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Effects of Concentrated Ambient Particles on Normal and Hypersecretory Airways in Rats

Jack R Harkema, Gerald Keeler, James Wagner, Masako Morishita, Edward Timm, Jon Hotchkiss, Frank Marsik, Timothy Dvonch, Norbert Kaminski, and Edward Barr

Includes a Commentary by the Institute's Health Review Committee

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The Health Effects Institute was chartered in 1980 as an independent and unbiased research organization to provide high quality, impartial, and relevant science on the health effects of emissions from motor vehicles, fuels, and other environmental sources. All results are provided to industry and government sponsors, other key decisionmakers, the scientific community, and the public. HEI funds research on all major pollutants, including air toxics, diesel exhaust, nitrogen oxides, ozone, and particulate matter. The Institute periodically engages in special review and evaluation of key questions in science that are highly relevant to the regulatory process. To date, HEI has supported more than 220 projects at institutions in North America, Europe, and Asia and has published over 160 Research Reports and Special Reports.

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STATEMENT

Synopsis of Research Report 120

Effects of Exposure to Concentrated Ambient Particles from Detroit Air on Healthy Rats and Rats with Features of Asthma or Mild Bronchitis

INTRODUCTION

Epidemiologic studies in diverse locations have reported that short-term increases in low levels of particulate matter (PM) are associated with short-term increases in morbidity and mortality. These associations are particularly evident in some groups of people, including those with compromised airway function. For people with asthma, a chronic disease of the lower airways, short-term exposure to PM appears to exacerbate symptoms and decrease lung function. Based on this and other evidence, the US Environmental Protection Agency in 1997 promulgated National Ambient Air Quality Standards for particles 2.5 µm or smaller in aerodynamic diameter ($PM_{2.5}$ or "fine" particles).

Despite the existing evidence, a number of questions remained about these effects. The Health Effects Institute issued Request for Applications 98-1, "Characterization of Exposure to and Health Effects of Particulate Matter," to address critical questions about the effects of particles. A key component of that RFA was to evaluate the health effects of ambient particles in humans and in animals that mimic relevant human conditions. One area of interest involved controlled exposures to concentrated ambient particles (CAPs) because such exposures might better reflect actual exposures to the particle mixture and because a technology to concentrate fine particles and expose humans and other species to them had recently become available.

APPROACH

HEI funded Dr Jack Harkema and colleagues to conduct a 2-year study with rats to evaluate the short-term effects of inhaling CAPs derived from the air in an area of Detroit, Michigan that has a high incidence of childhood asthma. They would assess CAPs effects on two key features of asthma: airway inflammation and hypersecretion of mucus. The investigators evaluated CAPs-associated airway effects in healthy BN and F344 rats; in BN rats that had been sensitized with the allergen ovalbumin to induce some features of asthma; and in F344 rats pretreated with endotoxin to have some features of mild bronchitis, which is also characterized by hypersecretion of mucus. Animals were exposed to CAPs 10 hours/day for 1 day or for 4 or 5 consecutive days in July or September, times of the year when the researchers thought mass concentrations of PM would be high. To conduct the study, HEI provided the investigators with the instrument to concentrate fine particles from ambient air. The investigators used non-HEI funds to build a mobile air research laboratory, a trailer that could be moved to the study site and contained equipment for monitoring ambient air, inhalation exposure chambers, and animal laboratory facilities.

Harkema and colleagues also explored whether the metal elements in the particles had toxic effects. First, the investigators determined whether metals were retained in the lungs of animals after inhaling CAPs. Second, they collected particles on filters during the exposure period and resuspended them in an aqueous solution. Using intratracheal instillation with a different set of healthy and ovalbumin-sensitized rats, the investigators compared the ability of different fractions of resuspended particles to induce airway inflammation: the soluble fraction, considered to be the fraction rich in metals; the insoluble fraction; and the total particles.

RESULTS

As expected, during the exposures ambient PM characteristics varied over the course of a day, from

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr Jack R Harkema at Michigan State University, East Lansing MI. The following Research Report contains both the detailed Investigators' Report and a Commentary on the study prepared by the Institute's Health Review Committee.

day to day, and in different seasons. The investigators concentrated ambient fine particles by an average of 19-fold, varying between 10-fold and 30-fold depending on weather conditions and ambient PM levels. Particles in the range 0.6 µm to 1 µm were preferentially concentrated. Several major PM components—including sulfate, nitrate, ammonium, and crustal elements—were found in the same proportions in CAPs as in ambient PM. In addition, the trace elements iron, vanadium, and antimony were all concentrated to the same extent in CAPs compared with ambient levels. Mass concentrations of CAPs were similar during the July and September series of exposures.

