0.785	−13.4	−34.7	7.8	0.187
Lymphocytes	−15.4	−34.8	3.88	0.11	−14.4	−57	28.2	0.465
Neutrophils	−0.34	−10.9	10.2	0.947	11.8	−1.1	24.7	0.069

[a] Change is averaged over the exposure and control sessions. Analyses are stratified by asthma severity using single-pollutant models.

[b] This table copied from Appendix Table C.8.

Table 33. Differences in Health Endpoints Between Exposure and Control Sessions Predicted by Exposure–Response Relations[a]

Endpoint	Group	$PM_{2.5}$	EC	UFP	NO_2
FEV_1	All subjects		−2.9	−4.2	−2.4
	Mild			−4.2	
	Moderate		−3.7	−4.3	
FVC	All subjects			−3.3	
	Mild			−10.4	
$FEF_{25–75}$	All subjects	−7.7	−6.5	−13	−9.6
	Mild	−13	−7.1	−18	
	Moderate				−19.2
eNO	All subjects		14.2		
	Mild		18.3		
EBC					
Iron	All subjects	−144			
	Moderate	−368			
pH	All subjects			−1.5	−1.6
	Mild				−1.3
	Moderate			−2.2	−2.1
PC_{20}	All subjects				−95
	Mild				−200
Sputum					
IL-8	All subjects				119
	Moderate				197
MPO	All subjects			1650	
	Moderate			2739	

[a] When values of statistical significance were available at multiple time points of measurements, the largest values are given. The estimates were based on the results from the single-pollutant model.

The results from two-pollutant models are shown in Tables 34–37. After an adjustment for copollutants, the UFP effects on FEF_{25-75} were no longer significant (Table 34). An adjustment for EC resulted in significant UFP effects on FEV_1 and FVC at fewer time points than an adjustment for either $PM_{2.5}$ or NO_2. After an adjustment for EC or $PM_{2.5}$, the UFP effect on EBC pH at hour 3 remained significant, and the main effect estimate (absolute value) increased from −0.32 (no adjustment) to −0.56 (EC adjustment) and −0.37 ($PM_{2.5}$ adjustment). However, the adjustment for NO_2 diminished the UFP effect on pH (effect estimate = −0.07, $P = 0.69$) (see Tables 29 and 34).

An adjustment for either NO_2 or $PM_{2.5}$ (Table 35) did not significantly change the effects of EC on FEV_1 as shown in Figure 7, panel B and Table 27. However, an adjustment for UFP resulted in substantially reduced effect estimates

Table 34. Copollutant-Adjusted Analyses for Percentage of Change in Health Endpoints per 10,000-UFP/cm^3 Increase[a,b]

Endpoint / Hour of Measurement	Adjusted for EC				Adjusted for $PM_{2.5}$				Adjusted for NO_2			
	Point Estimate	95% CI Lower	95% CI Upper	P[c]	Point Estimate	95% CI Lower	95% CI Upper	P[c]	Point Estimate	95% CI Lower	95% CI Upper	P[c]
FEV_1												
2	−1.15	−2.09	−0.21	**0.016**	−0.74	−1.33	−0.15	**0.014**	−0.70	−1.31	−0.10	**0.023**
3	−0.66	−1.69	0.37	0.209	−0.75	−1.40	−0.10	**0.023**	−0.84	−1.51	−0.18	**0.013**
5	−0.98	−2.09	0.13	0.082	−0.77	−1.47	−0.07	**0.031**	−1.01	−1.73	−0.29	**0.006**
7	−0.88	−2.01	0.25	0.125	−0.55	−1.27	0.16	0.131	−0.74	−1.48	−0.01	**0.047**
22	−1.41	−2.55	−0.27	**0.016**	−0.56	−1.29	0.16	0.129	−0.48	−1.23	0.26	0.199
FVC												
2	−1.10	−2.21	0.01	0.051	−0.69	−1.41	0.02	0.057	−0.67	−1.41	0.06	0.072
3	−1.03	−2.20	0.14	0.084	−0.83	−1.59	−0.06	**0.034**	−0.92	−1.70	−0.13	**0.022**
5	−1.22	−2.43	−0.02	**0.047**	−0.82	−1.62	−0.02	**0.045**	−1.14	−1.96	−0.32	**0.007**
7	−1.66	−2.87	−0.44	**0.008**	−0.37	−1.18	0.44	0.375	−0.53	−1.36	0.30	0.207
22	−2.24	−3.46	−1.03	< **0.001**	−0.81	−1.63	−0.01	**0.050**	−0.78	−1.62	0.05	0.065
FEF_{25-75}												
2	−1.71	−4.91	1.50	0.297	−1.47	−3.75	0.82	0.207	−1.57	−3.96	0.83	0.199
3	−0.24	−3.46	2.97	0.883	−1.11	−3.45	1.22	0.350	−1.38	−3.80	1.05	0.266
5	−1.54	−4.75	1.68	0.348	−1.45	−3.80	0.90	0.227	−1.85	−4.28	0.59	0.137
7	0.18	−3.04	3.39	0.914	−1.33	−3.68	1.03	0.268	−1.56	−4.00	0.87	0.208
22	−0.47	−3.69	2.75	0.774	−1.21	−3.56	1.15	0.314	−0.75	−3.19	1.68	0.544
eNO												
3	−0.81	−4.13	2.52	0.633	−0.16	−2.20	1.89	0.881	−0.70	−2.82	1.42	0.517
4	−3.07	−6.64	0.51	0.093	0.36	−1.83	2.54	0.748	−0.40	−2.67	1.88	0.731
5	−1.82	−5.56	1.92	0.340	1.66	−0.61	3.93	0.152	0.66	−1.72	3.03	0.587
6	−3.27	−7.12	0.58	0.096	0.57	−1.76	2.90	0.630	0.91	−1.53	3.35	0.463
7	−2.72	−6.65	1.21	0.174	0.53	−1.84	2.89	0.662	0.09	−2.39	2.58	0.943
22	−0.94	−5.04	3.16	0.653	1.13	−1.31	3.56	0.365	1.81	−0.77	4.39	0.168
EBC pH												
3	−0.56	−1.09	−0.04	**0.037**	−0.37	−0.70	−0.040	**0.029**	−0.07	−0.39	0.26	0.69
6	−0.35	−0.94	0.25	0.250	−0.09	−0.46	0.282	0.639	0.06	−0.30	0.42	0.74

[a] Change is averaged over the exposure and control sessions. Estimates are adjusted for copollutants using two-pollutant models.

[b] This table is copied from Appendix Table C.9.

[c] Bolded values are significant at $P \leq 0.05$.

with no statistical significance (Table 35). In the single-pollutant model, the EC effect on $FEF_{25–75}$ was only significant at hours 5 and 7. The effect was no longer significant after an adjustment for copollutants (Tables 27 and 35). In the single-pollutant model, the EC effect on eNO was significant at all time points. The effect was no longer significant at all time points and the effects estimates were reduced to less than half after adjustment for NO_2. Except at hours 3 and 22, the adjustment for UFP did not significantly change the EC effect on eNO, whereas the adjustment for $PM_{2.5}$ resulted in reductions in the effect estimates between those from the UFP adjustment and those from the NO_2 adjustment (Tables 27 and 35).

In the single-pollutant model, we observed significant $PM_{2.5}$ effects on $FEF_{25–75}$ at hours 3 and 5 (Figure 7, panel A). These effects were no longer significant after an adjustment

Table 35. Copollutant-Adjusted Analyses for Percentage of Change in Health Endpoints per 1-μg/m^3 Increase in EC[a,b]

	Adjusted for UFP				Adjusted for $PM_{2.5}$				Adjusted for NO_2			
Endpoint / Hour of Measurement	Point Estimate	95% CI Lower	95% CI Upper	P[c]	Point Estimate	95% CI Lower	95% CI Upper	P[c]	Point Estimate	95% CI Lower	95% CI Upper	P[c]
FEV_1												
2	0.20	−0.33	0.74	0.456	−0.25	−0.60	0.10	0.164	−0.31	−0.64	0.02	0.068
3	−0.09	−0.68	0.50	0.755	−0.37	−0.76	0.02	0.064	−0.45	−0.82	−0.09	**0.016**
5	−0.01	−0.64	0.63	0.988	−0.34	−0.75	0.09	0.124	−0.53	0.93	−0.14	**0.008**
7	0.10	−0.55	0.75	0.764	−0.17	−0.60	0.26	0.446	−0.37	−0.77	0.03	0.071
22	0.57	−0.08	1.23	0.087	−0.04	−0.48	0.39	0.846	−0.14	−0.54	0.27	0.513
FVC												
2	0.28	−0.35	0.92	0.384	−0.24	−0.66	0.18	0.257	−0.29	−0.69	0.10	0.148
3	0.18	−0.49	0.86	0.590	−0.35	−0.79	0.10	0.126	−0.47	−0.89	−0.05	**0.029**
5	0.25	−0.44	0.94	0.482	−0.31	−0.77	0.15	0.185	−0.56	−0.99	−0.12	**0.012**
7	0.82	0.12	1.51	**0.022**	0.16	−0.31	0.62	0.506	−0.10	−0.54	0.33	0.644
22	1.14	0.44	1.83	**0.001**	0.02	−0.44	0.48	0.933	−0.08	−0.52	0.35	0.705
$FEF_{25–75}$												
2	0.10	−1.74	1.94	0.917	−0.44	−1.64	0.76	0.467	−0.50	−1.65	0.65	0.394
3	−0.75	−2.59	1.10	0.427	−0.51	−1.72	0.69	0.404	−0.52	−1.67	0.63	0.377
5	−0.37	−2.22	1.48	0.695	−0.50	−1.70	0.71	0.419	−0.71	−1.86	0.44	0.227
7	−1.20	−3.04	0.65	0.203	−0.85	−2.05	0.36	0.168	−0.90	−2.05	0.25	0.124
22	−0.52	−2.37	1.33	0.581	−0.64	−1.84	0.57	0.300	−0.44	−1.59	0.71	0.455
eNO												
3	0.69	−1.22	2.60	0.478	0.09	−1.17	1.35	0.891	−0.24	−1.43	0.95	0.690
4	2.08	0.03	4.14	**0.047**	1.02	−0.35	2.40	0.144	0.47	−0.83	1.77	0.478
5	1.93	−0.22	4.08	0.079	1.68	0.23	3.13	**0.024**	0.91	−0.47	2.29	0.196
6	2.45	0.24	4.67	**0.030**	1.31	−0.20	2.82	0.090	1.05	−0.39	2.49	0.153
7	1.92	−0.34	4.18	0.095	1.07	−0.49	2.62	0.178	0.50	−0.99	1.98	0.513
22	1.09	−1.27	3.44	0.365	1.02	−0.66	2.71	0.234	0.85	−0.78	2.48	0.304
EBC pH												
3	0.18	−0.13	0.48	0.250	−0.11	−0.31	0.09	0.285	0.01	−0.17	0.19	0.947
6	0.17	−0.09	0.51	0.318	0.02	−0.20	0.25	0.839	0.06	−0.14	0.26	0.544

[a] Change is averaged over the exposure and control sessions. Estimates are adjusted for copollutants using two-pollutant models.

[b] This table is copied from Appendix Table C.10.

[c] Bolded values are significant at $P \leq 0.05$.

for EC at both time points and after an adjustment for UFP at hour 3. The effects remained significant after an adjustment for UFP at hour 5 and for NO_2 at hours 3 and 5 (Tables 25 and 36).

The effect of NO_2 on FEV_1 at hour 2 observed in the single-pollutant model was no longer significant after an adjustment for copollutants (Tables 31 and 37). Likewise, the NO_2 effects on FEF_{25-75} at several time points all became nonsignificant after an adjustment for copollutants. However, the adjustment for the copollutants did not significantly change the NO_2 effect on EBC pH (both the effect estimates and statistical significance).

Table 36. Copollutant-Adjusted Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in $PM_{2.5}$[a,b]

Endpoint / Hour of Measurement	Adjusted for UFP				Adjusted for EC				Adjusted for NO_2			
	Point Estimate	95% CI Lower	95% CI Upper	P[c]	Point Estimate	95% CI Lower	95% CI Upper	P[c]	Point Estimate	95% CI Lower	95% CI Upper	P[c]
FEV_1												
2	0.03	−1.03	1.10	0.948	−0.39	−1.50	0.71	0.487	−0.57	−1.60	0.45	0.273
3	0.24	−0.94	1.41	0.694	−0.06	−1.27	1.16	0.928	−0.48	−1.61	0.66	0.410
5	−0.17	−1.44	1.09	0.789	−0.61	−1.92	0.70	0.363	−0.86	−2.11	0.35	0.163
7	0.13	−1.17	1.42	0.850	−0.32	−1.66	1.03	0.643	−0.44	−1.71	0.82	0.490
22	0.44	−0.87	1.75	0.511	−0.32	−1.68	1.04	0.642	0.13	−1.15	1.41	0.839
FVC												
2	0.40	−0.90	1.69	0.546	−0.16	−1.48	1.16	0.809	−0.25	−1.51	1.01	0.634
3	0.84	−0.55	2.22	0.237	0.24	−1.16	1.63	0.738	0.07	−1.29	1.42	0.924
5	0.54	−0.92	1.99	0.470	−0.10	−1.54	1.34	0.893	−0.47	−1.88	0.94	0.512
7	0.45	−1.02	1.92	0.547	−0.40	−1.85	1.05	0.585	−0.01	−1.44	1.41	0.985
22	1.50	0.03	2.97	**0.046**	0.41	−1.04	1.87	0.576	0.95	−0.57	2.28	0.239
FEF_{25-75}												
2	−2.00	−6.12	2.13	0.343	−0.71	−4.46	3.04	0.710	−2.92	−6.99	1.15	0.159
3	−0.377	−8.00	0.45	0.080	−1.59	−5.35	2.17	0.406	−4.39	−8.54	−0.23	**0.039**
5	−4.28	−8.53	−0.02	0.049	−2.88	−6.64	0.88	0.133	−4.71	−8.89	−0.53	**0.027**
7	−2.10	−6.36	2.16	0.333	−0.54	−4.30	3.22	0.778	−2.91	−7.09	1.27	0.172
22	−2.05	−6.31	2.21	0.345	−0.89	−4.65	2.88	0.645	−2.08	−6.26	2.10	0.329
eNO												
3	0.74	−2.93	4.41	0.692	0.82	−3.06	4.71	0.677	−0.89	−4.53	2.76	0.633
4	−1.51	−5.42	2.41	0.450	−2.29	−6.53	1.94	0.288	−2.04	−6.02	1.93	0.313
5	−1.91	−5.98	2.17	0.358	−2.51	−7.00	1.98	0.273	−1.55	−5.76	2.65	0.468
6	−1.08	−5.25	3.10	0.612	−2.26	−6.93	2.41	0.342	−0.76	−5.13	3.61	0.734
7	−1.43	−5.67	2.81	0.510	−2.25	−7.05	2.55	0.358	−1.65	−6.15	2.84	0.471
22	0.44	−3.93	4.81	0.844	−0.18	−5.38	5.02	0.945	1.60	−3.25	6.46	0.517
EBC pH												
3	0.11	−0.49	0.70	0.720	0.01	−0.62	0.63	0.988	0.21	−0.34	0.76	0.459
6	−0.09	−0.75	0.57	0.785	−0.19	−0.88	0.50	0.587	0.08	−0.52	0.68	0.795

[a] Change is averaged over the exposure and control sessions. Estimates are adjusted for copollutants using two-pollutant models.

[b] This table is copied from Appendix Table C.11.

[c] Bolded values are significant at $P \leq 0.05$.

In summary, Tables 34–37 show that after an adjustment for copollutants, UFP was still significantly associated with FEV_1, FVC, and EBC pH at one or more time points (except when adjusted for NO_2). EC was significantly associated with eNO (except when adjusted for NO_2), FVC (except when adjusted for $PM_{2.5}$), and FEV_1 (except when adjusted for UFP or $PM_{2.5}$). NO_2 was significantly and consistently associated with EBC pH at 3 hours. In contrast, $PM_{2.5}$ was significantly associated with FEF_{25-75} only when adjusted for NO_2 but not when adjusted for UFP or EC.

Table 37. Copollutant-Adjusted Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in NO_2[a,b]

	Adjusted for UFP				Adjusted for $PM_{2.5}$				Adjusted for EC			
Endpoint / Hour of Measurement	Point Estimate	95% CI Lower	95% CI Upper	P[c]	Point Estimate	95% CI Lower	95% CI Upper	P[c]	Point Estimate	95% CI Lower	95% CI Upper	P[c]
FEV_1												
2	−0.08	−0.30	0.13	0.448	−0.15	−0.36	0.05	0.148	−0.18	−0.37	0.02	0.079
3	0.04	−0.19	0.28	0.717	−0.09	−0.31	0.14	0.464	−0.05	−0.27	0.17	0.646
5	0.04	−0.21	0.30	0.740	−0.07	−0.31	0.18	0.598	−0.07	−0.31	0.16	0.544
7	0.03	−0.23	0.30	0.808	−0.06	−0.31	0.19	0.642	−0.06	−0.30	0.18	0.614
22	−0.03	−0.30	0.24	0.821	−0.15	−0.40	0.11	0.264	−0.14	−0.38	0.10	0.256
FVC												
2	0.04	−0.23	0.30	0.782	−0.07	−0.32	0.18	0.588	−0.07	−0.31	0.16	0.535
3	0.11	−0.17	0.39	0.440	−0.05	−0.32	0.22	0.717	−0.02	−0.27	0.23	0.855
5	0.19	−0.11	0.48	0.214	0.04	−0.24	0.33	0.768	0.03	−0.23	0.29	0.806
7	0.05	−0.25	0.35	0.742	−0.03	−0.32	0.25	0.811	−0.07	−0.33	0.19	0.601
22	0.13	−0.17	0.43	0.390	−0.07	−0.36	0.21	0.620	−0.05	−0.31	0.21	0.713
FEF_{25-75}												
2	−0.47	−1.33	0.39	0.282	−0.48	−1.30	0.34	0.251	−0.43	−1.11	0.26	0.221
3	−0.45	−1.33	0.42	0.309	−0.50	−1.34	0.33	0.236	−0.30	−0.98	0.39	0.393
5	−0.52	−1.40	0.36	0.247	−0.55	−1.39	0.29	0.199	−0.47	−1.15	0.22	0.181
7	−0.29	−1.17	0.59	0.515	−0.37	−1.21	0.47	0.391	−0.22	−0.90	0.46	0.529
22	−0.59	−1.47	0.29	0.190	−0.66	−1.50	0.18	0.124	−0.49	−1.18	0.20	0.162
eNO												
3	0.36	−0.41	1.12	0.363	0.42	−0.32	1.16	0.270	0.32	−0.39	1.03	0.378
4	0.07	−0.76	0.89	0.876	0.32	−0.49	1.13	0.440	−0.04	−0.81	0.74	0.925
5	0.03	−0.83	0.88	0.953	0.45	−0.41	1.30	0.303	0.04	−0.78	0.87	0.916
6	−0.50	−1.38	0.38	0.263	−0.20	−1.09	0.69	0.660	−0.49	−1.35	0.36	0.258
7	−0.22	−1.12	0.67	0.626	0.03	−0.88	0.95	0.941	−0.25	−1.14	0.63	0.573
22	−0.74	−1.68	0.19	0.116	−0.38	−1.36	0.61	0.455	−0.59	−1.56	0.38	0.233
EBC pH												
3	−0.14	−0.26	−0.02	**0.020**	−0.19	−0.30	−0.08	**< 0.001**	−0.15	−0.25	−0.04	**0.007**
6	−0.07	−0.20	0.06	0.270	−0.09	−0.21	0.04	0.164	−0.07	−0.19	0.05	0.230

[a] Change is averaged over the exposure and control sessions. Estimates are adjusted for copollutants using two-pollutant models.

[b] This table is copied from Appendix Table C.12.

[c] Bolded values are significant at $P \leq 0.05$.

DISCUSSION

EXPOSURE CHARACTERISTICS

Diesel exhaust is a complex mixture, comprised of both particulate and gaseous species. EC is a major component of DE and constitutes 60% to 80% of diesel particles by weight (Amann and Siegla 1982; Zaebst et al. 1991). Most of the atmospheric EC associated with vehicular traffic has been attributed to DE, and the contribution from other sources (e.g., gasoline exhaust and tire debris) appears to be small (Hildemann et al. 1991; Zaebst et al. 1991; Schauer et al. 1996). Based on our measurements made during the intensive air pollution characterization campaigns (see Appendix F), EC concentrations ranged from 2.0 to 24.2 μg/m^3 at the exposure site and from 0.5 to 2.8 μg/m^3 at the control site. The EC concentrations measured at the control site were comparable to typical urban and suburban background concentrations (Chow et al. 1994), whereas the EC concentrations at the exposure site were similar to, and sometimes higher than concentrations reported for other urban roadsides and roadways that were heavily traveled by diesel vehicles (Kinney et al. 2000; van Vliet et al. 1997).

Our during-session measurements showed that the mean 2-hour average EC concentration during exposure sessions (when subjects walked in the exposure site) was 4.8 times the mean EC concentration during control sessions (when subjects walked in the control site) (Table 6). The mean EC content in $PM_{2.5}$ during exposure sessions was 27%, substantially higher than the mean EC content of 11% in $PM_{2.5}$ during control sessions.

While the mean EC concentrations at the exposure site during exposure sessions were nearly 5 times those at the control site during control sessions, the mean $PM_{2.5}$ concentrations at the exposure site during exposure sessions were < 2 times those at the control site during control sessions. The $PM_{2.5}$ concentrations measured at the exposure site during exposure sessions were similar to the pedestrian exposure concentrations of $PM_{2.5}$ (37.7 ± 16.4 μg/m^3) measured in a previous study (Kaur et al. 2005a) at Marylebone Road, a major road in Central London. The $PM_{2.5}$ pedestrian exposure concentrations in Northampton, United Kingdom of 15.06 ± 16.15 μg/m^3 (Gulliver and Briggs 2004) were lower than those found at the exposure site, but only slightly higher than those found at the control site. Some of the differences in $PM_{2.5}$ exposure concentrations between Northampton and Oxford Street in London can be attributed to dissimilarities in traffic density, urban design, and meteorologic conditions. A number of other studies examining $PM_{2.5}$ concentrations at static points on streets have found concentrations of 42.9 ± 23.0 μg/m^3 in Arnhem, Netherlands (Janssen et al. 1997), 37 to 47 μg/m^3 on sidewalks in Harlem, New York City (Kinney et al. 2000), 18.2 to 29.9 μg/m^3 at intersections in Hunts Point, New York City (Lena et al. 2002), and 12 to 86 μg/m^3 at nine roadside sites in Boston, Massachusetts (Levy et al. 2002). Therefore, $PM_{2.5}$ concentrations measured at the exposure site, although filled with diesel-powered vehicles, were not atypical compared with other urban streets or other sites.

When airborne particles are counted, the majority (> 80%) are in the ultrafine range in urban areas where motor vehicle emissions are a dominant pollution source. However, because these particles are extremely small, their mass is also extremely small. Therefore, it is more accurate to quantify these particles by particle number concentrations (counts) than by mass.

The UFP number concentrations at the exposure site during the exposure sessions were similar to those measured at Marylebone Road in London (mean = 65 × 10^3/cm^3) by Kaur and colleagues (2005b). A number of studies have reported varying UFP number concentrations in the urban environment. Junker and colleagues measured UFP concentrations from 5,690 ± 2,200 UFP/cm^3 to 19,300 ± 8,580 UFP/cm^3 for particles with sizes ranging from 0.018 μm to 0.421 μm at three sites in Basel (Junker et al. 2000); 180,000 to 350,000 UFP/cm^3 for particles ranging from 0.06 to 0.22 μm at a site 17 meters away from a freeway in Los Angeles (Zhu et al. 2002); and 18,000 UFP/cm^3 for particles ranging from 0.01 to 2.5 μm in Erfurt, Germany (Cyrys et al. 2003). Hitchins and colleagues (2000) observed a clear decline in UFP concentrations as the distance from the road increased, suggesting that the particles were related to vehicle exhaust emission. This was also observed by Levy and colleagues (2002) and Weijers and colleagues (2004). In another study, Zhu and colleagues (2002) found an exponential decay in black carbon (a proxy for EC) and particle numbers with distance from a freeway. Thus the greater difference in UFP number concentration than in $PM_{2.5}$ mass concentration that we found between the exposure site and the control site is not unexpected (see Table 6). Zhu and colleagues (2002) also found that wind speed and direction had a large impact on UFP concentration, suggesting that day-to-day variations in meteorologic conditions were largely responsible for the day-to-day variations in the UFP concentrations observed for the exposure and the control sites in our study.

Oxides of nitrogen, including NO_2, are formed through high-temperature combustion. The NO_2 concentrations are highest close to busy roads and in large urban areas. NO_2 values found by other studies are more difficult to

contrast, because they are usually expressed as annual or daily average values, while NO_2 concentrations in our study were measured for 2 hours around midday. Lewne and colleagues (2004) found that annual average NO_2 concentrations for different sampling sites were between 15.9 and 50.6 μg/m^3 (mean 28.8 μg/m^3) in Germany, between 12.1 and 50.8 μg/m^3 (mean 28.9 μg/m^3) in the Netherlands, and between 6.1 and 44.7 μg/m^3 (mean 18.5 μg/m^3) in Sweden. Daytime 2-hour averaged NO_2 concentrations measured in our study at the exposure site (143 μg/m^3) were considerably higher than those longer-term averages. However, our averaged 2-hour NO_2 concentrations measured at the control site were comparable to the concentrations reported for urban background sites by Lewne and colleagues (2004).

Interestingly, we observed a steep increase in NO_2 concentration measured during exposure sessions in the second study winter over those in the first winter (Figure 5). In contrast, there appeared to be a decreasing trend in exposure-session EC concentration over the study period. Similar patterns were observed during the two winters' intensive air pollution characterizations; although the pattern on NO_2 was less clear (see Appendix Figure F3). These changes may be ascribed to the introduction of particle filters on London buses, which needs to be further examined with additional ambient air monitoring data.

In summary, the exposure site on Oxford Street, with its high volume of diesel traffic, had higher concentrations of $PM_{2.5}$ mass, EC, UFP number, and NO_2, than did the control site at Hyde Park, where these pollutants were found at typical urban background concentrations (see Appendix F). Across all the 60 subjects, a 2-hour walk in the exposure site resulted in an average increase in exposure to EC, NO_2, UFP, and $PM_{2.5}$, compared with exposure to these pollutants during a 2-hour walk at the control site, by a factor of 4.8, 4.0, 3.4, and 2.0 respectively (see Table 6).

High concentrations of DE may exist in other locations, such as bus depots, diesel-powered train stations, and certain road tunnels. Although DE concentrations in these places are possibly even higher than those at the exposure site, it would be more difficult for the subjects to walk or behave naturally in these other locations. Therefore, Oxford Street provides an unusual opportunity, a natural exposure chamber, to investigate DE health effects.

EFFECTS ON ASTHMA SYMPTOMS

In the present study, we observed an increase in asthma symptoms score in the hours and days (up to a week) after a 2-hour session at the exposure site, although none of the scores were statistically significant at any of the time points (see Tables 19 and 20). The maximum control-adjusted percentage change from baseline was 46.9% for all the subjects combined, 47.8% for the subjects with mild asthma, and 46.6% for the subjects with moderate asthma, all occurring at day 2. The scores were combined from the five assessed symptoms: shortness of breath, chest tightness, wheezing, cough, and sputum production. Among the five symptoms, chest tightness and sputum production appeared to change the most. However, because the baseline symptom score was 1.33 (combined), 1.08 (mild asthma group), and 1.63 (moderate asthma group) on a scale of 0 (no symptoms at all) to 15 (severe on each of the 5 symptoms), a 50% increase in the combined symptom score would still yield a score less than 2.4. This explains why we did not receive any serous complaints from the subjects about their symptoms during and after exposure. The symptom scoring system may look arbitrary, but it is a respectable and simple way to measure symptoms. For the assessment of breathlessness, this scoring system has been validated against other physiologic parameters of breathing (e.g. Mahler et al. 1984). The subjects recorded their use of medication that provided an immediate effect on asthma symptoms (asthma reliever medication) on their symptom forms, but we did not ask them to record other medications that are taken regularly but are less likely to have an immediate impact on symptoms. In this study, asthma reliever medication refers to short-acting β-agonists, such as albuterol, that are delivered by inhaler.

Consistent with our findings on asthma symptoms, the daily frequency of asthma reliever medication use generally increased after exposure sessions. This increase was more apparent in the moderate asthma group, with a maximum increase of 41% (see Figure 6 and Table 22).

One of the inherent shortcomings of the present study is that subjects could not be blinded to exposure-versus-control sessions, which could potentially bias their reports. However, it is difficult for subjects to consistently and intentionally over report or under report symptoms or medication use on a daily basis for 7 days.

We were unable to find previous studies reporting short-term DE exposure effects on symptoms or medication use in asthmatic patients. In two previous studies, healthy adult subjects complained of irritation to the eyes and nose or an unpleasant smell during exposure to DE (Rudell et al. 1996, 1999).

EFFECTS ON LUNG FUNCTION

One of the most important findings of the present study is the observed effects on lung function. The exposure resulted in up to a 5% reduction in FEV_1 during the 2-hour walk at the exposure site and 5 hours after it. The reductions were

statistically significant at all time points. Reduction in FVC, up to 7%, was also observed but was not statistically significant at as many time points as FEV_1. Although not statistically significant, $FEF_{25–75}$ values also showed decreases after exposure. These lung function indices were measured up to 22 hours after exposure. At the last time point of measurements for FEV_1, FVC, and $FEF_{25–75}$, the group mean values of the three lung function indices, except FVC for the mild asthma group, still appeared to be lower after the exposure session than after the control session (Figure 6). Therefore it is possible that the effect may last beyond 22 hours after exposure, especially considering that the effect on daily PEFR trend was observed for up to 7 days after exposure.

We observed no statistical significance in the group mean values of $FEF_{25–75}$ between the exposure and control sessions. This may be due to the large intersubject difference in $FEF_{25–75}$; a larger sample size is usually required to detect a statistically significant effect for a more variable endpoint than for a less variable endpoint. Among the lung function indices measured in the present study (FEV_1, FVC, $FEF_{25–75}$, and PEFR), $FEF_{25–75}$ had the largest intersubject variability (see CV values in Table 11). From our individual-subject-based regression analyses using single-pollutant models, however, postsession changes in $FEF_{25–75}$ from baseline were significantly associated with each of the four pollutants ($PM_{2.5}$, EC, UFP, and NO_2) (see Figure 7 and Tables 25–37), indicating that an increase in during-session pollutant concentration led to a significant reduction in $FEF_{25–75}$. Interestingly, $FEF_{25–75}$ appears to be the most sensitive lung function index in these pollutant-specific analyses, as FEV_1 showed significant associations with only three of the four pollutants (EC, UFP, NO_2), and FVC showed a significant association only with $PM_{2.5}$.

In contrast, previous exposure chamber studies with human subjects failed to show any significant change in lung function after 1 to 2 hours of DE exposure for either healthy or asthmatic subjects (Rudell et al. 1996; Nightingale et al. 2000; Salvi et al. 2000; Nordenhäll et al. 2001; Holgate et al. 2003; Stenfors et al. 2004). Because the DE concentrations (> 100 μg/m^3 as PM_{10} or $PM_{2.5}$) used in the exposure chamber studies were substantially higher than $PM_{2.5}$ concentrations in our exposure sessions (37.1 ± 13.8 μg/m^3), the amount of exposure to DE does not seem to explain the discrepancies. The exposure chamber studies, however, had fewer subjects (8–25) and thus might not have sufficient statistical power to detect effects on lung function.

A small number of exposure chamber studies have employed whole-body plethysmography as a more sensitive measure of lung function (Rudell et al. 1996; Nordenhäll et al. 2001; Stenfors et al. 2004). Short-term DE exposure led to increased airway resistance in all three studies. Stenfors and colleagues (2004) found that the increase in resistance was more apparent, although not statistically significant, in the asthmatic subject group than in the healthy subject group, suggesting that people with asthma may be more sensitive to the effects of DE.

The effects of DE on pulmonary function have also been investigated in a variety of species, including hamster, rat, cat, and monkey (Heinrich et al. 1986; Lewis et al. 1986; Vinegar et al. 1986). Both restrictive and obstructive patterns of pulmonary dysfunction have been reported after chronic exposure. Decreases in lung volume and compliance have been observed, suggesting a restrictive disorder compatible with parenchymal fibrosis. Reduced expiratory flow rates, implying obstructive airflow limitation, have also been described in chronically exposed animals. Although these animal studies provided evidence of adverse effects of DE exposure on lung function, they typically involved exceptionally high concentrations (350–12,000 μg/m^3) of DE particles delivered over prolonged periods of time (3–24 months). Thus, it is difficult to compare the animal study results with findings from the present study.

We also examined the effects of the 2-hour DE exposure on bronchial reactivity of asthmatic subjects by measuring PC_{20} 5 hours after the end of each exposure and control session. We did not observe a significant PC_{20} difference between the exposure and control sessions. The only PC_{20} finding that came close to statistical significance was its association with NO_2 ($P = 0.064$), showing an increase in bronchial reactivity (a decrease in PC_{20} value) with an increase in NO_2 exposure. In a previous exposure chamber study, Nordenhäll and colleagues (2001) demonstrated significantly increased bronchial reactivity after DE exposure among people with mild asthma. There are a few reasons for the discrepancy between the two studies. The exposure chamber study used significantly higher DE concentrations and assessed bronchial reactivity at 24 hours after exposure as opposed to 5 hours after exposure in the present study. The exposure chamber study may be more prone to a Type I error due to the small number of subjects ($n = 14$).

EFFECTS ON AIRWAY INFLAMMATION AND ACIDIFICATION

Airway inflammation is considered a major factor in asthma pathogenesis. Airway inflammation may be assessed by invasive procedures such as fiberoptic bronchoscopy with bronchoalveolar lavage, or bronchial biopsies, but in the present study we used three less-invasive

techniques to assess airway inflammation: measurement of EBC pH and eNO, and induction of sputum.

EBC Markers

The measurement of EBC has been proposed as a simple, safe, and noninvasive technique to assess airway inflammation from the lower respiratory tract of children and adults (Mutlu et al. 2001). Several inflammatory mediators and markers of oxidative stress have been detected in EBC from healthy subjects or subjects with various illnesses (e.g., asthma, chronic obstructive pulmonary disease, bronchiectasis, cystic fibrosis, and acute respiratory distress syndrome). These include hydrogen peroxide, 8-ISP, serotonin, cytokines (IL-1, IL-8, TNF-α), nitrotyrosine, TBARS, leukotrienes, nitrite (or total nitrite and nitrate), prostaglandin E, and pH. However, important methodologic issues surrounding EBC collection and assay have been raised. Different methods used by different investigators may have resulted in conflicting data being reported or results that cannot be reproduced (Horvath et al. 2005). Furthermore, the exact site of the upper airway being sampled by EBC is disputed, as are the dilutional effects of pulmonary epithelial lining fluid with condensed water vapor (Effros et al. 2004). Oral contamination with ammonia has been used as an argument that EBC pH cannot provide reliable information regarding epithelial lining fluid pH. However, this is disputed (Hunt 2006). In the present study, we were able to consistently measure pH and iron concentration, but were unable to assay 8-ISPs (see Methods).

EBC pH We observed a significant difference ($P \leq 0.05$) in group mean EBC pH between the exposure and control sessions at both measurement times (hours 3 and 6). We observed that EBC pH at hour 3 was significantly and negatively associated with UFP and with NO_2 (see Figure 7). We also found that subjects with moderate asthma had lower EBC pH compared with subjects with mild asthma (see Table 11). This shows that the more severe the asthma, the greater the concentration of H^+ ions recovered in the EBC. This supports the findings of a previous report (Kostikas 2002). However, a more recent study found no differences in the EBC pH of asthmatic children compared with that of nonasthmatic children (Nicolaou et al. 2006). The asthmatic children probably had very mild asthma.

One of the most provocative findings to emerge from EBC studies has been the observation of Hunt and colleagues (2002) that EBC is acidified in patients with bronchial asthma, a phenomenon the authors designated as *acidopnea*. Acidopnea has also been described in other illnesses associated with airway inflammation (acute lung injury, chronic obstructive lung disease, bronchiectasis, and cystic fibrosis) (Kostikas et al. 2002; Tate et al. 2002; Gessner et al. 2003). Therefore, measurement of EBC pH has been described as a simple, noninvasive, inexpensive, and easily repeatable procedure for evaluating the inflammatory process in airway diseases (Kostikas et al. 2002). Although the mechanism underlying acidopnea remains unknown at present, EBC pH effects, when measured properly, have proven to be robust against artifacts of ventilatory pattern, storage conditions for samples, and orally-derived ammonia (bacteria related) (Vaughan et al. 2003).

Hunt and colleagues (2002) reported reductions in pH values of more than 2 logs (i.e., H^+ ions increased by a factor of more than 100) during an exacerbation of asthma. The pH values returned to normal when the patients were successfully treated with corticosteroids. The pH decrease that we observed from subjects breathing exposure-site air was only on the order of half a log (a 2-fold increase in H^+ ions). It was associated with significant reduction in lung function and with detectable but relatively small changes in symptom scores. The importance of the more marked fall in EBC pH after exposure, relative to control sessions, in the subjects with moderate asthma than in those with mild asthma remains unclear. If EBC pH is indeed a reflection of pH in the epithelial lining fluid of the lower airways, and decreased intracellular pH of inflammatory cells occurs during the process of activation (Hunt et al. 2000; Kostikas et al. 2002), then the fall in pH may indicate increased inflammatory cell activation within the lining of the airways. We found evidence of neutrophil recruitment and activation with the release of MPO in sputum, which would support the concept of neutrophilic inflammation.

It has been suggested that at lower pH values, that are observed during asthmatic exacerbations, an endogenous airway compound, nitrite, is converted to NO in quantities sufficiently large to account for the increased concentrations of eNO in expired air from asthmatic subjects (Hunt et al. 2002). This suggestion, however, is less likely to explain the findings in the current study because it is not supported by the following features noted in the present study: First, no significant correlations were found between EBC pH concentrations (or H^+ ion concentrations) and eNO concentrations. Second, no significant differences in eNO change from baseline were found between the exposure and control sessions. Finally, eNO was found to be significantly associated with EC, while EBC pH was significantly associated with NO_2 and with UFP, but not with EC.

EBC Free Iron This study appears to be the first in this research field to include measurements of iron concentration

in EBC. We reasoned that exposure to DE particles could place an iron burden on the lungs because the particles contain elemental iron (Karar et al. 2006). We also reasoned that inhaled DE could lead to changes in the binding capacity of iron in the fluid of the lung lining that would modulate the concentration of free iron. Iron is also an important pro-oxidant because it can catalyze ROS formation in the lung (Quinlan et al. 2002). Previous studies of iron in the lungs have resorted to assays of total iron in bronchoalveolar lavage fluid. Increased iron concentrations in the bronchoalveolar lavage fluid of current smokers compared with those of nonsmokers have been reported (Thompson et al. 1991). This increase was also found in the epithelial lining fluid. Iron-binding capacity in epithelial lining fluid is mediated mainly by specific iron-binding proteins such as transferrin, lactoferrin and ferritin; the iron not bound is considered free, although in practice it is usually bound to compounds with low molecular weight or is loosely bound by proteins such as albumin which are not specific for iron (Mateos et al. 1998). Thus, we assayed the relatively low molecular weight redox-active iron, which we thought would be most responsible for oxidative stress in the lungs. Because we did not assay iron-binding protein concentration, we were not able to determine whether pollutant exposure could lead to the release of free iron in the lungs. On the other hand, particle inhalation is likely to increase the burden of iron and other metals on the lungs (Mutti et al. 2006).

We observed a 96.4% average increase from baseline in EBC iron at hour 6 after exposure sessions, relative to hour 6 after control sessions ($P = 0.099$; Table 38). This increase might have been due to inhalation of particles at the exposure site which were expected to have higher iron content than particles at the control site. On the contrary, this marker, at both hours 3 and 6, was significantly and negatively associated with $PM_{2.5}$ but not with any of the other three pollutants. This suggests that $PM_{2.5}$ has a deleterious effect on EBC free iron, a possibility that should be investigated more carefully in future studies.

Table 38. EBC Iron From Nonstratified Analysis[a]

Time (Hr)	Baseline (nmol/L)	β_3 (nmol/L)	β_3/ Baseline (%)	SE	*P*
3	148	6.32	4.26	64.81	0.923
6	148	143.04	96.37	85.88	0.099
Overall		*df* = (2, 101)		*F* = 2.32	0.103

[a] This table was copied from Appendix Table B.9.

eNO

eNO is often used as a noninvasive marker of airway inflammation (Kharitonov and Barnes 2001). Compared with healthy controls, asthmatic subjects have higher airway eNO concentrations (Hamid et al. 1993; Kharitonov et al. 1997). Some evidence suggests a relation between eNO and the clinical signs and symptoms of asthma, especially during acute exacerbations. However, concentrations of eNO are affected by a number of other subject-specific factors that remain to be studied.

Studies have shown positive associations between eNO concentrations and $PM_{2.5}$ exposure in asthmatic children (Koenig et al. 2003) and in adults with asthma or COPD (Jansen et al. 2005); the subjects in both groups lived in Seattle, Washington. However, we did not find a statistically significant association between eNO and $PM_{2.5}$ in the present study, and did not find a significant difference in mean eNO concentration between the exposure and control sessions. The Seattle studies, which used a panel design, repeatedly measured the subjects' eNO on consecutive days over a period of several weeks (10 days in a winter season and 10 days in a spring season in the study with children; 12 days in the study with adults), whereas the present study measured the subjects' eNO concentration only twice. Given the large intersubject variability in eNO concentration we found in the current study (CV 74%–85%; see Table 11), the previous studies, albeit with a different study design and smaller sample sizes, might have been expected to have sufficient statistical power to detect an effect (19 in the study with children and 16 in the study with adults).

Induced Sputum Markers

The induction of sputum provides a sample of the inflammatory cells (e.g., neutrophils, macrophages, epithelial cells, eosinophils, lymphocytes) and soluble markers (e.g., IL-8, ECP, MPO) present in the airway lumen of the bronchial tree. The technique was described about half a century ago by Bickerman and colleagues (1958) for the diagnosis of lung cancer and later respiratory infections. Over the last 10 years, this technique has been applied as a noninvasive measure of inflammation in the airways, particularly involving airway conditions such as asthma and COPD. The reproducibility of differential cell counts (i.e., the percentage of total nonsquamous cells) and soluble mediators has been shown to be good using this method for samples obtained on different days from both healthy subjects and stable asthmatic subjects, irrespective of the

method of sputum processing employed (Pizzichini et al. 1996; Spanevello et al. 1997). Cell counts and supernatant measures differ among asthmatic, COPD, and healthy subjects (Cicutto et al. 2004). Asthmatic individuals have increases in sputum cell counts for eosinophils, mast cells, and neutrophils, as well as for markers of inflammatory cell activation, including ECP. The presence of eosinophils in sputum has been shown to be a more sensitive marker of airway inflammation than increased serum eosinophils or ECP concentration (Sorva et al. 1997). Sputum analysis can also detect changes in asthma control, such as increased cell counts during exacerbations and increased eosinophils and neutrophil numbers after an allergen challenge. Conversely, improved asthma control after treatment with inhaled or oral corticosteroids has been shown to be accompanied by decreases in sputum eosinophils and supernatant ECP concentrations (Keatings et al. 1997). Concentration of eosinophils in sputum has been used as a marker of severity to determine treatment, and controlling the concentrations of sputum eosinophils has been shown to provide the best measure of successful treatment (Saeed et al. 2002).

The major source of the MPO enzyme is neutrophils, but it is also produced by monocytes and tissue macrophages. Activated neutrophils release MPO from their cytoplasmic granules into the extracellular space and phagosomes, where it generates hypochlorous acid. MPO, therefore, serves as an indicator of neutrophil activity. It has been shown to increase in the sputum of healthy subjects exposed to DE (Nightingale et al. 2000; Nordenhäll et al. 2001). The present study demonstrated a group average increase of 521% from baseline in the concentration of sputum supernatant MPO after the exposure session compared with the control session ($P = 0.014$) (see Table 13).

Therefore, the sputum marker (MPO and neutrophils) results from the present study suggest a neutrophilic response after short-term DE exposure in asthmatic patients. The neutrophilic effect of short-term DE exposure concurred with somewhat consistent findings among previous exposure chamber studies that demonstrate increased neutrophil counts in sputum and bronchial lavage after exposure to DE or resuspended DE particles (Rudell et al. 1996, 1999; Salvi et al. 1999; Nightingale et al. 2000; Stenfors et al. 2004). Salvi and colleagues (1999) reported that exposure to DE, at 300 $\mu g/m^3$ PM_{10} for 1 hour, compared with exposure to clean air, resulted in increased numbers of neutrophils and B lymphocytes in the BAL fluid, as well as increased neutrophils, total T lymphocytes, $CD4^+$ lymphocytes, and mast cells in the epithelial submucosa, 6 hours after exposure (Salvi et al. 1999). Using the same DE exposure concentration and duration as those used by Salvi and colleagues (1999), Nordenhäll and colleagues (2001) observed a nonsignificant MPO increase in induced sputum 6 hours after exposure. Stenfors and colleagues (2004) observed a simultaneous decrease in submucosal neutrophils in bronchial biopsies 6 hours after DE exposure, suggesting movement of cells from the airway wall into the airway lumen (Stenfors et al. 2004). The influx of neutrophils to the airways may be a response to the release of inflammatory mediators.

In vitro studies demonstrated that exposure of human bronchial and alveolar epithelial cells to DE particles releases IL-8 (Bayram et al. 1998b; Seagrave et al. 2004). In vivo exposure chamber studies with human subjects have replicated this, demonstrating increased IL-8 concentrations in bronchial lavage and biopsies (Salvi et al. 2000; Nordenhäll et al. 2001) as well as a significant increase in the expression of IL-13 in the bronchial epithelial cells (Pourazar et al. 2004). The present study is the first to observe some evidence of increased IL-8 concentrations in induced sputum after exposure to DE in realistic environmental concentrations.

EFFECTS ON OXIDATIVE STRESS

Exposure to DE particles has been shown to generate a systemic response in healthy subjects, with significant increases in peripheral blood neutrophils and platelets (Salvi et al. 1999). The results from in vitro and animal studies suggest that DE particle exposure induces oxidative stress (Sagai et al. 1993; Lim et al. 1998; Hiura et al. 1999; Bonvallot et al. 2001). A study with human subjects demonstrated links between increased exposure to DE particles and increased production of an oxidative stress marker, CO, in exhaled breath and increased sputum neutrophil and MPO concentrations (Nightingale et al. 2000). To evaluate any local and systemic response (specifically oxidative stress) after DE exposure, we attempted to measure several EBC and blood markers of oxidative stress. Unfortunately, we were successful in measuring only TBARS in venous blood (see Methods). We did not detect any significant changes in blood TBARS concentration after the exposure sessions compared with the control sessions. One reason for not finding changes may be that those studies that demonstrated a systemic effect used significantly higher concentrations of DE or DE particles than those measured in our study. Another reason may be that we measured TBARS only once, 5 hours after the exposure, and so may have missed a rapidly-occurring oxidative-stress event.