At 24 hours after exposure to CAPs, no CAPs-associated inflammatory effects were found in the airways of healthy rats or of rats with mild bronchitis (the bronchitis had resolved by 4 days after endotoxin pretreatment). In CAPs-exposed rats with features of asthma, levels of some measures of mucus secretion and other markers of airway inflammation were higher than in control animals. These increases were modest, however (range 20% to 50%). Inflammatory responses to the instilled, water-soluble fraction of resuspended particles were small and variable. Some trace metals—lanthanum and vanadium in particular—were found in the lungs of rats exposed to CAPs in September. This finding is interesting because it indicates that some elements contained in PM may be retained in the lungs immediately after exposure, although it is not clear for how long. These findings are difficult to interpret: Trace metals were retained in the lungs of animals exposed in September but not those exposed in July, although the levels of trace metals in the CAPs during the two different exposure protocols appeared to be similar.

CONCLUSIONS

This study was the first to examine the effects of inhaled CAPs on the airways of rats conditioned to model asthma. In those animals, few inflammatory endpoints were affected and the changes that were detected were small in magnitude. The study found no inflammatory responses in healthy rats or rats with features of mild bronchitis. Some earlier CAPs exposure studies had detected airway inflammatory effects in healthy rats and in rats with a different, longer-lasting model of bronchitis. Differences in exposure protocols, strains of rats, models of bronchitis, and characteristics of the concentrated particles at the various study sites make comparing the results of these different studies challenging. The results of the current study did not find clear evidence of the toxicity of metal components in $PM_{2.5}$.

Harkema and colleagues showed that they could concentrate and characterize fine particles under different weather conditions in a mobile laboratory, expose rats to the concentrated aerosol, and evaluate many of the CAPs-associated responses on site. Thus, the mobile laboratory can be used to conduct similar studies of the effects of fresh, real-world, fine particles in many different locations.

However, the current study also underscores some of the limitations of the CAPs exposure approach: First, the fact that the composition of fine PM varies over the course of a single exposure day and from day to day makes identifying a potentially toxic PM component difficult. Second, a particular size range of fine particles is preferentially concentrated, which limits the conclusions that can be drawn about the characteristics of particles responsible for toxicity. In addition, because of this preferential concentration of particles based on size, some elements or compounds in the particles may be preferentially concentrated and others not.

Carefully designed epidemiologic studies and controlled exposures with consistent animal models and endpoints are needed in this and other locations to assess the health effects of particles outside the size range concentrated in this study.

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Research Report 120

Effects of Concentrated Ambient Particles on Normal and Hypersecretory Airways in Rats

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HEI STATEMENT

This Statement is a nontechnical summary of the Investigators' Report and the Health Review Committee's Commentary.

INVESTIGATORS' REPORT

When an HEI-funded study is completed, the investigators submit a final report. The Investigators' Report is first examined by three ouside technical reviewers and a biostatistician. The report and the reviewers' comments are then evaluated by members of the HEI Health Review Committee, who had no role in selecting or managing the project. During the review process, the investigators have an opportunity to exchange comments with the Review Committee and, if necessary, revise the report.

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COMMENTARY Health Review Committee

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ABSTRACT

Epidemiological studies have reported that elevated levels of particulate air pollution in urban communities are associated with increases in attacks of asthma based on evidence from hospital admissions and emergency department visits. Principal pathologic features of chronic airway diseases, like asthma, are airway inflammation and mucous hypersecretion with excessive amounts of luminal mucus and increased numbers of mucus-secreting cells in regions of the respiratory tract that normally have few or no mucous cells (ie, mucous cell metaplasia). The overall goal of the present project was to understand the adverse effects of urban air fine particulate matter ($PM_{2.5}$; ≤ 2.5 µm in aerodynamic diameter)* on normal airways and airways compromised with airway inflammation and excess mucus. Our project was specifically designed to (1) examine the chemical and physical characteristics of $PM_{2.5}$ and other airborne pollutants in the outdoor air of a local Detroit community with a high incidence of childhood asthma; (2) determine the effects of this community-based $PM_{2.5}$ on the airway epithelium in normal rats and rats compromised with preexisting hypersecretory airway diseases (ie, animal models of human allergic airway disease—asthma and chronic bronchitis); and (3) identify the chemical or physical components of $PM_{2.5}$ that are responsible for $PM_{2.5}$ -induced airway inflammation and epithelial alterations in these animal models. Two animal models of airway disease were used to examine the effects of $PM_{2.5}$ exposure on preexisting hypersecretory airways: neutrophilic airway inflammation induced by endotoxin challenge in F344 rats and eosinophilic airway inflammation induced by ovalbumin (OVA) challenge in BN rats.

A mobile air monitoring and exposure laboratory equipped with inhalation exposure chambers for animal toxicology studies, air pollution monitors, and particulate collection devices was used in this investigation. The mobile laboratory was parked in a community in southwestern Detroit during the summer months when particulate air pollution is usually high (July and September 2000). We monitored the outdoor air pollution in this community daily, and exposed normal and compromised rats to concentrated $PM_{2.5}$ from this local urban atmosphere. Rats in the inhalation studies were exposed for 1 day or for 4 or 5 consecutive days (10 hours/day) to either filtered air (controls) or concentrated ambient particles (CAPs) delivered by a Harvard ambient fine particle concentrator.