THE ROLE OF WHOLE DE VERSUS DE COMPONENTS

Diesel exhaust is a complex mixture of particles and gases. Investigating the relative toxicity of separate DE components to observed adverse effects of DE exposure has long been an important research goal. For example, in vitro studies have shown effects of whole DE on a diverse range of cytokines; however, such effects were not observed when the particles were removed from the exhaust or when inert particles were substituted for DE particles (Boland et al. 1999; Abe et al. 2000). Furthermore, DE particles scrubbed of their adsorbed organic components elicited a substantially diminished effect on cytokine production (Boland et al. 1999).

Utilizing natural day-to-day variations in concentrations of the four DE-related pollutants that we measured, we attempted to explore the relative role of these pollutants in affecting the health of asthmatic patients. The results from the single-pollutant and two-pollutant model analyses showed that UFP and EC were most consistently associated with one or more lung function indices: FEV_1, FVC, and $FEF_{25–75}$. UFP also had a significant effect on PC_{20} and on sputum MPO. In contrast, among all the health endpoints measured in the present study, an increase in during-session $PM_{2.5}$ concentration was associated only with a decrease in $FEF_{25–75}$ and with a decrease in EBC iron (see Table 33). In addition, the $PM_{2.5}$ effect on $FEF_{25–75}$ was no longer significant after adjustment for EC or UFP.

Particles freshly released from combustion sources are predominantly ultrafine. These particles do not have much mass because they are extremely small. Measurements of particle number may be more accurate than measurements of mass for quantifying these particles. Therefore, the stronger effects of UFP than those of $PM_{2.5}$ observed in the present study may simply reflect that UFP number concentration is a more sensitive measure of DE particles than is $PM_{2.5}$. We indeed observed a substantially larger gradient in during-session concentrations of UFP than in those of $PM_{2.5}$ across all the study subjects. Likewise, we observed a larger gradient in exposure concentrations of EC which may have served as a better proxy of DE than did $PM_{2.5}$.

Although few UFP epidemiologic studies have been performed to date, the existing results suggest an association of UFPs with certain respiratory health endpoints that is at least as strong as those with PM_{10} and $PM_{2.5}$ (Peters et al. 1997; Penttinnen et al. 2001). Another important characteristic of UFPs is that because they are extremely small, they have a large surface area per unit mass that is capable of carrying relatively large amounts of toxic substances and free radicals (Oberdörster et al. 1995; Nel et al. 2006). This provides an opportunity for the surface chemistry of the particles to have a biologic effect on cells that come in contact with them (Li et al. 2003).

Researchers increasingly recognize that particle composition plays an important role in particle toxicity. Animal chronic exposure studies (~ 6 months) have shown that different types of particles (e.g., ambient, inert carbon, wood combustion, gasoline exhaust, and DE particles) differentially affect lung responses (Baulig et al. 2003; Inoue et al. 2005; Seagrave et al. 2005). In general, results from these studies suggest that DE particles produce lung inflammation to a larger extent than other types of particles (Baulig et al. 2003; Inoue et al. 2005).

In the present study, we found that eNO concentrations were significantly associated with EC concentrations, indicating that increased EC exposure led to increased production of eNO (see Figure 7, panel B and Appendix C). DE particles have a characteristic carbon core onto which numerous organic chemicals and transition metals are adsorbed. EC is a proxy of this carbon core and is often regarded as an indicator of DE particles. Previous in vitro studies have shown that DE particles and their organic extracts were capable of inducing ROS in macrophages and epithelial cells (Sagai et al. 1993; Hiura et al. 1999; Bonvallot et al. 2001). However, inert carbon black did not increase ROS production (Hiura et al. 1999; Baulig et al. 2003). Mechanistic studies further suggested that PAHs and quinones adsorbed onto DE particles (or the carbon core) are specifically responsible for increased oxidative stress (Li et al. 2002; Takano et al. 2002). Increased oxidative stress, resulting from the instillation of DEP into the trachea of mice, was accompanied by increased production of NO, CO, and mucus (Lim et al. 1998). This may help explain why eNO was significantly associated with only EC in the present study. However, the EC effect on eNO did not appear to be independent from NO_2, because adjustment for NO_2 in the two-pollutant model significantly attenuated the EC effect. This suggests that complex gas-particle interactions are involved in eNO induction (and other related effects), which warrants future mechanistic investigations.

Compared with UFP or EC, NO_2 appeared to have a weaker effect on lung function. However, the inclusion of NO_2 in two-pollutant models significantly reduced the UFP effect on EBC pH and the EC effect on eNO. Interestingly, NO_2 was significantly associated with EBC pH, and this association was not affected by copollutants. In addition, NO_2 was the only pollutant that had a significant effect on bronchial reactivity (PC_{20}) and on IL-8 in induced sputum (see Table 37). It is known that at high exposure concentrations, NO_2 irritates and inflames the airways,

which increases the severity of respiratory symptoms for those with respiratory conditions (Strand et al. 1997, 1998; Chauhan et al. 1998). Findings from the present study demonstrate the health importance of NO_2 as a component of traffic pollution in city streets.

EFFECTS MODIFIERS

The main effects modifier, which forms the basis for one of the hypotheses of the present study, is background asthma severity. To our knowledge, this is the first study that examines the contribution of background asthma severity to an individual's respiratory response to DE exposure. Previous human studies, using exposure chambers, have been carried out either with healthy subjects or with asthmatic subjects whose background asthma severity levels were not considered (Rudell et al.1996, 1999; Salvi et al. 1999; Nightingale et al. 2000; Stenfors et al. 2004). In an exposure chamber study comparing airway resistance between subjects with mild asthma and healthy subjects, the investigators reported a larger increase in airway resistance in the asthmatic group, suggesting that asthmatic people may be more sensitive to the effects of DE exposure than healthy people (Stenfors et al. 2004).

The present study included study subjects with mild (n = 31) or moderate (n = 29) asthma. This allowed us to analyze data stratified by asthma severity. Our results show stronger effects of diesel traffic exposure in the moderate asthma group than in the mild asthma group on FEV_1, FVC, EBC pH, and sputum MPO (see Appendix B). In the pollutant-specific exposure–response analyses (Appendix C), we found that EC had stronger effects on FEV_1 in subjects with moderate asthma than in those with mild asthma, while UFP and NO_2 had similar effects on FEV_1 in subjects with mild or moderate asthma. We also found that both UFP and NO_2 had stronger effects on EBC pH in the moderate asthma group. UFP had stronger effects on FVC and sputum MPO in that group as well. The effects of EC on eNO appeared to be stronger for people with mild asthma. Interestingly, the effects of $PM_{2.5}$, EC, and UFP on $FEF_{25–75}$ all appeared to be stronger in the mild asthma group. NO_2 appeared to have a stronger effect on bronchial reactivity (PC_{20}) in the mild asthma group and a stronger effect on sputum IL-8 in the moderate asthma group. The biologic basis for these observed modifying effects of background asthma severity needs to be further studied. On the other hand, several limitations should be considered regarding analyses stratified by asthma severity. First, the differences described above appear to be based more on statistical significance than on effects estimates (differences in central tendency). Second, the sample sizes were relatively small. Finally, some misclassification of severity status was likely, because severity status was self-reported by the subjects during the screening visits. People's perception of their own asthma severity may change for reasons not apparent to the researcher.

One potential effects modifier is atopy status. Among the 60 asthmatic subjects, 42 were confirmed to be atopic, 8 were nonatopic, and the status of 10 was unknown. Because only a small fraction of the subjects were confirmed to have nonatopic asthma, the present study lacks the statistical power to test the effect of atopy. In a subset of analyses, however, we constrained our analyses by including only the atopic subjects. The results from this set of constrained analyses were similar to those from the unconstrained analysis. Therefore, the findings we reported did not appear to be confounded by atopy status.

Another potential effects modifier is preventive corticosteroid medication use. The study was designed to account for this by including within-subject comparisons (the same subject's exposure versus control sessions). Among the 60 subjects, 23 were corticosteroid naive, and the remaining 37 subjects were taking inhaled corticosteroids. There was no difference in response, after the exposure sessions compared with after the control sessions, between those taking inhaled corticosteroids and those not. This applies to changes in FEV_1, FVC, $FEF_{25–75}$, symptom scores, EBC pH, EBC iron, eNO, sputum markers, PC_{20}, and blood TBARS concentration. Therefore, our findings were not affected by whether subjects were taking inhaled corticosteroids.

Among the 37 subjects who were taking preventive corticosteroid medication, 25 (86%) had moderate asthma and 12 had mild asthma. As expected, the more severe asthma group had more subjects who used inhaled corticosteroids. If there had been an effect of preventive medication use, one would have expected less of an effect on endpoints in this group. However, for many parameters (e.g., FEV_1), the pollutant had a greater effect on subjects with more severe asthma, as discussed earlier. This makes it even less likely that preventive medication use might have influenced the response.

STUDY STRENGTHS AND LIMITATIONS

The present study is unique in its utilization of a London street and a nearby public park as natural exposure settings with proven differences in ambient concentrations of pollutants released from diesel-powered vehicle engines. The study included subjects with a range of ages and approximately the same numbers of males and females. No subjects encountered any adverse event or dropped out after starting the exposures, indicating that the study protocol was safe, practical, and reliable.

The study offers several advantages over exposure chamber studies for studying the effects of pollution on people. Previous exposure chamber studies have used a considerably smaller number of subjects (typically 10–15), few of whom had asthma. Previous studies with asthmatic subjects included only subjects with mild asthma, in contrast to the present study in which an approximately equal number of subjects with mild and with moderate asthma were compared regarding their responses to DE exposure.

The dose (exposure duration × exposure concentration) of DE pollutants was significantly smaller in the present study than in the exposure chamber studies. The realistic exposure condition used in the present study is frequently experienced by much of the general public. Exposure chamber studies have used a single fuel type, engine type, and a constant engine load, effectively delivering a controlled DE profile. It is well established that these factors alter exhaust emissions' attributes such as particle number, size distribution, and chemical composition. In the real world, DE is generated from engines of differing age, size, running temperature, and load, thus producing a cocktail of diesel exhausts. The exposure environments in the present study are not artificial and so are more familiar and comfortable to the study subjects than an enclosed laboratory chamber would be. Therefore, studies conducted under real-life situations are more clinically relevant than in vitro studies or exposure chamber studies involving controlled animal exposure studies or human chamber studies.

Variations in traffic volume, traffic flow, and weather conditions led to session-to-session fluctuations in pollutant concentrations at both the exposure and control sites. This enabled the analysis of exposure–response relations. In contrast, exposure chamber studies have usually involved a single dose due to constraints on resources and the subjects' time commitment. However, pollutant concentrations during the exposure sessions typically were substantially higher than during the control exposures. As a result, the scatter plots of pollutant concentrations showed two clusters (modes) of data. This kind of data distribution is not ideal for analyses based on linear regression models, but the present study was not primarily designed to examine the exposure–response relation. The pollutant-specific analysis presented here should be considered exploratory, even though the repeated-measure mixed-effects models were used in our analysis to maximize the appropriateness of the analysis to the extent possible. We also constrained the analysis within the exposure sessions and within the control sessions. However, this reduced the number of data points by half and also greatly narrowed the range of pollutant concentrations for the regression analysis, producing results of no statistical significance for almost all pollutant-endpoint pairs.

One of the most obvious limitations of the present study is that the subjects could not be blinded to the site location. Some may have been inclined to report increased symptoms or produce submaximal effort when performing spirometry tests during and after the exposure session relative to the control session. However, the spirometer software used to measure and record lung function (FEV_1, FVC, and $FEF_{25–75}$) would have rejected a suboptimal spirometric effort. This would appear as a lag response on the flow-volume graph. In addition, biologic samples were coded in such a way that sample types could not be recognized by the lab staff during the sample analysis.

Because this was a crossover study, a learning effect could possibly have changed the subjects' behavior after the first visit. Because the order of sessions for each subject was determined by randomization, any learning effect should have been eliminated. Other practical limits to the present study were the subjects' personal exposure in the time before and between exposure visits, including exposure during travel to the base hospital and study sites. To some extent, this was dealt with by monitoring personal NO_2 exposure and time spent on various activities the week before each session. On each session day, subjects were transported from the base hospital to the exposure site or to the control site in a gasoline-powered taxi, a journey of less than 10 minutes. They were transported in the same manner to the base hospital for postsession measurements.

Another limitation of the present study is that we could not measure some potentially confounding exposures. For example, a walk in the exposure site is likely to be a more stressful experience than a walk in a quiet park. It is possible that some of the responses we measured were induced by factors associated with stress, particularly noise (Ising et al. 2003). In terms of pollutant characterization, we did not and could not possibly measure all chemical constituents that were present in the atmospheres of the exposure and control sites.

We did not study a referent group without asthma. Thus we cannot be sure that our findings are specific to people with asthma, although the greater responses in those with more severe disease would suggest that they are. Finally, we did not study exposure to gasoline-driven traffic and cannot conclude that diesel traffic is more toxic than other types.

CONCLUSIONS

The present study utilized two natural city locations as the exposure and control sites. Due to a high prevalence of traveling and idling diesel-powered vehicles, the exposure site exhibited higher concentrations of $PM_{2.5}$, EC, UFP, NO_2, formaldehyde, and several PAHs than the control site. These pollutants were found to be present at typical urban background concentrations at the control site. Sixty asthmatic participants, walking for 2 hours at the exposure site, were exposed to concentrations of EC, NO_2, UFP, and $PM_{2.5}$ that were, on average, 4.8, 4.0, 3.4, and 2.0 times, respectively, higher than the concentrations of these pollutants encountered while they walked for 2 hours at the control site.

The participants' asthma symptom scores were higher at hours 2 and 7 and up to 7 days after the exposure session than after the control session. A similar trend was observed for the frequency of daily asthma reliever medication use. However, the magnitude of the increase in either symptom score or medication use was relatively small and did not reach statistical significance.

Compared with walking at the control site, walking at the exposure site resulted in significantly larger reductions in spirometric lung function indices after the exposure session. The reductions after the exposure sessions were from 3.0% to 4.1% for FEV_1 and from 2.8% to 3.7% for FVC compared with control sessions. In addition, decreases in daily PEFR, although not statistically significant, were also observed during the 1-week measurement period after exposure.

The significant reductions in lung function were accompanied by acidification and inflammation of the airways, supported by the following evidence: (1) relative to the control, the exposure led to a significant reduction in the pH of EBC; (2) the exposure led to a significant neutrophilic response with increased neutrophils and increased myeloperoxidase concentration in induced sputum; (3) reductions in FEV_1 at one or more time points were correlated with changes in the inflammatory markers EBC pH, EBC iron, and sputum IL-8. However, no effect was found on TBARS, a marker of oxidative stress, in venous blood drawn 5 hours after exposure.

The changes in lung function indices (FEV_1, FVC, and $FEF_{25–75}$) were most consistently associated with UFP and EC exposure concentrations, whereas changes in EBC pH were most consistently associated with NO_2 exposure concentrations. In addition, NO_2 had a significant effect on bronchial reactivity (PC_{20}) and on IL-8 in induced sputum. NO_2 also modified the UFP effect on EBC pH and the EC effect on eNO. However, our findings cannot be interpreted as demonstrating a causal association with any measured pollutant. The measured pollutant concentrations may simply represent the entire roadside diesel-traffic exposure that comprises not only the pollutants measured in this study but also other pollutants in the complex DE mixture, and resuspended coarse particles from road dust, engine debris, and tire debris.

Analyses stratified by asthma severity showed stronger effects of DE exposure for subjects with moderate asthma than for those with mild asthma for FEV_1, FVC, EBC pH, and sputum MPO. This suggests that background asthma severity contributes to individuals' susceptibility to diesel traffic exposure.

RECOMMENDATIONS

The findings from the present study help to explain the epidemiologic evidence that associates diesel traffic exposures with asthma severity and of the symptoms that many asthmatic subjects report after exposure to DE. The changes in lung function were small and unaccompanied by significant symptoms, but would be more important for patients with more compromised lung function. This study by itself should not deter most people with asthma from visiting or working in busy urban environments. Our design has considerable advantages over conventional exposure chamber studies and could readily be adapted to assess therapeutic strategies for prophylaxis of adverse reactions to traffic for people with asthma or other cardiovascular or respiratory diseases.

Increasingly, the adverse health effects of traffic-related air pollution are being recognized (Peters et al. 2005). Exhaust from diesel-powered vehicles, due to its unique physicochemical properties, has been of particular concern (HEI 1995). The research on the contribution of DE to these adverse health effects is mostly derived from population studies and in vitro studies. Exposure chamber studies with human subjects have generated useful data, but the studies are few and are burdened with unavoidable limitations. The present study used natural settings to study the health effects of short-term DE exposure.

The exposure model of the present study could be used to investigate health outcomes in different types of subjects (e.g., healthy subjects, subjects with COPD, or asthmatic children). Ambient particulate exposure is also linked with adverse cardiovascular events, most notably acute coronary syndrome and cardiac arrhythmias (Peters

et al. 2000; Maitre et al. 2006). With a similar exposure protocol, future studies can be carried out to measure cardiovascular endpoints and biomarkers in asthmatic or other populations.

The exposure model can be modified to address scientific questions related to the ones addressed in the present study. For example, it is interesting and important to know whether exhaust from gasoline-powered vehicles, at similar $PM_{2.5}$ or UFP concentrations, could lead to effects in asthmatic subjects similar to those observed in the present study. Some other London location could provide a matching site to Oxford Street to examine nondiesel automotive exhaust while still using Hyde Park as the control site.

The present study was carried out during the winter to minimize the potential effect of pollen on asthmatic subjects. Previous studies have shown the health effects of interaction between air pollution exposure and allergen exposure (e.g., Svartengren et al. 2000). It would be interesting to examine whether short-term DE exposure could enhance pollen- or allergen-induced asthma symptoms using the present study model.

ACKNOWLEDGMENTS

We thank the following colleagues for their contribution to this study: Jim L. Stewart-Evans, Eleni Malliarou, Magnus Svartengren, and Weichung Joe Shih. We thank the 60 subjects for their voluntary participation in the study. We thank Mr. Steve Neville of Westminster Council for his assistance in arranging field experiments. We are grateful to many individuals for their kind assistance with this project, including the following: Drs. Greg Quinlan and Sharon Mumby (Intensive Care Department, Royal Brompton Hospital, Imperial College London) provided the analysis of EBC iron. Dr. Serpil C. Erzurum (Cleveland Clinic Foundation, in Cleveland, Ohio) analyzed nitrite and nitrate in the EBC samples. Environmental and Occupational Health Sciences Institute (EOHSI), in New Jersey, provided travel funds for Dr. Zhang to collect the pilot study data. Dr. Lin Zhang and Mr. Jason Herrington (UMDNJ) assisted in analyzing PAHs and carbonyls. Ms. Jian Tong (UMDNJ) assisted in preparing this report. Dr. Frank Speizer (Harvard University) provided helpful comments on the study protocol and on this report. The project is funded by HEI through a research agreement with the University of Medicine and Dentistry of New Jersey.

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APPENDIX A. Baseline Health and Exposure Questionnaires

HEALTH EFFECTS OF DIESEL EXHAUST IN ASTHMATIC PATIENTS:
A REAL-WORLD STUDY IN LONDON:

PART 1: BASELINE HEALTH QUESTIONNAIRE

Study No.:	
Name:	
Date of birth:	
Sex:	M F
Home address:	
Telephone: home	
work	
e-mail	

1.	Have you had wheezing or whistling in your chest at any time *in the past 12 months*?	Y	N
2.	Have you woken up with a feeling of tightness in your chest at any time *in the past 12 months*?	Y	N

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3. Have you had an attack of asthma *in the past 12 months*? Y N

4a. Have you ever been told by a doctor that you have asthma? Y N

4b. At the moment, what treatment are you taking for your asthma?

reliever inhaler	Y	N	name and dose:
preventer inhaler(s)	Y	N	1. name and dose: 2. name and dose:
steroid tablets (prednisolone)	Y	N	daily dose:
other tablets	Y	N	1. name and dose: 2. name and dose: 3. name and dose:
other treatments (including complementary treatments)	Y	N	1. name and dose: 2. name and dose: 3. name and dose:

4c. if you are **not** taking steroid tablets at present, have you:

done so in the past 12 months?	Y	N	no. of times:
done so at any time in the past?	Y	N	

5. Have you been admitted as a hospital patient because of your asthma:

in the past 12 months?	Y	N	no. of times:
at any time in the past?	Y	N	

6. Do you currently attend a hospital clinic for your asthma?:

Y	N	name of hospital:

7. How far can you walk before you have to stop for breath?

Less than 50 yards	
50-100 yards	
Less than half a mile	
More than a mile	
As far as I want to	

8. Do any of the following make your asthma worse?

Colds	Y	N	Not sure
Dust in the home	Y	N	Not sure
Cats	Y	N	Not sure
Dogs	Y	N	Not sure
Aerosols in the home	Y	N	Not sure
Fumes, dusts or gases at work	Y	N	Not sure
Traffic fumes	Y	N	Not sure
Cleaning fluids	Y	N	Not sure
Cigarette smoke	Y	N	Not sure
Pollen	Y	N	Not sure
Cold weather	Y	N	Not sure
Exercise such as running	Y	N	Not sure
Perfumes	Y	N	Not sure
Foods	Y	N	Not sure
Stress or strong emotions	Y	N	Not sure

9. Do you have any medical conditions other than asthma which make it difficult to walk?

Y	N	please specify:

10a. Do you regularly smoke cigarettes? Y N

10b. Have you smoked any cigarettes in the past year? Y N

10c. Have you ever been a regular cigarette smoker? Y N

11a. In the last 12 months, have you had a runny or blocked nose, accompanied by sneezing or itchy eyes (when you did not have a cold)? Y N

11b. If **yes**, in which month(s) did this happen?

Jan:		Apr:		July:		Oct:	
Feb:		May:		Aug:		Nov:	
Mar:		June:		Sept:		Dec:	

12a. Have you ever been told by a doctor that you have hay fever? Y N

12b. If **yes**, at the moment are you taking any treatment for hay fever?

antihistamine tablets Y N name and dose:

nasal spray or drops Y N 1. name and dose:

2. name and dose

13. Do you have any other allergies than those mentioned above?

Y N please state:

14. Do you have any other important health-related conditions than those mentioned above?

Y	N	please state:

15. Are you taking any other treatments than those mentioned above?

Y	N	names(s) and dose(s):

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PART 2: BASELINE EXPOSURE QUESTIONNAIRE

1. **Which of the following best describes the building in which you live now** (check only one)**?**

____ One family house, detached from any other house.
____ One family house, attached to one or more houses.
____ Building for only two families.
____ Building for three to or more families.
____ Boat tent or van
____ Other, specify _____

2. **What is the age of your home?**

About ____ years old
____ Don't know.

3. **How many rooms are in your home (do not count bathrooms, porches, balconies, foyers, halls or half rooms?** _____*rooms*

4. **Does your home have an attached garage?**

_____Yes _____No

5. **Does your home have any of the following** (check all that apply)**? If you currently use any of the items, indicate how often you have used each in the last week?**

Item	Yes	No	Uncertain	Frequency used during last week
Fireplace				
Wood / Coal burning stove				
Radiant heating				
Forced air heating				
Portable kerosene heater				
Other portable or unvented gas heater				
Other				

6. **What fuels are used for heating your home?**

Fuel	Yes	No
Natural gas		
Electricity		
Fuel oil (e.g. kerosene)		
Coal or Coke		
Wood		
Solar energy		
Other (specify):		
No Fuel or heating used		
Uncertain		

7. **Does the room you spend most of your time in at home have either of the following** (check all that apply)?

_____Wall to wall carpeting

_____Rugs

8. **Have you ever seen cockroaches inside your home?**

_____Yes _____No _____Uncertain

If yes, have you seen cockroaches in your home in the last 12 months?

_____Yes _____No _____Uncertain

9. **Do you have any animals / pets inside your home** (check all that apply)**?**

Animal / Pet	Yes	No	How Many?
Cats			
Dogs			
Birds			
Rodents			
Other (specify):			

10. Do you regularly use any of the following in your home? If yes, then specify the frequency:

Item	Yes	No	Uncertain	Frequency (times per week)
Air fresheners				
Carpet fresheners				
Incense				
Scented candles				
Mothballs				
Deodorizers				
Other				

11. Does anyone currently smoke in the home?

_____Yes _____No _____Uncertain

If yes, please go to Question 12, if no, please go to Question 13.

12. On average during the past week, how many cigarettes, cigars, or pipes-full of tobacco per day were smoked in your home?

_____cigarettes _____pipes-full _____cigars

13. Do <u>you</u> cook in your home?

_____Yes _____No _____Uncertain

14a. What kind of stove/oven do you have (check one)**?**

Gas __ electric ___ other___

14b. If your answer to question 14a. is 'gas', does your gas stove/oven have a pilot light?

_____Yes _____No _____Uncertain

15. Do you work away from your home?

Yes ___ No___

If yes, please go to Question 16, otherwise please stop – this is the end.

16. Where do you work?

Street address: _____

_____

17. What is your job title?

_____

18. What are the four main tasks you are required to perform at work?

1.) _____

2.) _____

3.) _____

4.) _____

19. What method(s) of transportation do you usually use to get to work?

by car/taxi _____ by bus _____

by underground (Tube)_____ by foot _____

by train _____ by bicycle_____

by motorcycle _____ other (specify)_____

APPENDIX B. Summary of Results from Repeated-Measure Mixed-Effects Models to Compare Health Endpoints Between Exposure and Control Sessions

Repeated-measures mixed-effects linear regression models were used to model the average values of the health outcomes for each exposure at each time point. These models included the health outcome (FEV_1, $FEF_{25–75}$, and FVC) as the response (dependent variable), with *time* and *exposure* or *control* as categorical fixed effects, and individual subject as a random effect. Additional covariates, including ambient temperature and relative humidity, were entered for all the modeled health endpoints. Baseline NO_2 exposure was not included in the final models because we found no significant effects when included. In addition, age, sex, and ethnicity were entered as covariates for all the endpoints and biomarkers except FEV_1, FVC, and $FEF_{25–75}$, all of which were already reported as percentage-predicted values using height, age, and sex. In addition to the random effect for individual *subject* used to account for similarities of measures within an individual, a repeated-measure correlation structure was introduced to model correlations between measurements taken across time on the same day for each individual subject. The form of the model is as follows:

$$Y_{ijk} = \beta_0 + \alpha_i + \tau_j + \tau(\alpha)_{ij} + \beta_1 \mathrm{Temp} + \beta_2 \mathrm{RH} + \gamma_k + D_{ik}\phi + \epsilon_{j(ik)}$$

such that Y_{ijk} is the value of the health endpoint for the kth subject during the ith session ($i = 1$ for exposure, and $i = 0$ for control) at the jth time; τ_j represents the effect of the jth time ($j = 0, 1, 2, 3, 5, 7, 22$) within an exposure session (with $\tau_0 = 0$); α_i represents the effect of the site at baseline (with $\alpha_0 = 0$); $\tau(\alpha)_{1j}$ for $j = 1, 2, 3, 5, 7, 22$ measures the differences in health endpoint relative to baseline between the exposure site and the control site; γ_k represents the subject-specific random effect to adjust for the correlation within a subject between sites; and ϵ_{ijk} represents the random error, which is assumed to be normally distributed. The regression coefficients β_1 and β_2 represent the linear effects of temperature (Temp) and relative humidity (RH), respectively. An additional random effect for day was added to the model since multiple subjects had exposure sessions on the same day. The vector D_{ik} represents a series of indicator variables, identifying the day on which the kth subject experienced the ith session, with ϕ being a vector of random effects for days. The variance covariance structure for the ϵ_{ijk} was found to be best represented by a spatial power law structure using the Akaike Information Criteria and the Schwarz Bayesian Criterion. Specifically, the correlation between measurements taken at hours j and j' within session (i) and within person (k) are modeled as

$$\mathrm{cov}\left(\epsilon_{j(ik)}, \epsilon_{j'(ik)}\right) = \rho^{|j-j'|}\sigma^2$$

where ρ is the correlation between measurements taken one hour apart. In this model, $\tau(\alpha)$ represents the interaction between exposure (session) and time point. A test of this term reveals whether there are significant differences between exposures in the changes in health endpoints over time.

The models were created, parameters estimated and statistical tests performed using PROC MIXED in SAS Version 9.1.3. Using these models we performed both nonstratified analyses (for all subjects combined) and asthma-severity stratified analyses.

For each health endpoint measured in the study, we report the regression coefficient $\tau(\alpha)$, representing the exposure versus control difference in change from baseline (postsession minus presession). To translate these values into the difference in percentage changes from baseline, we divide by the baseline value for a particular health endpoint. For the endpoints that were measured at multiple time points, we also report F test results on the difference in overall time-series trend between exposure and control. The results are summarized in Tables B.1–B.20.

Table B.1. FEV_1 from Nonstratified Analysis

Time (Hr)	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	P
1	93.01	−0.88	−0.95	0.83	0.286
2	93.01	−3.00	−3.23	1.04	0.004
3	93.01	−3.06	−3.29	1.15	0.008
5	93.01	−3.82	−4.11	1.25	0.002
7	93.01	−2.81	−3.02	1.28	0.028
22	93.01	−1.87	−2.01	1.30	0.151
Overall		$df = (6, 530)$		$F = 2.19$	0.043

Table B.2. FEV_1 from Stratified Analysis

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*
1	95.7	−0.61	−0.64	1.17	0.602	90.1	−1.19	−1.32	1.19	0.318
2	95.7	−2.54	−2.66	1.49	0.089	90.1	−3.48	−3.86	1.45	0.017
3	95.7	−1.84	−1.92	1.66	0.269	90.1	−4.38	−4.86	1.58	0.006
5	95.7	−2.93	−3.07	1.82	0.109	90.1	−4.81	−5.34	1.67	0.004
7	95.7	−1.88	−1.97	1.88	0.318	90.1	−3.88	−4.30	1.70	0.023
22	95.7	−0.51	−0.53	1.92	0.792	90.1	−3.35	−3.72	1.71	0.051
Overall		*df* = (6, 272)		*F* = 0.86	0.525		*df* = (6, 244)		*F* = 1.84	0.093

Table B.3. FVC from Nonstratified Analysis

Time (Hr)	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*
1	103.19	1.06	1.03	1.22	0.384
2	103.19	−3.16	−3.06	1.45	0.029
3	103.19	−3.63	−3.52	1.54	0.018
5	103.19	−3.79	−3.68	1.59	0.018
7	103.19	−2.91	−2.82	1.61	0.070
22	103.19	−1.96	−1.90	1.61	0.226
Overall		*df* = (6, 528)		*F* = 2.76	0.012

Table B.4. FVC from Stratified Analysis

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*
1	104.5	0.20	0.19	1.52	0.895	101.8	1.97	1.94	1.93	0.309
2	104.5	−0.53	−0.50	1.82	0.772	101.8	−6.10	−6.00	2.27	0.008
3	104.5	−0.33	−0.32	1.94	0.864	101.8	−7.25	−7.12	2.38	0.003
5	104.5	−0.60	−0.58	2.01	0.765	101.8	−7.31	−7.18	2.44	0.003
7	104.5	−2.05	−1.96	2.03	0.315	101.8	−3.87	−3.80	2.45	0.116
22	104.5	1.30	1.24	2.04	0.525	101.8	−5.54	−5.45	2.46	0.025
Overall		*df* = (6, 271)		*F* = 0.49	0.814		*df* = (6, 243)		*F* = 4.36	0.000

Table B.5. $FEF_{25–75}$ from Nonstratified Analysis

Time (Hr)	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ baseline (%)	SE	*P*
1	64.52	−2.30	−3.56	1.80	0.203
2	64.52	−2.23	−3.45	2.09	0.286
3	64.52	−0.98	−1.53	2.18	0.651
5	64.52	−3.21	−4.97	2.22	0.150
7	64.52	−2.54	−3.94	2.23	0.255
22	64.52	−2.54	−3.94	2.24	0.257
Overall		*df* = (6, 528)		*F* = 0.59	0.737

Table B.6. $FEF_{25–75}$ from Stratified Analysis

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*	Baseline (% Predicted)	β_3 (% Predicted)	β_3/ Baseline (%)	SE	*P*
1	68.95	−1.01	−1.46	2.26	0.656	59.78	−3.69	−6.17	2.82	0.193
2	68.95	−4.46	−6.47	2.77	0.109	59.78	0.12	0.19	3.03	0.969
3	68.95	−3.21	−4.65	3.02	0.289	59.78	1.42	2.38	3.05	0.641
5	68.95	−4.77	−6.92	3.20	0.137	59.78	−1.58	−2.64	3.06	0.606
7	68.95	−0.96	−1.39	3.25	0.768	59.78	−4.50	−7.52	3.06	0.143
22	68.95	−3.04	−4.40	3.29	0.357	59.78	−2.06	−3.45	3.06	0.501
Overall		*df* = (6, 271)		*F* = 0.84	0.538		*df* = (6, 243)		*F* = 1.03	0.407

Table B.7. eNO from Nonstratified Analysis

Time (Hr)	Baseline (ppb)	β_3 (ppb)	β_3/ Baseline (%)	SE	*P*
3	45.43	−1.98	−4.36	1.39	0.154
4	45.43	−1.36	−2.99	1.51	0.369
5	45.43	0.56	1.24	1.60	0.725
6	45.43	−0.01	−0.03	1.66	0.994
7	45.43	0.18	0.40	1.71	0.916
22	45.43	0.55	1.22	1.84	0.765
Overall		*df* = (6, 652)		F = 1.14	0.339

Table B.8. eNO from Stratified Analysis

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline (ppb)	β_3 (ppb)	β_3/ baseline (%)	SE	*P*	Baseline (ppb)	β_3 (ppb)	β_3/ baseline (%)	SE	*P*
1	48.58	−0.22	−0.46	1.94	0.909	42.07	−3.41	−8.11	2.08	0.102
2	48.58	−1.20	−2.48	2.24	0.592	42.07	−1.21	−2.87	2.39	0.615
3	48.58	0.72	1.49	2.50	0.773	42.07	0.78	1.86	2.67	0.769
5	48.58	0.99	2.04	2.74	0.718	42.07	−0.83	−1.98	2.91	0.775
7	48.58	1.26	2.60	2.95	0.670	42.07	−0.63	−1.50	3.14	0.841
22	48.58	2.01	4.13	5.15	0.697	42.07	−0.77	−1.82	5.37	0.887
Overall		*df* = (6, 318)		*F* = 0.64	0.697		*df* = (6, 317)		*F* = 1.75	0.109

Table B.9. EBC Iron From Nonstratified Analysis

Time (Hr)	Baseline (nmol/L)	β_3 (nmol/L)	β_3/ Baseline (%)	SE	*P*
3	148	6.32	4.26	64.81	0.923
6	148	143.04	96.37	85.88	0.099
Overall		*df* = (2, 101)		*F* = 2.32	0.103

Table B.10. EBC Iron from Stratifed Analysis

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline (nmol/L)	β_3 (nmol/L)	β_3/ Baseline (%)	SE	*P*	Baseline (nmol/L)	β_3 (nmol/L)	β_3/ Baseline (%)	SE	*P*
3	103	37.60	36.55	54.09	0.490	209	−39.24	−18.82	134.57	0.772
6	103	93.66	91.06	55.00	0.094	209	207.49	99.52	181.33	0.259
Overall		*df* = (2, 55)		*F* = 1.47	0.238		*df* = (2, 41)		*F* = 1.69	0.197

Table B.11. EBC pH from Nonstratified Analysis

Time (Hr)	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
3	7.98	−0.16	−1.99	0.05	0.002
6	7.98	−0.11	−1.33	0.05	0.052
Overall		*df* = (2, 179)		*F* = 4.95	0.008

Table B.12. EBC pH from Stratifed Analysis

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline	β_3	β_3/ Baseline (%)	SE	*P*	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
3	8.04	−0.10	−1.19	0.06	0.112	7.91	−0.23	−2.92	0.08	0.006
6	8.04	−0.09	−1.11	0.07	0.184	7.91	−0.12	−1.53	0.08	0.147
Overall		*df* = (2, 91)		*F* = 1.34	0.267		*df* = (2, 82)		*F* = 3.96	0.023

Table B.13. PEFR from Nonstratified Analysis

Time (Hr)	Baseline (L/min)	β_3 (L/min)	β_3/ Baseline (%)	SE	*P*
1	487	−0.24	−0.05	5.63	0.966
2	487	−5.81	−1.19	6.55	0.375
3	487	−3.69	−0.76	6.84	0.590
4	487	−9.58	−1.97	6.94	0.168
5	487	−5.92	−1.22	6.99	0.398
6	487	−8.68	−1.78	6.99	0.215
7	487	−3.13	−0.64	7.04	0.657
Overall		*df* = (7, 558)		*F* = 0.56	0.785

Table B.14. PEFR from Stratified Analysis

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline (L/min)	β_3 (L/min)	β_3/ Baseline (%)	SE	*P*	Baseline (L/min)	β_3 (L/min)	β_3/ Baseline (%)	SE	*P*
1	507	−1.14	−0.23	7.95	0.886	465	0.75	0.16	7.96	0.925
2	507	−10.70	−2.11	8.65	0.217	465	0.04	0.01	9.75	0.997
3	507	−3.19	−0.63	8.78	0.717	465	−4.08	−0.88	10.53	0.699
4	507	−11.25	−2.22	8.80	0.202	465	−7.28	−1.57	10.90	0.505
5	507	−15.51	−3.06	8.84	0.081	465	5.60	1.21	11.08	0.614
6	507	−17.35	−3.42	8.80	0.050	465	1.47	0.32	11.17	0.896
7	507	−9.99	−1.97	8.80	0.257	465	4.57	0.98	11.34	0.687
Overall		*df* = (7, 291)		*F* = 1.00	0.433		*df* = (7, 251)		*F* = 0.44	0.878

Table B.15. Asthma Symptom Score from Nonstratified Analysis

Time	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
Hr					
2	1.33	0.19	14.46	0.31	0.534
7	1.33	0.07	5.11	0.40	0.864
Day					
1	1.33	0.51	38.08	0.41	0.213
2	1.33	0.62	46.88	0.41	0.125
3	1.33	0.00	0.37	0.41	0.990
4	1.33	−0.19	−14.22	0.41	0.643
5	1.33	0.14	10.17	0.41	0.740
6	1.33	−0.27	−20.59	0.41	0.502
7	1.33	0.10	7.40	0.41	0.809
Overall		*df* = (9, 717)		*F* = 0.94	0.486

Table B.16. Asthma Symptom Score from Stratified Analysis

	Mild Asthma					Moderate Asthma				
Time	Baseline	β_3	β_3/ Baseline (%)	SE	*P*	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
Hr										
2	1.08	−0.01	−0.70	0.43	0.986	1.63	0.44	27.12	0.38	0.240
7	1.08	−0.27	−24.87	0.50	0.589	1.63	0.47	28.82	0.57	0.408
Day										
1	1.08	0.50	46.48	0.50	0.314	1.63	0.49	30.19	0.65	0.450
2	1.08	0.52	47.84	0.50	0.300	1.63	0.76	46.55	0.65	0.247
3	1.08	0.01	0.47	0.50	0.992	1.63	0.03	1.56	0.65	0.969
4	1.08	0.08	7.69	0.50	0.868	1.63	−0.48	−29.21	0.66	0.470
5	1.08	−0.02	−2.26	0.50	0.961	1.63	0.35	21.20	0.66	0.600
6	1.08	−0.02	−2.26	0.50	0.961	1.63	−0.60	−36.93	0.66	0.361
7	1.08	0.14	13.34	0.50	0.773	1.63	0.12	7.38	0.66	0.855
Overall		*df* = (9, 376)		*F* = 0.47	0.893		*df* = (9, 321)		*F* = 0.95	0.486

Table B.17. Asthma Reliever Medication Use from Nonstratified Analysis

Time (Hr)	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
1	0.55	0.07	13.28	0.17	0.676
2	0.55	0.01	1.15	0.20	0.975
3	0.55	0.12	21.36	0.21	0.570
4	0.55	0.04	8.14	0.21	0.831
5	0.55	0.07	13.29	0.21	0.728
6	0.55	−0.04	−7.16	0.21	0.851
Overall		*df* = (6, 525)		*F* = 0.19	0.981

Table B.18. Asthma Reliever Medication Use from Stratified Analysis

	Mild Asthma					Moderate Asthma				
Time (Hr)	Baseline	β_3	β_3/ Baseline (%)	SE	*P*	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
1	0.39	−0.07	−17.58	0.19	0.718	0.74	0.25	33.78	0.32	0.435
2	0.39	−0.03	−7.33	0.21	0.893	0.74	0.05	6.76	0.36	0.891
3	0.39	0.01	2.93	0.22	0.958	0.74	0.25	33.78	0.38	0.508
4	0.39	−0.11	−27.85	0.22	0.620	0.74	0.24	32.23	0.38	0.534
5	0.39	−0.11	−27.85	0.22	0.621	0.74	0.30	41.12	0.38	0.429
6	0.39	−0.11	−27.85	0.22	0.621	0.74	0.05	6.10	0.38	0.907
Overall		*df* = (6, 288)		*F* = 0.12	0.994		*df* = (6, 225)		*F* = 0.29	0.940

Table B.19. Blood TBARS, PC_{20}, and Sputum Biomarkers from Nonstratified Analysis

	Baseline	β_3	β_3/Baseline (%)	SE	*P*
Blood TBARS	2.31 (µmol/L)	0.15 (µmol/L)	6.38	0.26	0.573
PC_{20}	2.82 (mg/mL)	−0.55 (mg/mL)	−19.6	1.1	0.612
Interleukin-8	90.1 (ng/mL)	34.68 (ng/mL)	38.5	50.8	0.498
Myeloperoxidase	6.08 (ng/mL)	31.70 (ng/mL)	521	13	0.014
Eosinophil cationic protein	24.41 (ng/mL)	7.50 (ng/mL)	30.7	14.2	0.600
Eosinophils[a]	1.68	0.42	25.0	0.8	0.591
Macrophages[a]	55.9	8.56	15.3	5.9	0.154
Epithelial cells[a]	3.03	−0.51	−17	0.7	0.438
Lymphocytes[a]	0.77	0.09	11.6	0.3	0.725
Neutrophils[a]	38.58	8.98	23.3	6	0.142

[a] Values for baseline and β_3 are the percentage of the cell type to total nonsquamous cells.

Table B.20. Blood TBARS, PC_{20}, and Sputum Biomarkers from Stratified Analysis

	Mild Asthma					Moderate Asthma				
	Baseline	β_3	β_3/ Baseline (%)	SE	*P*	Baseline	β_3	β_3/ Baseline (%)	SE	*P*
Blood TBARS	2.31 (μmol/L)	0.74 (μmol/L)	32.07	0.46	0.141	2.31 (μmol/L)	−0.3 (μmol/L)	−12.92	0.23	0.230
PC_{20}	2.73 (mg/mL)	−0.89 (mg/mL)	−32.5	1.7	0.608	2.92 (mg/mL)	−0.22 (mg/mL)	−7.69	1.32	0.866
Interleukin-8	89.03 (ng/mL)	6.34 (ng/mL)	7.12	62.5	0.920	91.47 (ng/mL)	18.37 (ng/mL)	20.1	88.5	0.837
Myeloperoxidase	5.69 (ng/mL)	8.29 (ng/mL)	146	4	0.067	6.53 (ng/mL)	46.86 (ng/mL)	718	24	0.065
Eosinophil cationic protein	13.67 (ng/mL)	−9.59 (ng/mL)	−70.1	21.9	0.665	38.94 (ng/mL)	15.13 (ng/mL)	38.8	18.3	0.417
Eosinophils[a]	1.53	0.64	41.6	1.1	0.566	1.92	−0.68	−35.3	1.2	0.573
Macrophages[a]	54.82	4.68	8.53	8.79	0.598	57.79	−7.66	−13.3	9.5	0.431
Epithelial cells[a]	3.14	−0.42	−13.5	0.7	0.56	2.83	−0.42	−15	1.3	0.741
Lymphocytes[a]	0.62	0.28	45.2	0.5	0.553	1.03	0.16	16	0.3	0.51
Neutrophils[a]	39.86	4.35	10.9	9	0.632	36.35	−9.45	−26	9.7	0.339

[a] Values for baseline and β_3 are the percentage of the cell type to total nonsquamous cells.

APPENDIX C. Summary of Results from Pollutant-Specific Exposure–Response Relations

We used repeated-measures mixed-effects linear regression models to explore pollutant-specific exposure–response relations. These models included the health endpoint, or change in health endpoint from baseline, as the response (dependent variable), and pollutant concentration, time point of measurement and appropriate covariates as independent (fixed-effects) variables. The model used in these analyses is the same as that in Appendix B. Instead of site as the measure of exposure, we used the actual concentrations measured for each session.

The form of the model is as follows:

$$Y_{ijk} = \beta_0 + \alpha C_{ik} + \tau_j + \tau(\alpha)_j C_{ik} + \beta_1 \mathrm{Temp} + \beta_2 \mathrm{RH} + \gamma_k + D_{ik}\phi + \epsilon_{j(ik)}$$

such that Y_{ijk} is the value of the health endpoint for the kth subject during the ith session ($i = 1$ for exposure and $i = 0$ for control) at the jth time; C_{ik} represents the 2-hour during-session average concentration of a pollutant (the continuous fixed-effects variable); τ_j represents the effect of the jth time ($j = 0, 1, 2, 3, 5, 7, 22$) within an exposure session (with $\tau_0 = 0$); α represents the effect of concentration at baseline (presumably insignificant); $\tau(\alpha)_j$ for $j = 1, 2, 3, 5, 7, 22$ measures the effect of C_{ik} on changes in health endpoints and biomarkers relative to baseline; γ_k represents the subject-specific random effect to adjust for the correlation within a subject between sites; and ϵ_{ijk} represents the random error, which is assumed to be normally distributed. The regression coefficients β_1 and β_2 represent the linear effects of temperature (Temp) and relative humidity (RH), respectively. Additional covariates were incorporated for all endpoints and biomarkers except FEV_1, FVC, and $FEF_{25–75}$ including age, sex, and ethnicity. An additional random effect for day was added to the model since multiple subjects had exposure sessions on the same day. The vector D_{ik} represents a series of indicator variables, identifying the day on which the kth subject experienced the ith session, with ϕ being a vector of random effects for days. The variance covariance structure for the ϵ_{ijk} was found to be best represented by a spatial power law structure using the Akaike Information Criteria and the Schwarz Bayesian Criterion. Specifically, the correlation between measurements taken at hours j and j' within session (i) and within person (k) are modeled as

$$\mathrm{cov}\left(\epsilon_{j(ik)}, \epsilon_{j'(ik)}\right) = \rho^{|j-j'|}\sigma^2$$

where ρ is the correlation between measurements taken one hour apart. In this model, $\tau(\alpha)$ represents the interaction between exposure (session) and time point. A test of this term reveals whether there are significant differences between exposures in the changes in health endpoints over time.