Rats were killed 24 hours after the end of the exposure. Biochemical, morphometric, and molecular techniques were used to identify airway epithelial and inflammatory responses to CAPs. Lung lobes were also either intratracheally lavaged with saline to determine cellular composition and protein in bronchoalveolar lavage fluid (BALF) or removed for analysis by inductively coupled plasma–mass spectrometry (ICPMS) to detect retention of ambient $PM_{2.5}$ – derived trace elements. The Harvard concentrator effectively concentrated the fine ambient particles from this urban atmosphere (10–30 times) without significantly changing the

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

This Investigators' Report is one part of Health Effects Institute Research Report 120, 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 Jack R Harkema, Department of Pathobiology and Diagnostic Investigation, Michigan State University, 212 Food, Safety and Toxicology Building, East Lansing MI 48824; *harkemaj@msu.edu*.

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major physicochemical features of the atmospheric particles. Daily CAPs mass concentrations during the 10-hour exposure period (0800–1800) in July ranged from 16 to 895 $\mu g/m^3$ and in September ranged from 81 to 755 $\mu g/m^3$.

In general, chemical characteristics of ambient particles were conserved through the concentrator into the exposure chamber. Single or repeated exposures to CAPs did not cause adverse effects in the nasal or pulmonary airways of healthy F344 or BN rats. In addition, CAPs-related toxicity was not observed in F344 rats pretreated with bacterial endotoxin. Variable airway responses to CAPs exposure were observed in BN rats with preexisting allergic airway disease induced by OVA sensitization and challenge. Only OVA-challenged BN rats exposed to CAPs for 5 consecutive days in September 2000 had significant increases in airway mucosubstances and pulmonary inflammation compared to saline-challenged/air-exposed control rats. OVA-challenged BN rats that were repeatedly exposed to CAPs in July 2000 had only minor CAPs-related effects. In only the September 5-day exposure protocol, $PM_{2.5}$ trace elements of anthropogenic origin (La, V, and S) were recovered from the lung tissues of CAPs-exposed rats. Recovery of these specific trace elements was greatest in rats with OVA-induced allergic airway disease.

Additional laboratory experiments using intratracheal instillations of ambient $PM_{2.5}$ samples were performed to identify bioactive agents in the CAPs to which rats had been exposed in the inhalation exposure component. Because the most pronounced effects of CAPs inhalation were found in BN rats with OVA-induced allergic airways exposed in September, we used ambient $PM_{2.5}$ samples that were collected on 2 days during the September CAPs inhalation exposures to use for instillation. Ambient $PM_{2.5}$ samples were collected, fractionated into soluble and insoluble species, and then compared with each other and with total $PM_{2.5}$ for their effects in healthy BN rats and those with OVA-induced allergic airway disease. Intratracheal instillation of the insoluble fraction of $PM_{2.5}$ caused mild neutrophilic inflammation in the lungs of healthy rats. However, total $PM_{2.5}$ or the soluble or insoluble fractions instilled in rats with OVA-induced airway inflammation did not enhance the inflammation or the airway epithelial remodeling that was evident in some of the BN rats exposed to CAPs by inhalation. Therefore, the results from this instillation component did not suggest what fractions of the CAPs may have been responsible for enhancing OVA-induced airway mucosubstances and pulmonary inflammation observed in the inhalation exposure component.

In summary, inhaled CAPs-related pulmonary alterations in the affected OVA-challenged rats appeared to be related to the chemical composition, rather than the mass concentration, to which the animals were exposed. Results of the trace element analysis in the lungs of CAPs-exposed BN rats exposed in September suggested that air particles derived from identified local combustion sources were preferentially retained in allergic airways. These results demonstrate that short-term exposures to CAPs from this southwestern Detroit community caused variable responses in laboratory rats and suggest that adverse biological responses to ambient $PM_{2.5}$ may be associated more closely with local sources of particles and weather patterns than with particle mass.

BACKGROUND

People with preexisting respiratory tract disease (eg, asthma, chronic bronchitis) may be at increased risk of airway injury from particulate air pollution. Because of their existing functional limitations, any further damage to the respiratory tract caused by particulate exposure may lead to further decrements in airway function that would result in a greater overall decline in health. The US federal statute mandates that National Ambient Air Quality Standards be set at a level that protects sensitive subgroups within the population (US Environmental Protection Agency [EPA] 1991; Bascom et al 1996). Therefore, research must be designed to identify human populations that are susceptible to major air pollutants like fine particulate matter. Human and animal studies must be conducted to explore possible factors (such as preexisting disease, diet, and genetic makeup) that may increase an individual's sensitivity to particulate pollution.

Asthma is the most common chronic disease of childhood in the developed world, affecting approximately 9 million children under the age of 18 in the United States (American Lung Association 2003). Many studies indicate that the rates of incidence, morbidity, and mortality due to pediatric asthma have been on the increase in recent years. There is growing concern that populations in urban settings appear to be at significantly increasing risk, with minority populations showing rates of disease that are disproportionate to the remainder of the population (Gergen et al 1988). These national trends are reflected in the pediatric asthma rates in Detroit, MI (Joseph et al 1996).