The models were created, parameters estimated and statistical tests performed using PROC MIXED in SAS Version 9.1.3. Using these models, we performed both nonstratified analyses (for all subjects combined) and asthma-severity stratified analyses.

For each endpoint and biomarker measured in the study, we report the regression coefficients $\tau(\alpha)$, representing the exposure versus control difference in change from baseline (postsession minus presession). To translate these values into the difference in percentage changes from baseline, we divide by the baseline value for a particular endpoint or biomarker. For the endpoints that were measured at multiple time points, we also report F test results on the difference in overall time-series trend between exposure and control.

We compared results from modeling the raw health endpoint value at a given time with those from modeling the change in health endpoint from baseline and found similar results in terms of statistical significance. For more straightforward interpretation of the results, especially for an easier comparison across different endpoints, we report the results from modeling changes from baseline. In Tables C1–C8, we report the point estimates and 95% CIs of regression coefficients (β_3), that is, the percentage changes in health endpoints and biomarkers from baseline that are associated with a unit change of pollutant concentration (as specified in the table titles).

Because the pollutants measured were correlated, we also preformed analyses using two-pollutant models in which two of the four pollutants were analyzed at a time. Otherwise the two-pollutant model had the same structure as the single-pollutant model described earlier. We analyzed only for the health endpoints that showed significant effects in the single-pollutant models. No asthma-severity stratified analyses were performed using the two-pollutant models. The results from two-pollutant models are shown in Tables C9–C12.

Table C.1. Nonstratified Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in $PM_{2.5}$[a]

Endpoint / Hour of Measurement	Point Estimate	95% CI		*P*
		Lower	Upper	
FEV_1				
2	−0.65	−1.52	0.21	0.139
3	−0.46	−1.43	0.51	0.349
5	−0.89	−1.96	0.18	0.103
7	−0.39	−1.50	0.72	0.490
22	−0.08	−1.22	1.05	0.885
Overall		*df* = (5, 412)	*F* = 0.94	0.457
FVC				
2	−0.25	−1.30	0.79	0.635
3	0.07	−1.08	1.22	0.911
5	−0.23	−1.47	1.01	0.715
7	0.11	−1.16	1.38	0.865
22	0.75	−0.54	2.03	0.254
Overall		*df* = (5, 410)	*F* = 0.65	0.658
$FEF_{25–75}$				
2	−3.36	−6.58	−0.15	0.041
3	−4.81	−8.41	−1.21	0.009
5	−5.63	−9.59	−1.66	0.006
7	−3.34	−7.44	0.77	0.111
22	−3.20	−7.41	1.01	0.136
Overall		*df* = (5, 410)	*F* = 1.86	0.100
eNO				
3	0.98	−2.96	4.92	0.626
4	−0.79	−5.09	3.52	0.720
5	0.05	−4.53	4.62	0.985
6	−0.16	−4.92	4.61	0.949
7	−0.54	−5.45	4.37	0.828
22	1.89	−3.48	7.25	0.490
Overall		*df* = (6, 506)	*F* = 0.50	0.805
EBC				
Fe				
3	−144	−249	−38.2	0.008
6	−116	−223	−7.78	0.036
Overall		*df* = (2, 74)	*F* = 4.13	0.020
pH				
3	−0.24	−0.75	0.27	0.352
6	−0.17	−0.73	0.38	0.534
Overall		*df* = (2, 168)	*F* = 0.44	0.647
Blood TBARS (7)	−3.37	−16	9.21	0.586
PC_{20} (7)	−13.4	−59.1	32.4	0.562
Sputum (22)				
IL-8	28.3	−16.6	73.1	0.211
MPO	235	−424	894	0.477
ECP	43.6	−83.4	171	0.493
Eosinophils	−6.59	−65.5	52.4	0.820
Macrophages	12.6	−1.91	27.1	0.086
Epithelial cells	−1.17	−32.4	30.1	0.939
Lymphocytes	−9.42	−68.6	49.8	0.748
Neutrophils	−7	−34.8	20.8	0.611

[a] Change is averaged over the exposure and control sessions using single-pollutant models.

Table C.2. Stratified Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in $PM_{2.5}$[a]

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	Point Estimate	95% CI		*P*	Point Estimate	95% CI		*P*
		Lower	Upper			Lower	Upper	
FEV_1								
2	−1.14	−2.52	0.24	0.104	−0.17	−1.30	0.95	0.761
3	−1.11	−2.65	0.44	0.159	0.17	−1.08	1.42	0.786
5	−1.36	−3.05	0.34	0.117	−0.46	−1.82	0.90	0.508
7	−0.62	−2.38	1.14	0.488	−0.18	−1.58	1.23	0.806
22	−0.74	−2.53	1.06	0.420	0.61	−0.81	2.04	0.398
Overall		*df* = (5, 209)	*F* = 0.77	0.575		*df* = (5, 191)	*F* = 0.64	0.670
FVC								
2	0.08	−1.33	1.48	0.915	−0.56	−2.15	1.04	0.492
3	0.13	−1.41	1.67	0.866	0.08	−1.63	1.79	0.927
5	0.34	−1.32	1.99	0.690	−0.68	−2.48	1.11	0.452
7	0.50	−1.19	2.19	0.559	−0.20	−2.01	1.61	0.826
22	0.90	−0.81	2.61	0.299	0.69	−1.13	2.51	0.454
Overall		*df* = (5, 208)	*F* = 0.26	0.936		*df* = (5, 190)	*F* = 0.70	0.625
$FEF_{25–75}$								
2	−7.35	−11.7	−2.99	0.001	0.25	−3.49	3.99	0.895
3	−8.98	−14.0	−4.00	0.001	−1.25	−6.36	3.86	0.631
5	−9.56	−15.2	−3.89	0.001	−2.40	−7.21	2.42	0.328
7	−5.92	−11.9	0.08	0.053	−1.26	−5.96	3.45	0.599
22	−8.34	−14.7	−1.99	0.010	1.19	−3.46	5.83	0.615
Overall		*df* = (5, 208)	*F* = 3.43	0.005		*df* = (5, 190)	*F* = 0.48	0.793
eNO								
3	6.75	1.28	12.2	0.016	−3.89	−9.6	1.82	0.181
4	5.17	−0.79	11.1	0.089	−5.67	−11.9	0.54	0.073
5	5.88	−0.44	12.2	0.068	−4.60	−11.1	1.95	0.168
6	6.81	0.24	13.4	0.042	−6.21	−13.0	0.59	0.073
7	6.55	−0.21	13.3	0.057	−6.34	−13.3	0.63	0.075
22	11.00	3.69	18.3	0.003	−6.18	−13.6	1.28	0.104
Overall		*df* = (6, 257)	*F* = 1.98	0.069		*df* = (6, 235)	*F* = 0.90	0.493
EBC								
Fe								
3	−70.9	−213	70.7	0.317	−213	−366	−59.9	0.008
6	14.3	−127	156	0.839	−200	−368	−32.1	0.021
Overall		*df* = (2, 38)	*F* = 0.77	0.470		*df* = (2, 31)	*F* = 4.59	0.018
pH								
3	−0.46	−1.1	0.17	0.152	−0.1	−0.93	0.72	0.805
6	−0.46	−1.18	0.25	0.201	0	−0.9	0.90	0.998
Overall		*df* = (2, 86)	*F* = 1.13	0.329		*df* = (2, 77)	*F* = 0.05	0.724
Blood TBARS (7)	−8.39	−35.8	19	0.506	−4.54	−18.3	9.19	0.473
PC_{20} (7)	−21.2	−94.8	52.4	0.563	−20.8	−86.4	44.7	0.522
Sputum (22)								
IL-8	46.3	1.56	90.9	0.043	46.8	−47.3	141	0.310
MPO	12.9	−25.9	51.7	0.498	611	−896	2117	0.406
ECP	38.5	−168	245	0.704	−23.5	−167	120	0.732
Eosinophils	−21.5	−150	107	0.722	−34.2	−117	48.4	0.361
Macrophages	0.98	−17.4	19.4	0.912	24.7	−6.83	56.2	0.106
Epithelial cells	7.07	−37.7	51.9	0.743	−41	−113	31	0.220
Lymphocytes	−72.4	−157	12.7	0.09	−27.3	−122	67	0.516
Neutrophils	3.73	−43.4	50.8	0.87	−5.26	−52.1	41.5	0.798

[a] Change is averaged over the exposure and control sessions. Analyses are stratified by asthma severity using single-pollutant models.

Table C.3. Nonstratified Analyses for Percentage of Change in Health Endpoints per 1-μg/m^3 Increase in EC[a]

Endpoint / Hour of Measurement	Point Estimate	95% CI		*P*
		Lower	Upper	
FEV_1				
2	−0.36	−0.63	−0.09	0.010
3	−0.42	−0.72	−0.12	0.007
5	−0.49	−0.81	−0.16	0.004
7	−0.33	−0.67	0	0.053
22	−0.12	−0.46	0.23	0.497
Overall		*df* = (5, 427)	*F* = 2.31	0.043
FVC				
2	−0.26	−0.57	0.06	0.111
3	−0.32	−0.66	0.02	0.066
5	−0.35	−0.72	0.01	0.059
7	0	−0.37	0.37	0.986
22	0.04	−0.33	0.41	0.841
Overall		*df* = (5, 425)	*F* = 1.83	0.105
$FEF_{25–75}$				
2	−0.74	−1.65	0.18	0.116
3	−0.87	−1.85	0.12	0.084
5	−1.12	−2.14	−0.10	0.031
7	−1.11	−2.14	−0.08	0.034
22	−0.75	−1.78	0.28	0.152
Overall		*df* = (5, 425)	*F* = 1.19	0.315
eNO				
3	1.63	0.41	2.85	0.009
4	1.91	0.57	3.25	0.005
5	2.37	0.95	3.79	0.001
6	2.18	0.70	3.67	0.004
7	1.92	0.39	3.46	0.014
22	1.97	0.28	3.65	0.022
Overall		*df* = (6, 523)	*F* = 2.00	0.064
EBC				
Fe				
3	16.9	−16.5	50.4	0.316
6	17.4	−16.3	51.1	0.307
Overall		*df* = (2, 74)	*F* = 0.72	0.490
pH				
3	−0.1	−0.26	0.06	0.210
6	< 0.01	−0.17	0.17	0.985
Overall		*df* = (2, 175)	*F* = 1.43	0.243
Blood TBARS (7)	−1.54	−8.86	5.79	0.670
PC_{20} (7)	−5.52	−20.3	9.22	0.458
Sputum (22)				
IL-8	0.59	−13	14.2	0.931
MPO	137	−50.3	324	0.148
ECP	−4.84	−42.8	33.1	0.799
Eosinophils	−4.27	−19.7	11.2	0.577
Macrophages	1.15	−3.4	5.69	0.611
Epithelial cells	3.01	−5.29	11.3	0.467
Lymphocytes	−7.65	−24.7	9.36	0.368
Neutrophils	−1.08	−8.68	6.52	0.774

[a] Change is averaged over the exposure and control sessions using single-pollutant models.

Table C.4. Stratified Analyses for Percentage of Change in Health Endpoints per 1-μg/m^3 Increase in EC[a]

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	Point Estimate	95% CI		*P*	Point Estimate	95% CI		*P*
		Lower	Upper			Lower	Upper	
FEV_1								
2	−0.40	−0.75	−0.04	0.028	−0.35	−0.79	0.09	0.120
3	−0.33	−0.72	0.06	0.098	−0.63	−1.12	−0.14	0.012
5	−0.48	−0.91	−0.05	0.028	−0.53	−1.06	0	0.050
7	−0.31	−0.75	0.13	0.163	−0.40	−0.94	0.15	0.154
22	0.01	−0.44	0.46	0.962	−0.41	−0.97	0.14	0.141
Overall		*df* = (5, 219)	*F* = 1.81	0.113		*df* = (5, 196)	*F* = 1.41	0.224
FVC								
2	−0.08	−0.39	0.24	0.639	−0.63	−1.23	−0.02	0.043
3	−0.06	−0.47	0.34	0.758	−0.86	−1.51	−0.20	0.010
5	−0.14	−0.52	0.24	0.479	−0.79	−1.47	−0.11	0.023
7	0.07	−0.31	0.45	0.716	−0.14	−0.83	0.55	0.684
22	0.34	−0.04	0.71	0.078	−0.56	−1.25	0.14	0.115
Overall		*df* = (5, 218)	*F* = 1.81	0.113		*df* = (5, 195)	*F* = 2.13	0.064
$FEF_{25–75}$								
2	−1.10	−1.97	−0.22	0.014	−0.15	−1.61	1.30	0.834
3	−1.05	−2.05	−0.05	0.039	−0.48	−2.44	1.48	0.629
5	−1.24	−2.37	−0.12	0.031	−0.83	−2.68	1.02	0.377
7	−1.05	−2.24	0.13	0.082	−1.16	−2.97	0.65	0.208
22	−0.84	−2.09	0.41	0.185	−0.56	−2.35	1.23	0.536
Overall		*df* = (5, 218)	*F* = 1.44	0.210		*df* = (5, 195)	*F* = 0.39	0.855
eNO								
3	2.45	1.08	3.83	0.001	0.18	−2.09	2.45	0.875
4	2.86	1.37	4.36	0	0.17	−2.31	2.66	0.892
5	2.92	1.34	4.51	0	1.41	−1.23	4.05	0.295
6	2.92	1.28	4.57	0.001	0.96	−1.79	3.72	0.491
7	2.65	0.96	4.33	0.002	0.57	−2.26	3.41	0.690
22	3.10	1.28	4.91	0.001	0.15	−2.96	3.26	0.924
Overall		*df* = (6, 269)	*F* = 3.17	0.005		*df* = (6, 241)	*F* = 0.54	0.781
EBC								
Fe								
3	16.3	−18.0	50.5	0.342	21.8	−45.3	89.0	0.512
6	18.1	−16.1	52.4	0.290	20.6	−51.5	92.8	0.564
Overall		*df* = (2, 38)	*F* = 0.71	0.496		*df* = (2, 31)	*F* = 0.26	0.771
pH								
3	−0.02	−0.19	0.15	0.808	−0.24	−0.55	0.08	0.139
6	0.02	−0.17	0.21	0.817	−0.02	−0.36	0.33	0.925
Overall		*df* = (2, 88)	*F* = 0.19	0.831		*df* = (2, 79)	*F* = 1.84	0.166
Blood TBARS (7)	−2.61	−16.7	11.5	0.685	−0.93	−10	8.13	0.822
PC_{20} (7)	−15.2	−36.3	5.9	0.153	0.48	−22.7	23.7	0.966
Sputum (22)								
IL-8	1.11	−12.9	15.2	0.873	1.25	−28.6	31	0.931
MPO	4.86	−6.26	16	0.377	256	−177	689	0.231
ECP	−16.5	−77.1	44.1	0.581	3.37	−44.2	50.9	0.883
Eosinophils	−2.35	−27.6	22.9	0.846	−11.1	−35.6	13.3	0.325
Macrophages	−0.98	−6.44	4.48	0.712	6.55	−4.6	17.7	0.212
Epithelial cells	3.32	−6.44	13.1	0.486	6.16	−13.2	25.5	0.484
Lymphocytes	−18.1	−36.3	0	0.05	−12.1	−53.4	29.3	0.52
Neutrophils	−0.48	−10.9	9.94	0.925	−5.85	−19.7	7.98	0.358

[a] Change is averaged over the exposure and control sessions. Analyses are stratified by asthma severity using single-pollutant models.

Table C.5. Nonstratified Analyses for Percentage of Change in Health Endpoints per 10,000-UFP/cm^3 Increase[a]

Endpoint / Hour of Measurement	Point Estimate	95% CI		*P*
		Lower	Upper	
FEV_1				
2	−0.79	−1.25	−0.33	0.001
3	−0.73	−1.24	−0.21	0.006
5	−0.90	−1.46	−0.34	0.002
7	−0.66	−1.24	−0.08	0.026
22	−0.44	−1.04	0.15	0.141
Overall		*df* = (5, 437)	*F* = 2.86	0.015
FVC				
2	−0.56	−1.11	−0.01	0.046
3	−0.61	−1.21	0	0.049
5	−0.71	−1.37	−0.06	0.032
7	−0.34	−1.01	0.33	0.317
22	−0.42	−1.10	0.26	0.223
Overall		*df* = (5, 435)	*F* = 1.28	0.272
$FEF_{25–75}$				
2	−2.24	−3.94	−0.54	0.010
3	−2.22	−4.12	−0.32	0.022
5	−2.81	−4.88	−0.73	0.008
7	−2.12	−4.27	0.02	0.053
22	−1.71	−3.91	0.48	0.125
Overall		*df* = (5, 435)	*F* = 1.82	0.107
eNO				
3	1.01	−1.11	3.12	0.350
4	0.73	−1.58	3.05	0.534
5	1.74	−0.72	4.19	0.166
6	1.13	−1.44	3.70	0.388
7	0.89	−1.76	3.54	0.510
22	1.62	−1.30	4.53	0.276
Overall		*df* = (6, 535)	*F* = 0.72	0.632
EBC				
Fe				
3	36.3	−24.2	96.7	0.236
6	31.7	−28.9	92.4	0.301
Overall		*df* = (2, 78)	*F* = 0.87	0.422
pH				
3	−0.32	−0.58	−0.05	0.018
6	−0.1	−0.38	0.19	0.515
Overall		*df* = (2, 179)	*F* = 3.55	0.031
Blood TBARS (7)	−2.24	−9.91	5.42	0.552
PC_{20} (7)	−23.4	−48.3	1.49	0.065
Sputum (22)				
IL-8	13.1	−10.2	36.4	0.266
MPO	353	37	669	0.029
ECP	6.29	−59.7	72.3	0.849
Eosinophils	0.68	−29.6	31	0.964
Macrophages	1.89	−6.78	10.6	0.661
Epithelial cells	−0.84	−16.9	15.3	0.916
Lymphocytes	−14.3	−46.7	18.1	0.377
Neutrophils	0.39	−14.1	14.9	0.957

[a] Change is averaged over the exposure and control sessions using single-pollutant models.

Table C.6. Stratified Analyses for Percentage of Change in Health Endpoints per 10,000-UFP/cm^3 Increase[a]

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	Point Estimate	95% CI		*P*	Point Estimate	95% CI		*P*
		Lower	Upper			Lower	Upper	
FEV_1								
2	−0.84	−1.53	−0.15	0.017	−0.73	−1.34	−0.11	0.021
3	−0.51	−1.28	0.26	0.194	−0.92	−1.60	−0.24	0.008
5	−0.89	−1.75	−0.03	0.042	−0.90	−1.64	−0.16	0.017
7	−0.68	−1.57	0.22	0.137	−0.65	−1.40	0.11	0.092
22	−0.16	−1.08	0.76	0.728	−0.70	−1.46	0.07	0.075
Overall		*df* = (5, 224)	*F* = 1.84	0.107		*df* = (5, 201)	*F* = 1.70	0.137
FVC								
2	−0.20	−0.91	0.51	0.578	−0.91	−1.76	−0.06	0.037
3	−0.02	−0.79	0.76	0.965	−1.17	−2.08	−0.26	0.012
5	−0.16	−0.98	0.67	0.709	−1.25	−2.20	−0.29	0.011
7	−0.21	−1.05	0.63	0.620	−0.47	−1.43	0.5	0.343
22	0.31	−0.54	1.16	0.473	−1.12	−2.09	−0.15	0.024
Overall		*df* = (5, 223)	*F* = 0.45	0.815		*df* = (5, 200)	*F* = 2.16	0.060
$FEF_{25–75}$								
2	−3.45	−5.67	−1.23	0.003	−1.09	−3.11	0.94	0.292
3	−3.26	−5.80	−0.72	0.012	−1.19	−3.94	1.56	0.393
5	−3.81	−6.70	−0.92	0.010	−1.84	−4.43	0.75	0.163
7	−2.50	−5.56	0.56	0.109	−1.76	−4.30	0.77	0.171
22	−2.35	−5.59	0.89	0.155	−1.07	−3.58	1.43	0.399
Overall		*df* = (5, 223)	*F* = 2.22	0.053		*df* = (5, 200)	*F* = 0.53	0.755
eNO								
3	3.02	0.21	5.84	0.035	−0.93	−4.10	2.24	0.565
4	3.09	0.02	6.16	0.048	−1.49	−4.96	1.98	0.398
5	3.41	0.16	6.66	0.040	0.16	−3.53	3.84	0.932
6	3.24	−0.14	6.62	0.060	−0.92	−4.77	2.92	0.637
7	3.16	−0.32	6.64	0.075	−1.26	−5.22	2.7	0.532
22	3.85	0.08	7.61	0.045	−0.62	−4.96	3.73	0.780
Overall		*df* = (6, 275)	*F* = 1.08	0.373		*df* = (6, 247)	*F* = 0.66	0.680
EBC								
Fe								
3	35.4	−35.0	106	0.315	39.1	−64	142	0.446
6	29.7	−40.7	100	0.398	41.1	−69.2	151	0.454
Overall		*df* = (2, 40)	*F* = 0.62	0.546		*df* = (2, 33)	*F* = 0.39	0.680
pH								
3	−0.16	−0.49	0.16	0.323	−0.47	−0.91	−0.04	0.033
6	−0.09	−0.46	0.27	0.612	−0.10	−0.59	0.38	0.673
Overall		*df* = (2, 90)	*F* = 0.53	0.593		*df* = (2, 81)	*F* = 3.28	0.043
Blood TBARS (7)	−2.36	−19.2	14.5	0.759	−1.85	−9.69	6	0.607
PC_{20} (7)	−37.4	−77.4	2.62	0.066	−19.3	−52.7	14	0.247
Sputum (22)								
IL-8	8.49	−18.5	35.5	0.525	15.4	−26.3	57	0.451
MPO	5.18	−16.8	27.2	0.632	586	−23	1196	0.058
ECP	−16.4	−134	101	0.777	19.5	−43.2	82.1	0.521
Eosinophils	5.55	−47.8	58.9	0.828	−7.2	−48.4	34.1	0.702
Macrophages	−1.01	−12.5	10.5	0.857	6.72	−11.8	25.2	0.433
Epithelial cells	4.83	−15.8	25.4	0.630	−4.42	−40.7	31.8	0.789
Lymphocytes	−26.6	−66.6	13.5	0.182	−28.7	−93.1	35.7	0.340
Neutrophils	−1.45	−23.3	20.4	0.891	0.21	−24	24.5	0.985

[a] Change is averaged over the exposure and control sessions. Analyses are stratified by asthma severity using single-pollutant models.