Several epidemiological studies have suggested that increases in particulate air pollution levels are associated with increased hospital admissions and emergency department visits for respiratory disease, including asthma and other chronic obstructive pulmonary diseases in children

and adults (Dockery and Pope 1994). A highly significant correlation between total suspended particles and hospital admission rates for asthma was found for children between 1 year and 4 years old in Hong Kong (Tseng et al 1992). Schwartz and colleagues (1993) found a highly significant association between asthma admissions and PM_{10} concentrations even though the PM_{10} concentrations studied were quite low (mean 29.6 µg/m^3; maximum 103 µg/m^3). After reviewing the epidemiologic literature for the adverse effects attributed to acute particle exposure, Dockery and Pope concluded that for each 10-µg/m^3 increase in PM_{10}, hospital admissions and emergency hospital visits increased approximately 1% for all respiratory complaints, and 2% to 3% for asthma (Dockery and Pope 1994). Similar estimates of risk have recently been calculated by Jaffe and coworkers (2003), who report a 12% increase in emergency room admissions for asthma associated with a 50-µg/m^3 increase in PM_{10}, and by Atkinson and colleagues (2001) in a multi-city European study where daily hospital admissions for asthma increased 1.2% per 10-µg/m^3 increase.

The association of $PM_{2.5}$ with respiratory morbidity has also been the subject of more recent epidemiologic analyses. For example, a reassessment of the Harvard Six Cities Study suggested that fine particles have a great impact on asthma symptoms, whereas particles in the coarse fraction had no effect (Schwartz and Neas 2000). However, studies on asthmatic children in Seattle (Yu et al 2000) and Los Angeles (Ostro et al 2001) showed that adverse health effects related to increases in ambient $PM_{2.5}$ were similar to those caused by PM_{10}. By comparison, Lin and coworkers (2002) concluded that Toronto particles in the $PM_{2.5}$–PM_{10} size range (coarse particles) were associated with increased hospital admissions for pediatric asthma, whereas no similar correlations existed for $PM_{2.5}$ (Lin et al 2002). Taken together, these studies suggest that questions about the association between $PM_{2.5}$ and childhood asthma are unresolved and require further controlled investigation.

A principal pathologic feature of chronic asthma and other chronic airway diseases (like chronic bronchitis, chronic rhinitis, and cystic fibrosis) is excessive amounts of mucus in the airway lumina associated with increased numbers of mucous (goblet) cells in regions of the respiratory tract that normally have few or no mucus-secreting cells (ie, mucous cell metaplasia) (Reid 1954; Glynn and Michaels 1960; Esterly and Oppenheimer 1968; Dunnill et al 1969; Cutz et al 1978; Reynolds and Merrill 1981; Aikawa et al 1992). Strong evidence suggests that mucus impaction of the pulmonary airways contributes importantly to morbidity and mortality from asthma (Aikawa et al 1992). Excessive production of sputum is a common symptom in asthma and chronic bronchitis, and mucous plugging of the airways is consistently reported in postmortem examinations of airways from patients with fatal asthma.

Several animal studies from our laboratory and others have indicated that common ambient air pollutants (eg, ozone, sulfur dioxide [SO_2]) can significantly alter the mucous apparatus (the mechanism of synthesis, storage, and secretion of airway mucus) of the conducting airways by increasing the number of mucous cells in the luminal surface epithelium and associated increases in secreted mucus in the airway lumina (Reid 1963; Harkema et al 1987a,b, 1989, 1997; Gearhart and Schlesinger 1989; Schlesinger et al 1992). Our laboratory (Harkema et al 1987a,b) reported mucous cell metaplasia in the nasal airways of bonnet monkeys after acute and chronic exposures (8 hours/day for 6 or 90 days) to ambient concentrations of ozone (0.15 or 0.30 ppm). We have also reported that ozone-induced mucous cell metaplasia in the nasal epithelium can be induced in the F344 rat after acute (6 hours/day for 3 or 7 days) or prolonged (6 hours/day, 5 days/week for 20 months) exposures to 0.5 to 1.0 ppm ozone (Harkema et al 1989, 1992; Harkema and Hotchkiss 1994). Furthermore, the nasal mucous cell metaplasia induced after the prolonged exposure regimen was also associated with significant reductions in the flow rates of mucus in the rat nose 1 week after exposure (Harkema et al 1992, 1994).

Gearhart and Schlesinger (1989) reported that rabbits repeatedly exposed to acidic aerosols (250 µg/m^3 sulfuric acid, 1 hour/day, 5 days/week for up to 52 weeks) have marked increases in mucous secretory cells, especially in small pulmonary airways, the likely consequence of which would be an increase in production and hypersecretion of airway mucus. More recently it has been reported that rats exposed to residual oil fly ash (ROFA) particles (a combustion-generated urban particulate) had conspicuous increases in mucous cells in pulmonary airways along with airway inflammation and epithelial hyperplasia (Dreher et al 1997). However, the consequences of ambient $PM_{2.5}$ exposure on airway secretory cells and the mucous apparatus in the lung have not been investigated. Data are needed from animal studies to fill this gap.

OBJECTIVES AND SPECIFIC AIMS

The overall goal of the present project was to understand the adverse effects of $PM_{2.5}$ on normal and hypersecretory airways. Our investigation was specifically designed to (1) examine the chemical and physical characteristics of $PM_{2.5}$ and other airborne pollutants in the outdoor air of a local Detroit community with a high incidence

of childhood asthma; (2) determine the effects of this community-based $PM_{2.5}$ on the airway epithelium in normal rats and rats compromised with preexisting hypersecretory airway diseases (ie, animal models of allergic airway disease [asthma] and chronic bronchitis); and (3) identify the chemical and physical components of $PM_{2.5}$ that are responsible for $PM_{2.5}$-induced airway inflammation and epithelial alterations in these animal models.

We used two rodent models of airway epithelial injury and subsequent mucous cell metaplasia with mucous hypersecretion to examine the effects of $PM_{2.5}$ exposure on preexisting hypersecretory airways (ie, OVA-induced mucous cell metaplasia in BN rats and endotoxin-induced mucous cell metaplasia in F344 rats). The endotoxin-induced mucous cell metaplasia is associated with neutrophilic inflammation (bronchitis). BN rats that were systemically sensitized to OVA and then challenged with an aerosol of OVA also rapidly developed a mucous cell metaplasia in the pulmonary airways, but had an associated influx of eosinophilic rather than neutrophilic inflammatory cells. Others have used OVA-challenged BN rats as a rodent model of allergic (or asthmatic) airway disease in humans (Haczku et al 1996; Schneider et al 1997).

A mobile air monitoring and exposure laboratory equipped with two inhalation-exposure chambers for animal toxicology studies and state-of-the-art air pollution monitoring and $PM_{2.5}$-collection devices was used in our project. The mobile laboratory was parked in a community in southwestern Detroit during the summer months when particulate air pollution is high. We continuously monitored the outdoor air pollution in this community, and also exposed normal or compromised rats to CAPs from this local urban atmosphere. Rats were exposed for 1 day or 4 to 5 days (10 hours/day) to either filtered air (controls) or CAPs generated by a Harvard ambient fine particle concentrator. Rats were killed 24 hours after the end of the exposure and lung and nasal tissues were removed for analysis.

We used biochemical, morphometric, and molecular techniques to identify airway inflammation and alterations in epithelial cell populations, mucin gene expression, intraepithelial production of mucosubstances, and epithelial cell proliferation in rat pulmonary airways after exposure to CAPs.

In a subsequent intratracheal instillation component conducted in our laboratory at Michigan State University, we investigated the role of soluble and insoluble products of CAPs in the pathogenesis of the observed exposure-induced inflammatory and epithelial lesions. In these intratracheal instillation studies, ambient $PM_{2.5}$ samples were collected, fractionated into soluble and insoluble species, and then compared with each other and with total $PM_{2.5}$ for their effects in a selected rodent model of compromised airways. Selection of the rodent model for intratracheal instillation studies was based on the animal model that showed the most significant findings from our CAPs inhalation exposure component (ie, OVA-induced allergic airway disease in BN rats).

In summary, our specific aims were

Aim 1: To determine the chemical and physical characteristics of ambient urban air particulates in a local community of Detroit that has a population with a high incidence of childhood asthma and high levels of outdoor air particulate pollution during the summer months.

Aim 2: To compare the physical and chemical features of CAPs (used for animal inhalation exposures) with those of the ambient urban air particulates from which the CAPs were derived.

Aim 3: To test the hypothesis that exposure to CAPs from a southwest Detroit community would induce airway inflammation and associated airway epithelial hyperplasia and mucous cell metaplasia with overproduction and hypersecretion of airway mucus in normal healthy rats.

Aim 4: To test the hypothesis that exposure to CAPs would exacerbate airway epithelial hyperplasia, mucous cell metaplasia, and overproduction and hypersecretion of mucus in rats compromised with preexisting hypersecretory airways.

Aim 5: To test the hypothesis that the CAPs-induced airway inflammation and associated airway epithelial alterations (eg, hyperplasia, metaplasia) are dependent on soluble (leachable) components from the inhaled particles (eg, metals).

MATERIALS, METHODS, AND STUDY DESIGN

THE SOUTHWESTERN DETROIT COMMUNITY: SITE FOR INHALATION EXPOSURES

Between 1982 and 1992, the prevalence rate of pediatric asthma (under age 18) in the United States increased by 58% (American Lung Association 2003). The mortality rate from asthma for persons 19 years and under increased by 78% from 1980 to 1993. The national trends in the increase of asthma were also seen in Detroit. In a 1993–1994 study with two Detroit inner-city elementary schools, it was found that 17.4% of the 230 children in the sample had a physician diagnosis of asthma (Joseph et al 1996). Statistics from the Detroit area available from the Henry Ford Health System indicate a steady increase in pediatric

hospital admissions for asthma among African-American children: In 1986, 11.6% of pediatric hospital admissions were for asthma; this escalated to 17.5% in 1989. In addition, data from the Michigan Department of Community Health show that hospital discharges with childhood asthma as a diagnosis have a 5-year rate of 71.2 per 100,000 hospital discharges. Likewise, hospital discharge rates for asthma by zip code were 64.9 to 79.7 for the southwest Detroit communities. Communities on the southwest side of Detroit reflect a mix of moderately high to very high infant childhood asthma hospitalization rates and consistently high proportions of households living below the poverty level, a "cofactor" for asthma in urban populations.