Table C.7. Nonstratified Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in NO_2[a]

Endpoint / Hour of Measurement	Point Estimate	95% CI		*P*
		Lower	Upper	
FEV_1				
2	−0.22	−0.40	−0.05	0.012
3	−0.12	−0.32	0.07	0.217
5	−0.16	−0.37	0.06	0.152
7	−0.11	−0.34	0.11	0.310
22	−0.13	−0.35	0.10	0.270
Overall		*df* = (5, 402)	*F* = 1.53	0.180
FVC				
2	−0.09	−0.30	0.11	0.376
3	−0.07	−0.30	0.16	0.549
5	−0.04	−0.29	0.21	0.761
7	−0.06	−0.31	0.20	0.666
22	−0.02	−0.28	0.23	0.863
Overall		*df* = (5, 400)	*F* = 0.18	0.971
$FEF_{25–75}$				
2	−0.78	−1.44	−0.13	0.019
3	−0.73	−1.46	0	0.051
5	−0.88	−1.69	−0.08	0.031
7	−0.60	−1.43	0.23	0.155
22	−0.75	−1.60	0.10	0.084
Overall		*df* = (5, 400)	*F* = 1.46	0.202
eNO				
3	0.53	−0.27	1.32	0.191
4	0.30	−0.57	1.17	0.498
5	0.47	−0.45	1.40	0.315
6	0	−0.97	0.96	0.994
7	0.11	−0.88	1.11	0.827
22	−0.07	−1.16	1.03	0.906
Overall		*df* = (6, 493)	*F* = 0.98	0.438
EBC				
Fe				
3	−9.01	−38.6	20.6	0.546
6	−1.92	−31.6	27.7	0.897
Overall		*df* = (2, 69)	*F* = 0.20	0.823
pH				
3	−0.15	−0.25	−0.06	0.002
6	−0.06	−0.16	0.04	0.246
Overall		*df* = (2, 165)	*F* = 5.58	0.005
Blood TBARS (7)	−0.84	−2.45	0.77	0.294
PC_{20} (7)	−8.68	−17.9	0.52	0.064
Sputum (22)				
IL-8	10.9	2.63	19.3	0.011
MPO	69.9	−56.7	197	0.272
ECP	−1.72	−25.7	22.3	0.886
Eosinophils	−3.62	−18.8	11.5	0.629
Macrophages	0.78	−3.63	5.19	0.722
Epithelial cells	−1.46	−9.37	6.45	0.709
Lymphocytes	−7.58	−24	8.85	0.354
Neutrophils	1.14	−5.99	8.27	0.746

[a] Change is averaged over the exposure and control sessions using single-pollutant models.

Table C.8. Stratified Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in NO_2[a]

Endpoint / Hour of Measurement	Mild Asthma				Moderate Asthma			
	Point Estimate	95% CI		*P*	Point Estimate	95% CI		*P*
		Lower	Upper			Lower	Upper	
FEV_1								
2	−0.22	−0.53	0.09	0.167	−0.20	−0.41	0.01	0.064
3	−0.20	−0.56	0.15	0.253	−0.05	−0.29	0.18	0.668
5	−0.23	−0.62	0.16	0.242	−0.12	−0.38	0.13	0.348
7	−0.25	−0.65	0.15	0.220	−0.05	−0.31	0.21	0.714
22	−0.15	−0.57	0.26	0.462	−0.09	−0.36	0.18	0.496
Overall		*df* = (5, 190)	*F* = 0.49	0.785		*df* = (5, 201)	*F* = 1.20	0.310
FVC								
2	−0.14	−0.45	0.18	0.394	−0.08	−0.38	0.21	0.581
3	−0.23	−0.57	0.10	0.174	0.02	−0.30	0.34	0.885
5	−0.12	−0.47	0.24	0.520	−0.01	−0.35	0.32	0.938
7	−0.34	−0.70	0.01	0.060	0.08	−0.26	0.42	0.627
22	−0.15	−0.52	0.21	0.400	0.05	−0.30	0.39	0.793
Overall		*df* = (5, 189)	*F* = 0.95	0.449		*df* = (5, 200)	*F* = 0.29	0.916
$FEF_{25–75}$								
2	−0.93	−1.97	0.11	0.078	−0.59	−1.28	0.10	0.091
3	−0.81	−1.99	0.38	0.183	−0.60	−1.54	0.34	0.210
5	−0.88	−2.24	0.48	0.203	−0.89	−1.78	−0.01	0.048
7	−0.49	−1.94	0.96	0.508	−0.68	−1.55	0.19	0.123
22	−0.65	−2.21	0.91	0.413	−0.78	−1.63	0.08	0.075
Overall		*df* = (5, 189)	*F* = 0.73	0.601		*df* = (5, 200)	*F* = 1.14	0.339
eNO								
3	−0.09	−1.33	1.14	0.885	0.80	−0.29	1.88	0.149
4	−0.86	−2.20	0.48	0.208	0.94	−0.25	2.13	0.120
5	−0.74	−2.15	0.67	0.303	1.13	−0.13	2.39	0.080
6	−0.83	−2.30	0.63	0.264	0.29	−1.03	1.61	0.665
7	−0.72	−2.23	0.78	0.343	0.52	−0.84	1.88	0.451
22	−0.84	−2.45	0.76	0.301	0.04	−1.45	1.54	0.950
Overall		*df* = (6, 234)	*F* = 0.72	0.631		*df* = (6, 247)	*F* = 1.36	0.232
EBC								
Fe								
3	−16	−55.7	23.7	0.418	−9.58	−57.9	38.8	0.690
6	2.01	−37.7	41.7	0.919	−2.26	−53.4	48.9	0.929
Overall		*df* = (2, 32)	*F* = 0.48	0.622		*df* = (2, 33)	*F* = 0.09	0.915
pH								
3	−0.12	−0.25	0.01	0.072	−0.19	−0.34	−0.05	0.010
6	−0.11	−0.24	0.03	0.119	−0.05	−0.21	0.11	0.541
Overall		*df* = (2, 78)	*F* = 1.82	0.169		*df* = (2, 81)	*F* = 4.61	0.013
Blood TBARS (7 hr)	−1.4	−4.63	1.83	0.346	−0.4	−3.41	2.61	0.772
PC_{20} (7 hr)	−18.1	−34.3	−2.01	0.029	−6.12	−18	5.8	0.303
Sputum (22 hr)								
IL-8	6.65	−4.47	17.8	0.228	18.1	4.85	31.4	0.010
MPO	−0.51	−9.31	8.29	0.905	149	−90.1	387	0.209
ECP	3.22	−47.6	54	0.897	−7.89	−29.8	14	0.458
Eosinophils	−8.24	−36.4	19.9	0.538	12.6	−12.6	37.7	0.288
Macrophages	0.12	−5.68	5.93	0.965	2.61	−9.65	14.9	0.642
Epithelial cells	−1.33	−11.5	8.84	0.785	−13.4	−34.7	7.8	0.187
Lymphocytes	−15.4	−34.8	3.88	0.11	−14.4	−57	28.2	0.465
Neutrophils	−0.34	−10.9	10.2	0.947	11.8	−1.1	24.7	0.069

[a] Change is averaged over the exposure and control sessions. Analyses are stratified by asthma severity using single-pollutant models.

Table C.9. Copollutant-Adjusted Analyses for Percentage of Change in Health Endpoints per 10,000-UFP/cm^3 Increase[a]

Endpoint / Hour of Measurement	Adjusted for EC				Adjusted for $PM_{2.5}$				Adjusted for NO_2			
	Point Estimate	95% CI		P[b]	Point Estimate	95% CI		P[b]	Point Estimate	95% CI		P[b]
		Lower	Upper			Lower	Upper			Lower	Upper	
FEV_1												
2	−1.15	−2.09	−0.21	**0.016**	−0.74	−1.33	−0.15	**0.014**	−0.70	−1.31	−0.10	**0.023**
3	−0.66	−1.69	0.37	0.209	−0.75	−1.40	−0.10	**0.023**	−0.84	−1.51	−0.18	**0.013**
5	−0.98	−2.09	0.13	0.082	−0.77	−1.47	−0.07	**0.031**	−1.01	−1.73	−0.29	**0.006**
7	−0.88	−2.01	0.25	0.125	−0.55	−1.27	0.16	0.131	−0.74	−1.48	−0.01	**0.047**
22	−1.41	−2.55	−0.27	**0.016**	−0.56	−1.29	0.16	0.129	−0.48	−1.23	0.26	0.199
FVC												
2	−1.10	−2.21	0.01	0.051	−0.69	−1.41	0.02	0.057	−0.67	−1.41	0.06	0.072
3	−1.03	−2.20	0.14	0.084	−0.83	−1.59	−0.06	**0.034**	−0.92	−1.70	−0.13	**0.022**
5	−1.22	−2.43	−0.02	**0.047**	−0.82	−1.62	−0.02	**0.045**	−1.14	−1.96	−0.32	**0.007**
7	−1.66	−2.87	−0.44	**0.008**	−0.37	−1.18	0.44	0.375	−0.53	−1.36	0.30	0.207
22	−2.24	−3.46	−1.03	< **0.001**	−0.81	−1.63	−0.01	**0.050**	−0.78	−1.62	0.05	0.065
$FEF_{25–75}$												
2	−1.71	−4.91	1.50	0.297	−1.47	−3.75	0.82	0.207	−1.57	−3.96	0.83	0.199
3	−0.24	−3.46	2.97	0.883	−1.11	−3.45	1.22	0.350	−1.38	−3.80	1.05	0.266
5	−1.54	−4.75	1.68	0.348	−1.45	−3.80	0.90	0.227	−1.85	−4.28	0.59	0.137
7	0.18	−3.04	3.39	0.914	−1.33	−3.68	1.03	0.268	−1.56	−4.00	0.87	0.208
22	−0.47	−3.69	2.75	0.774	−1.21	−3.56	1.15	0.314	−0.75	−3.19	1.68	0.544
eNO												
3	−0.81	−4.13	2.52	0.633	−0.16	−2.20	1.89	0.881	−0.70	−2.82	1.42	0.517
4	−3.07	−6.64	0.51	0.093	0.36	−1.83	2.54	0.748	−0.40	−2.67	1.88	0.731
5	−1.82	−5.56	1.92	0.340	1.66	−0.61	3.93	0.152	0.66	−1.72	3.03	0.587
6	−3.27	−7.12	0.58	0.096	0.57	−1.76	2.90	0.630	0.91	−1.53	3.35	0.463
7	−2.72	−6.65	1.21	0.174	0.53	−1.84	2.89	0.662	0.09	−2.39	2.58	0.943
22	−0.94	−5.04	3.16	0.653	1.13	−1.31	3.56	0.365	1.81	−0.77	4.39	0.168
EBC pH												
3	−0.56	−1.09	−0.04	**0.037**	−0.37	−0.70	−0.040	**0.029**	−0.07	−0.39	0.26	0.69
6	−0.35	−0.94	0.25	0.250	−0.09	−0.46	0.282	0.639	0.06	−0.30	0.42	0.74

[a] Change is averaged over the exposure and control sessions. Estimates are adjusted for copollutants using two-pollutant models.

[b] Bolded values are significant at $P \leq 0.05$.

Table C.10. Copollutant-Adjusted Analyses for Percentage of Change in Health Endpoints per 1-μg/m^3 Increase in EC[a]

Endpoint / Hour of Measurement	Adjusted for UFP				Adjusted for $PM_{2.5}$				Adjusted for NO_2			
	Point Estimate	95% CI		P[b]	Point Estimate	95% CI		P[b]	Point Estimate	95% CI		P[b]
		Lower	Upper			Lower	Upper			Lower	Upper	
FEV_1												
2	0.20	−0.33	0.74	0.456	−0.25	−0.60	0.10	0.164	−0.31	−0.64	0.02	0.068
3	−0.09	−0.68	0.50	0.755	−0.37	−0.76	0.02	0.064	−0.45	−0.82	−0.09	**0.016**
5	−0.01	−0.64	0.63	0.988	−0.34	−0.75	0.09	0.124	−0.53	0.93	−0.14	**0.008**
7	0.10	−0.55	0.75	0.764	−0.17	−0.60	0.26	0.446	−0.37	−0.77	0.03	0.071
22	0.57	−0.08	1.23	0.087	−0.04	−0.48	0.39	0.846	−0.14	−0.54	0.27	0.513
FVC												
2	0.28	−0.35	0.92	0.384	−0.24	−0.66	0.18	0.257	−0.29	−0.69	0.10	0.148
3	0.18	−0.49	0.86	0.590	−0.35	−0.79	0.10	0.126	−0.47	−0.89	−0.05	**0.029**
5	0.25	−0.44	0.94	0.482	−0.31	−0.77	0.15	0.185	−0.56	−0.99	−0.12	**0.012**
7	0.82	0.12	1.51	**0.022**	0.16	−0.31	0.62	0.506	−0.10	−0.54	0.33	0.644
22	1.14	0.44	1.83	**0.001**	0.02	−0.44	0.48	0.933	−0.08	−0.52	0.35	0.705
$FEF_{25–75}$												
2	0.10	−1.74	1.94	0.917	−0.44	−1.64	0.76	0.467	−0.50	−1.65	0.65	0.394
3	−0.75	−2.59	1.10	0.427	−0.51	−1.72	0.69	0.404	−0.52	−1.67	0.63	0.377
5	−0.37	−2.22	1.48	0.695	−0.50	−1.70	0.71	0.419	−0.71	−1.86	0.44	0.227
7	−1.20	−3.04	0.65	0.203	−0.85	−2.05	0.36	0.168	−0.90	−2.05	0.25	0.124
22	−0.52	−2.37	1.33	0.581	−0.64	−1.84	0.57	0.300	−0.44	−1.59	0.71	0.455
eNO												
3	0.69	−1.22	2.60	0.478	0.09	−1.17	1.35	0.891	−0.24	−1.43	0.95	0.690
4	2.08	0.03	4.14	**0.047**	1.02	−0.35	2.40	0.144	0.47	−0.83	1.77	0.478
5	1.93	−0.22	4.08	0.079	1.68	0.23	3.13	**0.024**	0.91	−0.47	2.29	0.196
6	2.45	0.24	4.67	**0.030**	1.31	−0.20	2.82	0.090	1.05	−0.39	2.49	0.153
7	1.92	−0.34	4.18	0.095	1.07	−0.49	2.62	0.178	0.50	−0.99	1.98	0.513
22	1.09	−1.27	3.44	0.365	1.02	−0.66	2.71	0.234	0.85	−0.78	2.48	0.304
EBC pH												
3	0.18	−0.13	0.48	0.250	−0.11	−0.31	0.09	0.285	0.01	−0.17	0.19	0.947
6	0.17	−0.09	0.51	0.318	0.02	−0.20	0.25	0.839	0.06	−0.14	0.26	0.544

[a] Change is averaged over the exposure and control sessions. Estimates are adjusted for copollutants using two-pollutant models.

[b] Bolded values are significant at $P \leq 0.05$.

Table C.11. Copollutant-Adjusted Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in $PM_{2.5}$[a]

Endpoint / Hour of Measurement	Adjusted for UFP				Adjusted for EC				Adjusted for NO_2			
	Point Estimate	95% CI Lower	95% CI Upper	P[b]	Point Estimate	95% CI Lower	95% CI Upper	P[b]	Point Estimate	95% CI Lower	95% CI Upper	P[b]
FEV_1												
2	0.03	−1.03	1.10	0.948	−0.39	−1.50	0.71	0.487	−0.57	−1.60	0.45	0.273
3	0.24	−0.94	1.41	0.694	−0.06	−1.27	1.16	0.928	−0.48	−1.61	0.66	0.410
5	−0.17	−1.44	1.09	0.789	−0.61	−1.92	0.70	0.363	−0.86	−2.11	0.35	0.163
7	0.13	−1.17	1.42	0.850	−0.32	−1.66	1.03	0.643	−0.44	−1.71	0.82	0.490
22	0.44	−0.87	1.75	0.511	−0.32	−1.68	1.04	0.642	0.13	−1.15	1.41	0.839
FVC												
2	0.40	−0.90	1.69	0.546	−0.16	−1.48	1.16	0.809	−0.25	−1.51	1.01	0.634
3	0.84	−0.55	2.22	0.237	0.24	−1.16	1.63	0.738	0.07	−1.29	1.42	0.924
5	0.54	−0.92	1.99	0.470	−0.10	−1.54	1.34	0.893	−0.47	−1.88	0.94	0.512
7	0.45	−1.02	1.92	0.547	−0.40	−1.85	1.05	0.585	−0.01	−1.44	1.41	0.985
22	1.50	0.03	2.97	**0.046**	0.41	−1.04	1.87	0.576	0.95	−0.57	2.28	0.239
$FEF_{25–75}$												
2	−2.00	−6.12	2.13	0.343	−0.71	−4.46	3.04	0.710	−2.92	−6.99	1.15	0.159
3	−0.377	−8.00	0.45	0.080	−1.59	−5.35	2.17	0.406	−4.39	−8.54	−0.23	**0.039**
5	−4.28	−8.53	−0.02	0.049	−2.88	−6.64	0.88	0.133	−4.71	−8.89	−0.53	**0.027**
7	−2.10	−6.36	2.16	0.333	−0.54	−4.30	3.22	0.778	−2.91	−7.09	1.27	0.172
22	−2.05	−6.31	2.21	0.345	−0.89	−4.65	2.88	0.645	−2.08	−6.26	2.10	0.329
eNO												
3	0.74	−2.93	4.41	0.692	0.82	−3.06	4.71	0.677	−0.89	−4.53	2.76	0.633
4	−1.51	−5.42	2.41	0.450	−2.29	−6.53	1.94	0.288	−2.04	−6.02	1.93	0.313
5	−1.91	−5.98	2.17	0.358	−2.51	−7.00	1.98	0.273	−1.55	−5.76	2.65	0.468
6	−1.08	−5.25	3.10	0.612	−2.26	−6.93	2.41	0.342	−0.76	−5.13	3.61	0.734
7	−1.43	−5.67	2.81	0.510	−2.25	−7.05	2.55	0.358	−1.65	−6.15	2.84	0.471
22	0.44	−3.93	4.81	0.844	−0.18	−5.38	5.02	0.945	1.60	−3.25	6.46	0.517
EBC pH												
3	0.11	−0.49	0.70	0.720	0.01	−0.62	0.63	0.988	0.21	−0.34	0.76	0.459
6	−0.09	−0.75	0.57	0.785	−0.19	−0.88	0.50	0.587	0.08	−0.52	0.68	0.795

[a] Change is averaged over the exposure and control sessions. Estimates are adjusted for copollutants using two-pollutant models.

[b] Bolded values are significant at $P \leq 0.05$.

Table C.12. Copollutant-Adjusted Analyses for Percentage of Change in Health Endpoints per 10-μg/m^3 Increase in NO_2[a]

	Adjusted for UFP				Adjusted for $PM_{2.5}$				Adjusted for EC			
Endpoint / Hour of Measurement	Point Estimate	95% CI Lower	95% CI Upper	P[b]	Point Estimate	95% CI Lower	95% CI Upper	P[b]	Point Estimate	95% CI Lower	95% CI Upper	P[b]
FEV_1												
2	−0.08	−0.30	0.13	0.448	−0.15	−0.36	0.05	0.148	−0.18	−0.37	0.02	0.079
3	0.04	−0.19	0.28	0.717	−0.09	−0.31	0.14	0.464	−0.05	−0.27	0.17	0.646
5	0.04	−0.21	0.30	0.740	−0.07	−0.31	0.18	0.598	−0.07	−0.31	0.16	0.544
7	0.03	−0.23	0.30	0.808	−0.06	−0.31	0.19	0.642	−0.06	−0.30	0.18	0.614
22	−0.03	−0.30	0.24	0.821	−0.15	−0.40	0.11	0.264	−0.14	−0.38	0.10	0.256
FVC												
2	0.04	−0.23	0.30	0.782	−0.07	−0.32	0.18	0.588	−0.07	−0.31	0.16	0.535
3	0.11	−0.17	0.39	0.440	−0.05	−0.32	0.22	0.717	−0.02	−0.27	0.23	0.855
5	0.19	−0.11	0.48	0.214	0.04	−0.24	0.33	0.768	0.03	−0.23	0.29	0.806
7	0.05	−0.25	0.35	0.742	−0.03	−0.32	0.25	0.811	−0.07	−0.33	0.19	0.601
22	0.13	−0.17	0.43	0.390	−0.07	−0.36	0.21	0.620	−0.05	−0.31	0.21	0.713
$FEF_{25–75}$												
2	−0.47	−1.33	0.39	0.282	−0.48	−1.30	0.34	0.251	−0.43	−1.11	0.26	0.221
3	−0.45	−1.33	0.42	0.309	−0.50	−1.34	0.33	0.236	−0.30	−0.98	0.39	0.393
5	−0.52	−1.40	0.36	0.247	−0.55	−1.39	0.29	0.199	−0.47	−1.15	0.22	0.181
7	−0.29	−1.17	0.59	0.515	−0.37	−1.21	0.47	0.391	−0.22	−0.90	0.46	0.529
22	−0.59	−1.47	0.29	0.190	−0.66	−1.50	0.18	0.124	−0.49	−1.18	0.20	0.162
eNO												
3	0.36	−0.41	1.12	0.363	0.42	−0.32	1.16	0.270	0.32	−0.39	1.03	0.378
4	0.07	−0.76	0.89	0.876	0.32	−0.49	1.13	0.440	−0.04	−0.81	0.74	0.925
5	0.03	−0.83	0.88	0.953	0.45	−0.41	1.30	0.303	0.04	−0.78	0.87	0.916
6	−0.50	−1.38	0.38	0.263	−0.20	−1.09	0.69	0.660	−0.49	−1.35	0.36	0.258
7	−0.22	−1.12	0.67	0.626	0.03	−0.88	0.95	0.941	−0.25	−1.14	0.63	0.573
22	−0.74	−1.68	0.19	0.116	−0.38	−1.36	0.61	0.455	−0.59	−1.56	0.38	0.233
EBC pH												
3	−0.14	−0.26	−0.02	**0.020**	−0.19	−0.30	−0.08	**< 0.001**	−0.15	−0.25	−0.04	**0.007**
6	−0.07	−0.20	0.06	0.270	−0.09	−0.21	0.04	0.164	−0.07	−0.19	0.05	0.230

[a] Change is averaged over the exposure and control sessions. Estimates are adjusted for copollutants using two-pollutant models.

[b] Bolded values are significant at $P \leq 0.05$.

APPENDIX D. HEI Quality Assurance Report

The conduct of this study was subjected to independent audits by Mr. David Bush of T&B Systems, Inc. Mr. Bush is an expert in quality assurance for air quality monitoring studies and data management. The audits included on-site reviews of study activities for conformance to the study protocol and operating procedures, and selected performance audits of monitoring equipment. The dates of the audits are listed below with the phase of the study examined.

QUALITY ASSURANCE AUDITS

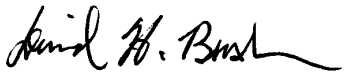
January 13–16, 2004: The auditor conducted an on-site audit at the Imperial College of Science Technology and Medicine and the Royal Brompton Hospital in London, England. Dr. Henry Gong and Ms. Kimberly Hudson from the Los Amigos Research and Education Institute also participated in this audit, providing expertise for the review of the clinical portions of the study. Several recommendations were presented for strengthening and documenting data collection procedures for both the air quality and clinical data efforts, and a significant issue regarding measured flows for particulate measurements was identified.

May 5–7, 2004: The auditor conducted an on-site audit at the University of Medicine and Dentistry of New Jersey, Piscataway, NJ, concentrating on analytical and data management activities conducted at the Environmental and Occupational Health Sciences Institute (EOHSI) laboratory. No problems significantly affecting study data were noted, though some recommendations were presented for improving quality control for some of the analytical activities.