We selected an elementary school, Maybury Multicultural School (4410 Porter Street), in one of the southwest Detroit communities (Mexican Town) (Figure 1) as the site for conducting our outdoor air monitoring and inhalation toxicology studies. The majority of the children living in this community and attending Maybury are Hispanic. The occurrence of childhood asthma at Maybury has been as

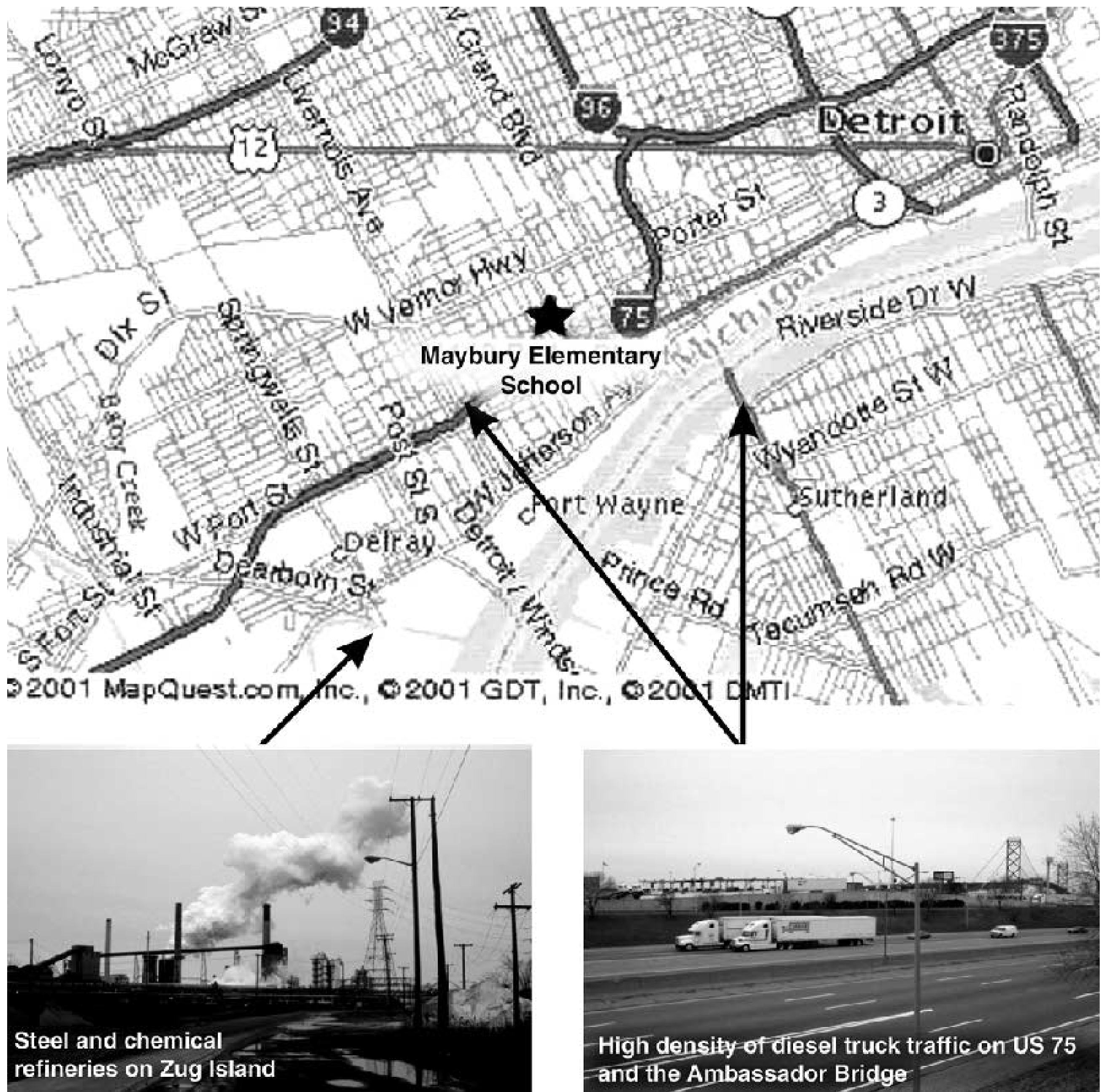


Figure 1. Maybury Multicultural School in southwest Detroit (site of AirCARE 1 during the inhalation toxicology studies).

high as 20% of the student population in recent years. Within 2.5 km from Maybury School are (1) the Ambassador Bridge, the major border crossing for diesel trucks between Windsor, Ontario, Canada and Detroit MI, USA; (2) steel and chemical refineries on Zug Island; (3) a municipal water treatment plant; (4) a municipal power plant; and (5) an interchange for major interstate highways (US 96 and 75). All of these sites are potential sources of particulate and gaseous outdoor air pollutants in this southwest Detroit community.

In 1995, an area of Wayne County including southern portions of Detroit, River Rouge, Ecorse, and Wyandotte were designated as nonattainment areas under the US National Ambient Air Quality Standards (Michigan Department of Environmental Quality 1995). On July 18, 1997, the EPA promulgated a new standard for particulate matter with an aerodynamic diameter of 2.5 µm or smaller. The final form of the standard provided an annual as well as a 24-hour average. The annual average may not exceed 15 $\mu g/m^3$, and the 98th percentile of the 24-hour standard must be less than or equal to 65 $\mu g/m^3$. After several years of being in nonattainment, the area surrounding this monitoring site was redesignated in October 1996 as being in attainment for PM_{10} (Michigan Department of Environmental Quality 1996). Measurements of the levels of total suspended particles and fine fraction mass were performed at a site on Livernois Avenue during a University of Michigan Air Quality Laboratory (UMAQL) Study in 1996. The $PM_{2.5}$ concentrations measured at the southwest Detroit location ranged from 4 $\mu g/m^3$ to higher than 60 $\mu g/m^3$ (Keeler, personal observation, July 1996). Furthermore, PM_{10} daily maximum concentrations exceeding 100 $\mu g/m^3$ have been observed in Detroit (annual average concentration of 39 $\mu g/m^3$ at the Dearborn site in Wayne County). Altogether, these data suggest that day-to-day fluctuations in particulate mass are considerable in the communities to be studied (Michigan Department of Environmental Quality 1996).

MOBILE AIR RESEARCH LABORATORY (AirCARE 1)

The mobile air research laboratory, AirCARE 1, used in this investigation is a unique, specially designed mobile laboratory constructed inside the confines of a 16.2-m semitrailer (Figure 2). The trailer contains on-board electric power, water, and heating, ventilation, and air conditioning (HVAC) systems. The trailer is electrically wired using a 400-A main breaker panel-board, powered by a 150-kVA, 3P-480-V delta primary 208Y/120-V secondary 60-Hz transformer located on the outside of the large trailer. The panel-board consists of one 208Y VAC 125A/3P circuit, one 208Y VAC 60A/3P circuit, and thirty-three 120VAC 20A/1P circuits. Fresh water can be stored in two 380-L storage tanks or connected directly to a pressurized line. The HVAC unit is located in the rear of the trailer in a room that is completely isolated from the rest of the laboratory space. On top of the trailer, an aluminum mezzanine platform with safety guardrails provides ample space for air sampling and monitoring equipment. A 10-m telescoping meteorological tower, attached to the side of AirCARE 1 is used to mount temperature, humidity, and wind direction and speed instruments. A hydraulic-lift gate mounted directly under the entrance door to the atmospheric monitoring laboratory provides a means to lift heavy equipment into the trailer.

AirCARE 1 contains three distinct rooms that provides over 37 m^2 of laboratory space. These three rooms are (1) a biomedical laboratory for animal necropsies or surgical procedures; (2) an inhalation exposure laboratory; and (3) an atmospheric monitoring laboratory (Figure 3). Each laboratory has its own separate HVAC control, which allows for

Figure 2. The mobile air research laboratory (AirCARE 1). Note the ambient air monitoring equipment mounted on top of the trailer.

Figure 3. Layout and design of laboratories within AirCARE 1.

careful monitoring of temperature within each room. Numerous electrical outlets on separate circuits in each room provides ample electrical power for computers and air sampling equipment.

The biomedical laboratory contains steel cabinets, stainless-steel counter tops, refrigerator, freezer, sink with hot and cold water, Millipore water purification system, and a self-contained fume hood (Paramount model 72 Filtered Enclosure). A 750-L holding tank for brown water is located in a storage bin fastened beneath the undercarriage of the trailer. In this room, laboratory rodents were anesthetized, killed, and necropsied, or underwent minor surgical procedures (eg, implantation of subcutaneous osmotic pumps).

The inhalation exposure laboratory contains a Harvard ambient fine particle concentrator, two reinforced–stainless-steel Hinners-type whole-body inhalation chambers (CAPs and filtered air), and a Hazelton 1000 chamber. The concentrator is a three-stage aerosol concentrator that utilizes the technology of virtual impactors to increase the concentration of particles (size range 0.1–2.5 µm) by a factor of 30 (Sioutas et al 1997). A $PM_{2.5}$ inlet is mounted on top of the trailer roof approximately 1.5 m above the aluminum mezzanine platform. A series of stainless-steel ducts 0.31 m in diameter connect the inlet to the concentrator.