March 13–15, 2006: The auditor conducted an on-site audit of the study's database at the National Heart & Lung Institute in London, where many of the original study forms were maintained. Several data points for each parameter were traced through the entire data processing sequence to verify the integrity of the database. Some minor inconsistencies between the data and original forms were noted, and suggestions were given for further validating lung function data.

November 14, 2007: The auditor reviewed the study final report. Some additional data and data presentation related issues were identified. All were addressed by the authors, and none affected the study findings.

Written reports of each inspection were provided to the HEI project manager, who transmitted the findings to the Principle Investigator. These quality assurance audits demonstrated that the study was conducted by an experienced team with a high concern for data quality. Study personnel were very responsive to audit recommendations, providing formal responses that adequately addressed all issues. The report appears to be an accurate representation of the study.



David H. Bush, Quality Assurance Officer

APPENDICES AVAILABLE ON THE WEB

The following materials may be requested by contacting the Health Effects Institute at 101 Federal Street, 5th Floor, Boston, MA 02110, +1-617 488 2300, fax +1-617-488-2335, or email (*pubs@healtheffects.org*). Please give (1) the first author, full title, and number of the Research Report and (2) title of appendix requested.

Appendix E. Pilot Study to Support the Selection of Diesel Exhaust Exposure and Control Sites

Appendix F. Intensive Characterization of Air Pollution in Hyde Park and Oxford Street

Appendix G. Symptom Monitoring Diary and Peak Flow Chart

Appendix H. Summary of Response to Baseline Health and Exposure Questionnaires

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OTHER PUBLICATIONS RESULTING FROM THIS RESEARCH

McCreanor JE, Cullinan P, Nieuwenhuijsen MJ, Stewart-Evans J, Malliarou E, Järup L, Harrington R, Svartengren M, Han I, Ohman-Strickland P, Chung KF, and Zhang J. 2007. Respiratory effects of exposure to diesel traffic in persons with asthma. N Engl J Med 357:2348–2358.

ABBREVIATIONS AND OTHER TERMS

1-OHP	1-hydroxypyrene
8-ISP	8-isoprostanes
β_3	the percentage changes in health endpoints and biomarkers from baseline that are associated with a unit change of pollutant concentration (as specified in Appendix C table titles).
CI	confidence interval
CO	carbon monoxide
CO_2	carbon dioxide
COC	chain of custody
CV	coefficient of variation
df	degrees of freedom
DE	diesel exhaust
DEP	diesel exhaust particle
DTT	dithiothreitol
EBC	exhaled breath condensate
EC	elemental carbon
ECP	eosinophil cationic protein
ELISA	enzyme-linked immunosorbent assay
eNO	exhaled nitric oxide

F	*F* test
FEF_{25-75}	forced expiratory flow during the middle half of the FVC
FEI	fluoroenzyme immunoassay
FEV_1	forced expiratory volume in the first second
FVC	forced vital capacity
GINA	Global Initiative for Asthma
HPLC	high performance liquid chromatography
IARC	International Agency for Research on Cancer
ICAM-1	intracellular adhesion molecule-1
IgE	immunoglobulin E
IL-8	interleukin-8
MPO	myeloperoxidase
NO	nitric oxide
NO_2	nitrogen dioxide
NO_x	oxides of nitrogen
PAH	polycyclic aromatic hydrocarbon
PBS	phosphate buffer solution
PC_{20}	dose of methacholine required to decrease FEV_1 by 20%
PEFR	peak expiratory flow rate
PM	particulate matter
$PM_{2.5}$	PM ≤ 2.5 μm in aerodynamic diameter
PM_{10}	PM ≤ 10 μm in aerodynamic diameter
ROS	reactive oxygen species
SD	standard deviation
SE	standard error
TBARS	thiobarbituric acid reactive substances
TLV	threshold limit value
UFP	ultrafine particle (PM ≤ 0.1 μm in aerodynamic diameter)
UMDNJ	University of Medicine and Dentistry of New Jersey
U.S. EPA	U.S. Environmental Protection Agency

Research Report 138, *Health Effects of Real-World Exposure to Diesel Exhaust in Persons with Asthma*, J. Zhang et al.

INTRODUCTION

As a result of improved engine performance and lower fuel costs, the use of light diesel-powered vehicles has risen in the past few decades in several countries. Although diesel engines emit less carbon dioxide (CO_2*) and carbon monoxide (CO) than gasoline engines, diesel engines have until recently released higher amounts of nitrogen oxides (NO_x) and particulate matter (PM). Concerns about the health effects of diesel exhaust (DE) and diesel exhaust particles (DEPs) have resulted in the funding of numerous epidemiologic and toxicologic studies, many of which have focused on evaluating the carcinogenic effects of long-term DE exposure (reviewed in Health Effects Institute 2006). However, only a few studies had evaluated the short-term effects of exposure to DE. These studies had either administered DEPs intranasally to healthy people (Diaz Sanchez et al. 1994, 1997) or had exposed healthy individuals or individuals with asthma to DE via inhalation (Salvi et al. 1999; Nightingale et al. 2000; Nordenhäll et al. 2001).

To more fully address the issue of whether exposure to DE is associated with changes in airway inflammation, asthma symptoms, or other allergic responses, HEI issued RFA 00-1, *Effects of Diesel Exhaust and Other Particles on the Exacerbation of Asthma and Other Allergic Diseases*, in January 2000. The RFA sought epidemiologic and experimental studies to address these questions. In response, Dr. Junfeng (Jim) Zhang of the University of Medicine and Dentistry of New Jersey–School of Public Health, proposed a study that would investigate the effects that walking on a street in Central London, United Kingdom, would have on two groups of people whose asthma differed in severity (moderate and mild). Vehicular traffic on this street is restricted to taxis and buses, the majority of which are powered by diesel engines. Dr. Zhang and his colleagues hypothesized that this exposure would exacerbate asthma symptoms, decrease lung function, and induce lung inflammation and oxidative stress responses. They also proposed to measure on-site concentrations of several air pollutants.

The HEI Health Research Committee had some concerns about the proposed study, particularly about whether differences in air pollutant concentrations would be found between the exposure site and the control site (a nearby park from which vehicles were prohibited). In addition, the Committee thought that factors such as meteorologic conditions, levels of pollen, or occupational exposures might confound any responses that were measured. To address these concerns, Zhang and colleagues conducted a pilot study at the two sites. That study showed that concentrations of two of the pollutants the investigators proposed to measure differed between the sites. In their revised application, they proposed to conduct the study during winter months to avoid high concentrations of aeroallergens. The study would exclude people who had high occupational or residential exposures to PM. The Research Committee recommended the study for funding, recognizing that the proposed research was not a clinical study in which the exposure atmosphere and other variables could be rigorously controlled. The Committee nonetheless valued the opportunity the study would provide to assess the acute effects of short-term exposure to components of traffic-derived ambient air pollution in a real-world setting in individuals with varying degrees of chronic asthma. Under the aegis of this same RFA, HEI is also funding a clinical study of exposure to inhaled DE, which is currently led by Dr. Richard Effros, Los Amigos Research and Education Institute, California (see accompanying Preface).

SCIENTIFIC BACKGROUND

TRAFFIC-DERIVED PM

DE is an important component of air pollution in urban environments. Emissions from diesel engines are a complex mixture of particulate and gaseous compounds that vary with engine type. DEPs are composed of a carbon core to

Dr. Junfeng Zhang's 3-year study, "Health Effects of Diesel Exhaust in Asthmatics: A Real-World Study in a London Street," began in October 2002. Total expenditures were $660,764. The draft Investigators' Report from Dr. Zhang and colleagues was received for review in July 2006. A revised report, received in March 2007, was accepted for publication in June 2007. Final changes to the report were submitted in October 2007. During the review process, the HEI Health Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in both the Investigators' Report and in the Review Committee's Commentary.

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

which nitrate, sulfate, metals, and organic compounds adhere. CO_2, CO, nitrogen and sulfur compounds, and low-molecular-weight hydrocarbons are the principal gaseous components of DE. DE from older engines contributes large amounts of NO_x and primary PM to urban air (although most urban PM is derived from city and regional background).

The particles emitted by diesel engines (as well as gasoline engines) fall into a bimodal size distribution: nuclei mode (particles < 50 nm in diameter, and thus falling in the ultrafine particle [< 100 nm] range), which accounts for the major number of particles, and accumulation mode (50–300 nm), which accounts for most of the most of the particles' mass. In its 2002 Diesel Health Assessment, the EPA determined that between 50% to 90% of the DEPs were below 50 nm in diameter (EPA 2002). Since then, the United States, Europe, and other countries have introduced low-sulfur diesel fuel and implemented tighter diesel PM emission standards for both light-duty and heavy-duty vehicles (reviewed in HEI Special Panel on the Health Effects of Traffic-Related Air Pollution 2009). These changes have resulted in the introduction of new PM control devices — primarily filters and catalysts — for diesel vehicles.

Laboratory testing of heavy-duty vehicles has shown that the combination of low sulfur fuel and a control device reduces PM mass emission rates by more than ninety percent. There are challenges in measuring the number, size, and composition of nuclei mode particles, especially from these new low-emitting engines. Nonetheless, studies indicate that overall the introduction of these new technologies and tighter emissions standards have either decreased — or not changed — the number of nuclei mode particles (e.g. Ntziachristos et al 2005, Ristovski et al 2006; Liu et al 2007; Biswas et al 2008). At the same time, the catalyzed trap, a common PM emission control device, has been shown to increase emissions of NO_2 relative to emissions without the filter. New even tighter NO_x emissions standards are being implemented to reduce these emissions further.

Given the durability of diesel engines, the transition to these newer technologies will take time, so that emissions from older diesel are likely to dominate the exposure of the population for several years.

Individuals in urban environments are also exposed to vehicle-derived pollutants that are not emitted from tailpipes. These include particles from brake and tire wear as well as resuspended road dust (Schauer et al. 2006). These mechanically generated particles are predominantly in the coarse particle range, ≤ 10 µm in aerodynamic diameter (PM_{10}), with most of the mass made up of particles in the 2.5 µm to 10 µm size range. Components of these emissions include metals (from brake wear) and organic compounds (from tires).

HEALTH EFFECTS ASSOCIATED WITH EXPOSURE TO TRAFFIC

HEI will publish a comprehensive and updated review of the health effects of traffic in early 2009 (HEI Special Panel on the Health Effects of Traffic-Related Air Pollution 2009). Before the current study started, several studies had found an association between the effects of short-term exposure to urban PM on the respiratory system and asthmatic responses (reviewed by Koren 1995; Pope et al. 1995). Other studies have suggested that exposure to traffic-related air pollution, which includes DE, is significantly associated with an increased risk of allergies and allergic rhinitis (Kramer et al. 2000; Wyler et al. 2000). These studies used the proximity of a residence to a major roadway to measure relative exposure to DE. In addition, Edwards and colleagues (1994) reported that hospital admissions for asthma were associated with higher traffic density on the main road nearest to a person's residence. Furthermore, van Vliet and colleagues (1997) found an increased risk of coughing, runny nose, and doctor-diagnosed asthma in children living within 100 meters of a major freeway. Other studies indicated that stronger associations with health endpoints were observed in subjects residing near roads with heavy truck traffic, rather than near roads with mostly automobile traffic (Brunekreef et al. 1997; Ciccone et al. 1998). However, as described in more detail later in the next section, some studies of short-term controlled exposure to traffic-derived air pollution have not shown major effects on health endpoints.

HEALTH EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO DE

Lung Function

Asthma is a chronic obstructive and inflammatory disease of the lungs that results in episodic wheezing, breathlessness, chest tightness, and cough. Asthma is associated with bronchial hyperreactivity, and several stimuli (e.g., viral and respiratory infections, pollen, dust, temperature changes, and pollutants) can trigger bronchoconstriction in people with asthma (Busse and Lemanske 2001). Some studies have investigated the effects of short-term exposures to DE or traffic emissions in both healthy and asthmatic subjects. Nordenhäll and colleagues (2001) found significant increases in airway hyperresponsiveness and airway resistance in 14 asthmatic participants exposed to DE in an exposure chamber for 1 hour. Lung-function measurements conducted before and after those sessions found

an increase in airway resistance, but no change in forced vital capacity (FVC) or forced expiratory volume in the first second (FEV_1). Two studies in healthy volunteers did not find associations between acute DE exposure and changes in lung function (Salvi et al. 1999; Nightingale et al. 2000). Lung-function tests conducted immediately before and after healthy participants underwent a 1-hour exposure to DE in a chamber study showed no significant differences in pulmonary function (Salvi et al. 1999). A study with 10 healthy participants, whose lung function was measured before exposure and every 30 minutes after exposure (up to 4 hours) to DEP in an exposure chamber, showed no significant changes in lung function from baseline after exposure (Nightingale et al. 2000).

Studies assessing the effect of DE exposure in asthmatic subjects are even rarer. In a study conducted by Svartengren and colleagues (2000), 20 asthmatic subjects were exposed to air pollution for 30 minutes in a road tunnel (or a control environment with less traffic) and were given an allergen challenge 4 hours later. The investigators measured asthma symptoms and early-phase (specific airway resistance) and late-phase (FEV_1 3 to 10 hours after allergen inhalation) asthmatic responses. The results suggested that the subjects exposed to pollutants in the tunnel had a marginally larger early reaction, with decreased lung function and more asthma symptoms appearing during the late phase.

Immunologic, Inflammatory, and Oxidative Stress Responses Associated with Exposure to DE

Before the current study, studies in both humans and rodents suggested that administration of DEP enhances features characteristic of an allergic pattern of immune response. Diaz-Sanchez and colleagues (1994) found that administering 300 µg of DEP into the noses of healthy and asthmatic individuals enhanced the total number of IgE-secreting B cells in the upper airways of both groups. They also found (Diaz-Sanchez et al. 1997) that administering DEP in combination with ragweed allergen enhanced the production of messenger RNA specific for interleukin-4 (IL-4) and IL-13 — cytokines associated with and the development of an allergic response. Several early studies in rodents (e.g., Takano et al. 1997; Fujimaki et al. 1997) reported that the administration of DEP with allergens such as ovalbumin enhanced Th2-cell cytokine production and antigen-specific IgE synthesis, key features of an allergic response.

Some controlled-exposure studies had evaluated immunologic and inflammatory endpoints in healthy and asthmatic volunteers exposed to DE. Salvi and colleagues (1999; 2000) reported an increase in neutrophil and B cell numbers and in levels of IL-8, fibronectin, and histamine in the bronchoalveolar lavage fluid from healthy participants. Rudell and colleagues (1999) also found an increase in neutrophil numbers in airway lavage fluid of healthy volunteers, but not in their fibronectin or myeloperoxidase (MPO) levels. Nightingale and colleagues (2000) found a significant increase in sputum neutrophils and MPO. Before the current study, only Nordenhäll and colleagues (2001) had investigated inflammatory responses in asthmatic people exposed to DE; their study found no significant changes in MPO, eosinophil cationic protein (ECP), or IL-8, but did find a significant increase in IL-6.

A voluminous literature exists on oxidative stress as a plausible mechanism to explain the induction of PM-mediated inflammatory responses. Studies in rodents and in vitro have linked controlled exposure to DE with the induction of oxidative stress (Sagai et al. 1993; Tsurudome et al. 1999, Hiura et al. 1999). However, before the current study, only Nightingale and colleagues (2000) had reported effects of DE on markers of oxidative stress in a controlled-exposure study with human subjects.

Thus, at the time the current study was funded, some effects of short-term exposure to traffic-derived pollution and DE had been reported in a few studies of healthy volunteers and people with asthma. However, the findings were not consistent across the studies, which may have resulted from the small number of subjects in each study group, as well as from differences between studies in the timing of health endpoint measurements after exposure. This study by Zhang and colleagues used a sample population of adequate size, which was exposed to urban air on a street where vehicles with diesel engines predominated. This allowed the investigators to evaluate the effects of pollutant exposures under real-world conditions on people with asthma. Some of the results of the study have been published (McCreanor et al. 2007).

SUMMARY OF STUDY

OBJECTIVES

The overall objective of the study was to determine the effects on the respiratory systems of volunteers with mild to moderate asthma, during and after short-term exposure to urban diesel traffic in a real-world setting. A secondary objective was to explore exposure–response relations for several DE components.

The study was designed to test three hypotheses:

1. Acute exposure to urban diesel traffic worsens asthma symptoms and reduces lung function in nonsmoking asthmatic adults performing moderate exercise;
2. Worsening of asthma symptoms is associated with increases in oxidative stress and inflammation in the airways; and
3. Baseline asthma severity as well as the level of exposure to diesel traffic determines the degree of exacerbation of the features of asthma to be measured.

STUDY DESIGN AND METHODS

Study Design

Study Population Zhang and colleagues addressed the study hypotheses with 60 participants (29 women and 31 men) who had either mild or moderate symptoms of asthma. The study population consisted of nonsmoking volunteers between 18 and 55 years old; 29 had moderate asthma and 31 had mild asthma. Twenty-five members of the group with moderate asthma and 12 members of the group with mild asthma used inhaled corticosteroids as a preventive medication. Details of how asthma was diagnosed and how participants were classified as having mild or moderate asthma, along with additional inclusion and exclusion criteria used in determining the study population, are given in the Methods and Study Design section of the Investigators' Report.

The investigators recruited volunteers from (1) a patient database of the asthma laboratory at the Royal Brompton Hospital, the Central London hospital at which clinical measurements were made; (2) Royal Brompton Hospital outpatient respiratory clinics; and (3) advertisements distributed through Royal Brompton Hospital, Imperial College London, a student newspaper, and an electronic student journal.

Zhang and colleagues evaluated pulmonary function, asthma symptoms, and markers of airway inflammation and oxidative stress in the participants during a screening visit at the Royal Brompton Hospital.

Exposure and Control Sessions On the day of an exposure or control session, the investigators made baseline, presession measurements of each participant's airway function (described below in the section entitled Health Endpoint Measurements) at the Royal Brompton Hospital. Participants were then taken by taxi to the exposure site, Oxford Street (approximately 3 miles), or to the control site, Hyde Park (approximately 1 mile from the hospital and 1 to 1.5 miles from Oxford Street). Oxford Street is a busy Central London thoroughfare; vehicular traffic is restricted to buses and taxis, which are powered predominantly by diesel engines. However, Oxford Street is intersected by smaller streets on which no restrictions are placed. All vehicular traffic is excluded from Hyde Park.

Each session took place during a weekday morning and lasted 2 hours. Subjects walked along predetermined paths in either the exposure site or the control site. Each 30 minutes of walking was followed by a 15-minute rest period. A field technician and physician accompanied participants during the session. At the end of the session, the participants were driven back to the hospital for further measurements. Table 3 of the Investigators' Report provides a detailed timeline of all the endpoints and pollutant measurements that were made in the study. Exposure and control sessions were separated by a minimum of three weeks and took place during two winter seasons (November 2003 to March 2004 and October 2004 to April 2005).

Pollutant and Meteorologic Measurements

The investigators measured five components of urban, combustion-source–derived air pollution: PM $\leq$ 2.5 μm in aerodynamic diameter ($PM_{2.5}$), UFP, CO, elemental carbon (EC), and nitrogen dioxide (NO_2). Pollutant samplers were placed on a cart that accompanied the participants during the sessions. Details of sampling methods are provided in the Investigators' Report. Measurements were collected throughout the exposure and control sessions. Because of data quality concerns, the investigators excluded CO concentrations from their analyses. To provide an indication of a participant's exposure to air pollution from combustion sources, personal NO_2 exposure was measured throughout the week preceding each session. Temperature and relative humidity were recorded throughout the exposure and control sessions.

Health Endpoint Measurements

Pulmonary Function The investigators used spirometry to assess the pulmonary function parameters FEV_1, FVC, and forced expiratory flow during the middle half of the FVC ($FEF_{25–75}$). These parameters were measured 2 hours before a 2-hour session (hour −2); at the start of a session (the baseline value at hour 0); at hours 1 and 2 (the middle and end of a session); and at hours 3, 5, 7, and 22 (after a session). Participants recorded peak expiratory flow rate (PEFR) values on diary cards four times daily for one week before and one week after each session. Bronchial reactivity, determined by the dose of methacholine required to decrease FEV_1 by 20% (PC_{20}), was measured during the screening visit and at hour 7.

Asthma Symptoms Participants used the diary cards to record nighttime asthma awakenings, shortness of breath, chest tightness, wheezing, cough, sputum production, and use of the asthma reliever medication albuterol (a bronchodilator). Daily entries for each item were made one week before and one week after each session. Asthma symptoms were also recorded during each session and 7 hours after the start of each session (hour 7).

Markers of Airway Inflammation and Oxidative Stress Zhang and colleagues measured several markers of airway inflammation and oxidative stress in exhaled breath, sputum, and blood that have been measured in previous studies (Pavord et al. 1997; Salvi et al. 2000; Sydbom et al. 2001; Nightingale et al. 2000). These markers were exhaled nitric oxide (eNO); expired breath condensate (EBC) pH; sputum IL-8, MPO, and ECP; and serum thiobarbituric acid reactive substances (TBARS). The investigators also measured free iron in EBC because particles in urban environments contain iron. The investigators thought that the inhaled iron might affect a person's iron metabolism.

EBC was collected 2 hours before each session, 1 hour after each session, and 5 hours after each session (hours −2, 3, and 7). eNO was measured 2 hours before each session, hourly for 5 hours after each session, and at hour 22 (hours −2, 3 through 7, and 22). Sputum samples, also analyzed for differential cell counts (including neutrophil number), were collected from participants during the screening visit and at hour 22. TBARS were measured in blood samples drawn before and after each session (hour −2 and hour 7).

Zhang and colleagues reported that as a result of technical problems they were unable to measure several markers of oxidative stress in EBC (nitrite and nitrate, 8-isoprostane, and nitrotyrosine) and did not report information on levels of 1-hydroxypyrene in urine (a potential marker of DE exposure) because the amount of 1-hydroxypyrene was below the detection limit in many of the samples.

STATISTICAL METHODS AND DATA ANALYSIS

The investigators used three types of statistical methods. In increasing order of complexity these were as follows: descriptive and correlations analysis, comparative analysis between the exposure and control sessions, and pollutant-specific exposure–response analysis.

Descriptive and correlations analyses included estimating summary statistics such as means, standard deviations, and correlation coefficients, as well as providing some plots of responses over time. This approach gave an overview of the distributional shape of the data, identified and isolated outliers, and provided relevant characteristics of the subjects and the pollution measures.

Comparative analyses between the exposure and control sessions took advantage of the paired design of the experiment to compare exposure responses with control responses within subjects. Baseline measurements were compared using paired *t* tests; responses over time were compared using mixed-effects linear models.

Pollutant-specific exposure–responses were estimated by adding a control for the actual amount of exposure to a pollutant measured for each subject from his or her personal monitor. For this analysis, the response was the change in a health endpoint from baseline. Covariates included measured pollutant concentration, time, and several other covariates including temperature and relative humidity, and subject characteristics such as age and sex. The first set of pollution-specific exposure–response analyses used just one of the four pollutants as a single covariate; a second set of analyses used two of the four pollutants as covariates in a set of copollutant models. All possible pairs of copollutants were examined in this way.

The mixed-effects models used for comparisons between exposure and control sessions and for pollution-specific exposure–response analyses included (1) random effects for subject; (2) random effects for time, within subjects; and (3) a random effect for date, across subjects. These comparisons were made in a combined analysis with all subjects (nonstratified) and then done separately for participants with either mild or moderate asthma (stratified).

RESULTS

Pollutant Concentrations During Exposure and Control Sessions

Participants were exposed to higher average pollutant concentrations during the exposure session (Oxford Street) than during the control session (Hyde Park). For $PM_{2.5}$ mass, the concentration was higher during the exposure session by a factor of 2.0 (mean ± SD; 32 ± 14 $\mu g/m^3$ vs. 16 ± 13 $\mu g/m^3$); for UFP it was higher by a factor of 3.4 (66.6 ± 13.8/cm^3 vs. 19.5 ± 6.4/cm^3); for EC it was higher by a factor of 4.8 (8.1 ± 3.2 $\mu g/m^3$ vs. 1.7 ± 1.3 $\mu g/m^3$); and for NO_2 it was higher by a factor of 4.0 (143 ± 78 $\mu g/m^3$ vs. 36 ± 37 $\mu g/m^3$). In addition, the average temperature was 2.4°C higher and humidity 5.5% lower during exposure sessions than during control sessions.

Comparison of Responses Between Exposure and Control Sessions

The investigators compared endpoints at different times during and after the exposure session with endpoints during and after the control session. Commentary Table 1 shows that several endpoints did not change significantly (asthma symptom scores, asthma reliever medication use, and PEFR — all three recorded 1 to 7 days post-session — as well as eNO, PC_{20}, blood TBARS, and $FEF_{25–75}$).

Some of these endpoints showed nonsignificant changes. $FEF_{25–75}$ was lower at all time points in the exposure versus control analyses (Investigators' Report Table 15 and Figure 6). Nonsignificant increases were also observed in symptoms and asthma reliever medication use. Nonsignificant decreases were observed in PEFR during the 7 days after exposure; nonsignificant increases were also noted in the sputum endpoints measured at hour 22 (IL-8 and percentage neutrophils, eosinophils, and lymphocytes). Total cell counts were not reported. Of the sputum parameters evaluated, only the change in MPO level was significant, with more than a 5-fold increase after the exposure session compared with after the control session.

Commentary Table 1 also shows that differences between exposure and control sessions in FEV_1, FVC, and EBC pH were statistically significant at one or more time points. Commentary Table 2 shows the range of changes in these endpoints that were found in both nonstratified analyses (including all subjects) and analyses stratified by asthma severity.

In nonstratified analyses the decrease in FEV_1 was greater at hours 2, 3, and 5 of the exposure session than of the control session; these differences were statistically significant. FVC was similarly lower at these time points for the exposure sessions compared with the control sessions,

Commentary Table 1. Differences Between Exposure and Control Sessions at One or More Measured Time Points

Change in Endpoint / Endpoint
Not Significant
Asthma symptom scores (+)
Asthma medication use (+)
Airway markers
PEFR (−)
FEF_{25-75} (−)
eNO
ECP
EBC iron
PC_{20}
Blood TBARS
Sputum markers
IL-8 (+)
Neutrophils (+)
Eosinophils (+)
Lymphocytes (+)
Statistically Significant
FEV_1 (−)
FVC (−)
MPO (+)
EBC pH (−)

(+) indicates increase in endpoint.

(−) indicates decrease in endpoint.

Commentary Table 2. Changes in Pulmonary Function and Airway Acidification in Stratified and Nonstratified Analyses

Population	Pulmonary Function		Airway Acidification
	FEV_1	FVC	EBC pH
Overall	↓ 3.0% (hour 7) to 4.1% (hour 5)	↓ 3.1% (hour 2) to 3.7% (hour 5)	↓ 2.0%[a]
Moderate asthma	↓ 3.9% (hour 2) to 5.3% (hour 5)	↓ 6.0% (hour 2) to 7.2% (hour 5)	↓ 2.9%[a]
Mild asthma	NS	NS	NS

NS indicates not significant.

↓ indicates significantly associated with decrease in endpoint.

[a] Significant only at hour 3.

Commentary Table 3. Associations that Reached Statistical Significance Between Specific Pollutants and Changes in Endpoints from Baseline at One or More Time Points in One-Pollutant Models

	Pulmonary Function			Airway Inflammation				
	FEV_1	FVC	FEF_{25-75}	eNO	IL-8	MPO	EBC pH	EBC iron
$PM_{2.5}$	NS	NS	↓ [a]	↑ [a]	↑ [a]	NS	NS	↓ [b]
EC	↓ [c]	↓ [b]	↓ [a]	↑ [a]	NS	NS	NS	NS
UFP	↓ [d]	↓ [c]	↓ [a]	NS	NS	↑ [b]	↓ [b]	NS
NO_2	↓	NS	↓	NS	↑ [b]	NS	↓ [b]	NS

NS indicates not significant.

↓ indicates significantly associated with decrease in endpoint.

↑ indicates significantly associated with increase in endpoint.

[a] Dominated by mild asthma group.

[b] Dominated by moderate asthma group.

[c] Dominated by mild asthma group at hour 2, moderate asthma group at hour 3, significant in both severity groups at hour 5.

[d] Dominated by moderate asthma group at hour 3, significant in both asthma severity groups at hours 2 and 5.

but the differences were not significant after the investigators applied a Bonferroni correction. EBC pH at hour 3 showed a greater reduction after the exposure session than after the control session; the 2% reduction correlated with a 2-fold increase in H^+ ions. Stratified analyses indicated that the reductions in FEV_1, FVC, and EBC pH were dominated by responses in the moderate asthma group. The 7.2% reduction in FVC at hour 5 in the group with moderate asthma was the largest reported relative change in pulmonary function.

Associations Between Individual Pollutants and Specific Endpoints

Zhang and colleagues evaluated associations between individual pollutants and changes from baseline in health or biomarker endpoints in one- and two-pollutant models.

One-Pollutant Models Commentary Table 3 and the subsequent paragraphs describe the associations reported using one-pollutant models in nonstratified analyses and analyses stratified by asthma severity. The table includes associations that were not significant in nonstratified analyses but achieved significance at one or more time points in stratified analyses.

In summary, Commentary Table 3 indicates that several changes in pulmonary function parameters were associated with the individual pollutants (except for $PM_{2.5}$) in these analyses, but fewer measures of airway inflammation were associated with specific pollutants. $FEF_{25–75}$, which did not differ significantly between the exposure and control sessions, was significantly associated with each of the individual pollutants when analyzed separately. Associations between individual pollutants and specific health endpoints were as described below:

$PM_{2.5}$ In nonstratified analyses, an increase in $PM_{2.5}$ was associated with a decrease in $FEF_{25–75}$ at two time points but not overall, and was associated with a decrease in EBC iron. In analyses stratified by asthma severity, $PM_{2.5}$ was associated at one or more time points with effects on $FEF_{25–75}$, eNO, and IL-8 in the group with mild asthma, and with effects on EBC iron in the group with moderate asthma.

EC In nonstratified analyses, an increase in EC was associated with changes in FEV_1, FVC, eNO, and $FEF_{25–75}$ (the latter at two time points) but was not significant overall. In analyses stratified by asthma severity, EC was associated at one or more time points with effects on $FEF_{25–75}$ and eNO that were dominated by responses in the group with mild asthma, and with effects on FEV_1 that were dominated by responses in different groups at different times.

UFP In nonstratified analyses, an increase in UFP was associated with changes in FEV_1, EBC pH, and MPO, and was marginally associated with a change in $FEF_{25–75}$. In analyses stratified by asthma severity, UFP was associated with changes in FEV_1 in both groups of asthma patients, in $FEF_{25–75}$ in the group with mild asthma, and in EBC and MPO in the group with moderate asthma.

NO_2 In nonstratified analyses, an increase in NO_2 was associated with changes in EBC pH, sputum IL-8, FEV_1, and $FEF_{25–75}$ at one or more time points, and marginally significantly associated with PC_{20} (not shown in Commentary Table 3). In analyses stratified by asthma severity, NO_2 was associated with changes in EBC pH and IL-8 in the group with moderate asthma, and with changes in PC_{20} (not shown) in the group with mild asthma.

Two-Pollutant Models The investigators evaluated the effects of individual pollutants on FEV_1, FVC, $FEF_{25–75}$, eNO, and EBC pH after incorporating a second pollutant into the model; the endpoints IL-8 and MPO were not included in this analysis. The results of these two-pollutant models are shown in Tables 34–37 of the Investigators' Report. In summary, several associations between the measured pollutants and changes in endpoints lost significance after the investigators controlled for other pollutants, but some associations were unaffected by the inclusion of a second pollutant. Associations of UFP with endpoints were not affected by adjusting for other pollutants, except NO_2. Adjusting for NO_2 generally appeared to reduce associations with the other pollutants.

HEI EVALUATION OF THE STUDY

The HEI Review Committee thought that Zhang and colleagues' study used an innovative approach for assessing the short-term health effects of a real-world exposure to a DE-enriched atmosphere among asthmatic subjects, a group that may be particularly sensitive to DE. The study design successfully combined measured exposure concentrations of individual air pollutants that were associated with traffic emissions (UFP, EC, NO_2, and $PM_{2.5}$) and noninvasive techniques that measured pulmonary function and airway inflammation and acidification. In addition, by using each person as his or her own control, the study design eliminated a range of potential individual-specific confounders. The use of exposure and control sessions in the same fall and winter months in successive years also reduced, but did not completely eliminate, the possibility that concentrations of aeroallergens would be high. These allergens might have been serious confounders of responses in asthmatic subjects.

The Committee thought that Zhang and colleagues successfully established that pollutant levels differed between the exposure and control sites, and that the investigators had reported original and potentially relevant airway responses. The findings in which the Committee had most confidence were the comparisons of responses associated with the exposure session on Oxford Street and those associated with the control session in Hyde Park.

The Committee considered the reported decreases in the lung-function parameters FEV_1 (with a range of 3.0% to 4.1% at hours 2 through 7) and FVC (with a range of 3.1% to 3.7% at hours 2 through 7) to have the most potential clinical relevance. A decrease of airway function in those ranges is likely to have the greatest impact on people with the most severe type of asthma, in which the capacity of the airways is diminished, and on people whose asthma is not appropriately controlled by medication. However, as described later in this section, the investigators reported that these changes did not affect the clinical status of the participants in the current study. Zhang and colleagues had noted that changes in FEV_1 and FVC had not been detected in previous studies of asthmatic participants exposed to DE in chamber studies (Salvi et al. 1999, Nightingale et al. 2000, Nordenhäll et al. 2001). Zhang and colleagues attributed their detection of changes in these endpoints as resulting either from the greater statistical power in their study compared with earlier studies — resulting from the larger group size studied — or from differences in exposure atmospheres in the different studies.

Comparing airway responses, the Committee agreed with the investigators' interpretation of the comparisons between the exposure and control sessions: the 5-fold increase in sputum MPO and 2.0% decrease in EBC pH (an approximately 2-fold increase in H^+ ions) were indicative of some exposure-associated effects on inflammation and acidification in the airways. The Committee pointed out, however, that Zhang and colleagues had found only these two significant changes in the set of airway inflammatory markers they had measured and had not found any change in the one marker of oxidative stress they had successfully measured. Thus, the evidence of effects on markers of airway inflammation and systemic oxidative stress was not entirely coherent. Given that MPO is predominantly found in neutrophils, and that the investigators reported nonsignificant increases in sputum IL-8 concentration and percentage of neutrophils, the Committee tentatively agreed with Zhang and colleagues' interpretation that the exposure session mildly enhanced airway acidification and neutrophil-mediated airway inflammation. Similar inflammatory responses have been reported in chamber studies of healthy people and of people with asthma who were exposed to DE (Nordenhäll et al. 2001; Nightingale et al. 2000; Salvi et al. 2000; Svartengren et al. 2000).

The Committee agreed with the investigators' interpretation of the comparisons between exposure and control sessions on symptoms and asthma reliever medication use: asthma symptom scores and asthma reliever medication

use up to 7 days after the exposure session were somewhat higher than, but not significantly different from, symptom scores and asthma reliever medication use after the control session. The Committee thought the lack of differences in asthma symptoms and asthma reliever medication use were important because the Committee had been concerned about an inherent drawback of the study design: because participants were aware of whether they were participating in an exposure or control session, their self-reported use of asthma reliever medication or changes in symptoms could have been biased. Because changes in symptoms and asthma reliever medication use after the sessions were not significant, this lack of blinding did not appear to affect the participants' perception of their clinical situation. More important, however, the Committee thought that the lack of statistical difference between asthma symptom scores and asthma reliever medication use after the exposure and control sessions strongly suggested that the statistically significant changes in endpoints (FEV_1, FVC, MPO, and EBC pH) had little or no impact on the clinical status of the participants up to 7 days after the exposure session.

The Committee thought Zhang and colleagues' exploratory one- and two-pollutant analyses, which were designed to identify associations between individual pollutants and specific health endpoints, were valid attempts to separate the effects of components in the air pollutant mix. The Committee noted that because the investigators conducted multiple tests, some associations that were reported as significant in those analyses were likely to have arisen by chance. In addition, the modification of an effect of one pollutant on another in two-pollutant models is difficult to interpret. For these reasons, the Committee thought that although the analyses of pollutants and health endpoints had provided intriguing preliminary data about the associations between individual pollutants and specific health endpoints, further studies would be needed to more convincingly establish these relations.

The Committee interpreted results from the pollutant-specific analyses of health endpoints to indicate that all four of the measured pollutants were associated with at least some change in one or more inflammatory or pulmonary function endpoints. EC, frequently used as a marker of diesel exposure, was associated with several changes. The Committee agreed with the investigators' general conclusions that UFP was associated with changes in several endpoints in one-pollutant analyses and that changes in endpoints associated with UFP were robust even after adjusting for copollutants in two-pollutant analyses. However, the Committee noted that NO_2 was associated with almost as many changes in endpoints as was UFP in the one-pollutant analyses, and that decreases in several associations after adjusting for NO_2 in two-pollutant models suggested that NO_2 is also associated with many of the endpoints measured.

The Committee thought the association with NO_2 was particularly intriguing, because as the investigators noted, concentrations of NO_2 in the air increased steeply over the two years of the study (2004 and 2005), but concentrations of the other pollutants did not increase. During this time, London buses were fitted with catalyzed particulate filters that decreased particulate pollutant emissions but increased the ratio of NO_2 to NO_x (Carslaw 2005). The NO_2 increase reported in this study is believed to be due to the introduction of these new filters in the buses. While NO_2 concentrations increased over the study period, EC concentrations decreased (see Figure 5 of the Investigators' Report), indicating a significant shift in emissions over the course of the study. To determine whether associations between individual pollutants and endpoints differed between the two years of the study, the Committee thought it would have been valuable to analyze associations between individual pollutants and endpoints by year. The Committee would also have liked the investigators to analyze the pollution data collected by London's central monitoring agency to determine whether the changes in pollutant concentration by year were reproduced across London. However, the investigators were unable to undertake these analyses in the time frame of the study.

The Committee thought that Zhang and colleagues' analysis of responses by asthma severity was also valuable. The Committee agreed with the investigators' interpretation of results for comparisons of the exposure and the control session: that larger decreases in FEV_1, FVC, and EBC pH were found in people with moderate asthma than in people with mild asthma. Based on these results, the investigators concluded that the background severity of asthma may increase an individual's susceptibility to diesel traffic exposure. This interpretation is biologically plausible because people with more severe asthma are already likely to have more changes to their airways than people with less severe asthma, so a further insult or challenge to their airways may have a greater impact. However, this clear-cut dichotomy of response pattern in people with moderate versus mild asthma was not found in analyses of associations between specific pollutants and endpoints: some associations were greater in people with mild asthma, some greater in people with moderate asthma, and some were dominated by one or another asthma-severity group at different time points. As Zhang and colleagues also point out, the diagnosis of mild or moderate asthma in a given person may change over time for unknown reasons.

Thus, background severity of asthma may be an important factor in responses to diesel traffic exposure, but further work is needed to confirm or disprove this hypothesis.

The Committee noted that a greater proportion of patients with moderate asthma than with mild asthma were using corticosteroid medication on a regular basis to control their asthma. The investigators interpreted these data to indicate that corticosteroid use did not affect responses, but the Committee believed that the investigators could not exclude the possibility that corticosteroid use might have masked a potentially greater response in the group of people with moderate asthma than was observed.

The Committee pointed out that the study design was intended to evaluate the effects of a single exposure to an environment dominated by DE, and that evaluating the effects of multiple exposures was not part of the experimental plan. Nonetheless, individuals who participated in the study had almost certainly been exposed previously to pollutant environments enriched for DE. Thus, the responses measured in the current study are not likely to be naive responses, but responses to a second or subsequent exposure. The Committee thought it was not possible to predict whether the responses measured in the current study would be enhanced, attenuated, or identical if the participants were exposed again to an atmosphere enriched for diesel traffic.

The Committee agreed with the investigators that the findings should not be interpreted to indicate that exposure to DE in and of itself explains the differences in findings between the exposure and control sites. The first reason was that the investigators did not evaluate an absolutely specific marker of diesel emissions. Of the pollutants evaluated in this study, EC may be the best marker of DE; as the investigators report, most of the atmospheric EC associated with vehicular traffic is derived from DE, with only small contributions from other sources (Hildemann et al. 1991; Schauer et al. 1996). UFPs constitute the majority of DE particles in fresh emissions, but are not specific to diesel engine emissions. Similarly, NO_2 is considered a marker of vehicular traffic emissions, but is not specific to diesel engine emissions.

A second reason for being cautious in attributing the effects reported in this study to DE exposure is that the participants were almost certainly exposed during their sessions to air pollutants that were not derived from diesel-powered engines. These include pollutants present in tailpipe emissions of gasoline-powered cars on streets that cross Oxford Street, as well as particles in the coarse particle size range derived from roadway dust and from tire and brake wear in all types of vehicles. Concentrations of several of these traffic-associated components of the pollutant mix — including CO, organic carbon compounds, metals, and coarse particles — were not assessed in the current study.

The exposure and control sites differed in other unmeasured characteristics. Oxford Street is a busy thoroughfare with heavy pedestrian and vehicular traffic during the day; walking on the sidewalk of this street would be noisier and likely to be more stressful than walking in bucolic Hyde Park (e.g., Ising et al. 2004). Thus, although the findings of the current study indicate that lung function is decreased and airway inflammation may be enhanced in persons with asthma who are exposed to ambient urban air that is especially high in DE, further studies are needed to provide direct evidence of the effects of DE and to identify the specific components of DE that may be responsible for health effects. A final consideration is that since the completion of the study, more stringent emissions and fuel standards have been implemented and new engine technologies introduced in both the United States and Europe. As older vehicles are replaced in the fleet, decreases in most traffic-related pollutant concentrations can be anticipated in the coming years; the health impact of these changes will need to be assessed in the future.

SUMMARY AND CONCLUSIONS

Zhang and colleagues conducted an innovative study to assess the short-term health effects of a real-world exposure to an urban atmosphere with high concentrations of pollutants derived from diesel traffic in subjects, with either mild or moderate asthma, who may be particularly sensitive to such an exposure. The study design successfully integrated measurements of several pollutant concentrations as well as noninvasive measurements of airway responses. The study compared concentrations of four traffic-associated air pollutants (UFP, EC, NO_2, and $PM_{2.5}$) with biologic and clinical endpoints assessed in participants before, during, and after an exposure session — a walk on Oxford Street, a busy thoroughfare in London, United Kingdom, in which vehicular traffic was restricted to buses and taxis that were predominantly diesel powered — and a control session, a walk in nearby Hyde Park, from which vehicular traffic was excluded. The investigators successfully established that concentrations of the measured air pollutants were higher at the exposure site than at the control site.

The study provided interesting new findings, particularly when comparing health endpoints between the exposure and control sessions. The effects with the most potential clinical significance were the relative decreases in FEV_1 (3.0%–4.1%) and FVC (3.1%–3.7%) during and several hours after the exposure ended. The magnitude of these

decrements in lung function may be clinically relevant for patients with severe or uncontrolled asthma, whose lung capacity is severely diminished compared with healthy people. Comparisons of responses from the exposure and control sessions also showed significant changes in one marker each of airway inflammation (MPO) and of airway acidification (EBC pH). In conjunction with sputum findings of marginal statistical significance, these findings suggest that the exposure session was associated with a mild increase in inflammatory response in the airways that was mediated by neutrophils.

Asthma symptoms and the use of asthma reliever medication increased only marginally after the exposure session, as compared with the control session. Thus, whereas exposure to a diesel-traffic–enriched environment may have produced changes in pulmonary function and inflammatory endpoints, the fact that these changes were not accompanied by significant changes in symptoms or the use of asthma reliever medication suggests that this single exposure did not affect the clinical status of asthmatic participants.

In analyses of exposure and control sessions with stratification by the severity of asthma, changes in FEV_1, FVC, and EBC pH were significant only in subjects with moderate asthma. Because the majority of subjects in the group with moderate asthma were taking corticosteroids, it is possible that corticosteroid use may have blunted responses in this group. The investigators concluded that the background severity of asthma affects an individual's susceptibility to diesel traffic exposure, an interpretation that is biologically plausible. However, this dichotomy of responses between mild and moderate asthma was not clear-cut in pollutant-specific analyses for many other endpoints. In addition, the distinction between mild and moderate asthma in a given person may change over time for unknown reasons. Thus, the background severity of asthma may be an important factor affecting responses to diesel traffic exposure, but further work is needed to confirm or disprove this hypothesis.

Exploratory one- and two-pollutant analyses to identify associations between specific components of the pollutant mix and changes in endpoints found that UFP and NO_2 were associated with the most endpoints, EC with fewer, and $PM_{2.5}$ with fewer still. All these pollutants are constituents of traffic emissions, and EC is frequently used as a marker of diesel emissions, but none is absolutely specific to diesel. Thus, because the pollutants measured are not specific to diesel emissions, the results are only suggestive of the effects of DE on the endpoints measured.

Explanations for the effects observed, other than exposure to DE, also need to be borne in mind. One is that participants were almost certainly concurrently exposed to air pollutants not associated with diesel-powered engines. These include pollutants derived from tailpipe emissions of gasoline-powered cars on streets that cross Oxford Street, as well as particles not derived from tailpipe emissions — such as those generated by tire and brake wear and roadway dust — that are produced by all vehicles. In addition, concentrations of several traffic-associated pollutants (including CO, organic carbon compounds, and particles in the coarse size range) were not measured in the current study and may have had stronger associations with the endpoints evaluated than did the pollutants that were measured. Furthermore, the exposure and control sites differed in other, unmeasured characteristics, particularly in noise levels and the amount of stress experienced by the subjects.

Although the findings of the current study indicate that lung function is slightly decreased and some markers of airway inflammation are increased in people with asthma who are exposed to ambient urban air in a roadside environment dominated by diesel vehicles, the study does not provide direct evidence that DE itself causes these effects. Additional studies would be needed to address that question, and to identify specific components of DE that might be responsible for any observed health effects. A final consideration is that since the study was completed, more stringent emissions and fuel standards have been implemented and new engine technologies introduced in both the United States and Europe. As older vehicles are replaced in the fleet, decreases in most traffic-related pollutant concentrations can be anticipated. The health impact of these changes will need to be assessed; this study may serve as a baseline analysis for future studies on the effects of such changes.

ACKNOWLEDGMENTS

The Health Review Committee thanks the ad hoc reviewers for their help in evaluating the scientific merit of the Investigators' Report. The Committee is also grateful to Maria Costantini for her oversight of the study, to Jacqueline Hayes, Marian Berkowitz, and Geoffrey Sunshine for their assistance in preparing its Commentary, to Carol A. Moyer for science editing of this Report and its Commentary, to Ruth Shaw for its composition, and to Bernard Jacobson, Flannery Carey McDermott, and Quentin Sullivan for their roles in preparing this Research Report for publication.

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Number 138
February 2009