The two inhalation chambers are located directly in-line with the outlet of the concentrator. These chambers have a volume of 0.32 m^3 and hold a single level of 16 rats. One chamber was used to expose the rats to CAPs and the other served as a control chamber providing only filtered air. The CAPs chamber inlet was connected directly in-line to the outlet of the concentrator and also to a high-efficiency particulate air (HEPA) filter. Airflow into the chamber could be switched between the concentrator or the HEPA filter by turning a gate valve. The filtered-air chamber was connected to an in-line HEPA filter and was maintained at the same pressure as the CAPs chamber. The Hazelton 1000 chamber is located in the corner of the exposure laboratory, but can be moved when loading animals or when it needed to be cleaned. A HEPA filter connected to the inlet of the Hazelton chamber provided filtered air to the rats during nonexposure times.

Air from each chamber was exhausted to the outside through a port located under each chamber. The airflow in each of the chambers could be adjusted using flow-control needle valves connected to the exhaust ports. Two plastic carboys located above the Hazelton chamber provided fresh water to the animals during the nonexposure periods.

The atmospheric monitoring laboratory is designed to monitor both outside ambient air conditions and particulate concentration levels within the exposure chambers in

the inhalation exposure laboratory. The atmospheric monitoring laboratory contains three portable instrumentation racks, a HEPA laminar-flow workstation, and ample countertop space and storage cabinets. Directly above the instrumentation racks are roof portholes for running sampling tubes and instrumentation cables. Sampling tubes can also be run through portholes located in the wall between the inhalation exposure laboratory and the atmospheric monitoring laboratory. The HEPA laminar-flow workstation provides a clean, positive airflow for installing and changing filter samples.

A small utility trailer is used to provide electrical power and 72 sq ft of isolated space for a 15-hp Roots Blower, a 3/4-hp and two 1/3-hp Gast rotary-vane vacuum pumps. These four vacuum pumps are used to run the ambient fine particle concentrator and provided airflow through the Hinners and Hazelton chambers housing the laboratory rats. The inlets to the vacuum pumps are plumbed to camlock F-couplings located on the side of an exterior wall. Lengths of flexible vacuum tube are used to connect the couplings on the auxiliary trailer to the exhaust lines on the particle concentrator and animal chambers. The trailer is power-ventilated to control the ambient heat generated from the four vacuum pumps. All of the vacuum pumps and roof ventilators are controlled remotely with wall-mounted switches located inside AirCARE 1's inhalation exposure laboratory. Sound-absorbing foam installed on the interior walls and ceiling help reduce noise generated by the vacuum pumps.

On July 9, 2000 the AirCARE 1 was moved to the designated urban site located at Maybury Multicultural Elementary School in southwestern Detroit. During the first week, air sampling equipment was installed and the Harvard ambient fine particle concentrator was run to compare the composition and concentration of particles and gases sampled within the chambers to those collected in the ambient air.

URBAN AMBIENT AEROSOL AND GASEOUS POLLUTANT MEASUREMENTS

Tapered Element Oscillating Microbalance

Levels of $PM_{2.5}$ were monitored continuously with a Rupprecht and Patashnick (Albany NY) Series 1400a tapered element oscillating microbalance (TEOM) equipped with a sharp-cut cyclone inlet to size-select the $PM_{2.5}$ particles (BGI, Waltham MA) in near real-time (30-minute intervals) (Table 1). The TEOM measures the mass collected on an exchangeable filter cartridge by monitoring the corresponding frequency changes of a tapered element that is oscillating. As more mass collects on the exchangeable filter, the tube's natural frequency of oscillation decreases. A direct relationship exists between the tube's change in frequency and mass on the filter. The TEOM filter was heated to 40°C to minimize interference from particle-bound water and semivolatile components. Rupprecht and Patashnick reported that estimated precision was ± 1.5 μg/m³ for 1-hour averages.

MOI and the $PM_{2.5}$ and PM_1 Cyclone Samplers

A six-stage MOI (MSP Corporation, Minneapolis MN) was used to collect size-fractionated samples. Particles were collected on 37-mm Teflon (polytetrafluoroethylene) filters in each stage of the impactor at a flow rate of 30 L/min. The stages included: first, > 5 μm; second, 5 to 2.5 μm; third, 2.5 to 1 μm; fourth, 1 to 0.6 μm; fifth, 0.6 to 0.18 μm; and sixth, < 0.18 μm. During the investigation, ultrafine particles (typically defined as < 0.1 μm) were estimated from this < 0.18–μm stage of the MOI and reported as $PM_{0.18}$.

In addition, $PM_{2.5}$ and PM_1 were each sampled onto cyclone samplers equipped with 47-mm Teflon membrane filters (Gelman Sciences, Ann Arbor MI). Another $PM_{2.5}$ sample was collected onto a quartz filter to analyze elemental carbon (EC) and organic carbon (OC). Vacuum pumps were used to draw air through Teflon-coated cyclone inlets (URG Corp, Chapel Hill NC) at a flow rate of 16.7 L/min. Analyses of these filter samples are shown in Table 1.

Samples from the MOI and the $PM_{2.5}$ and PM_1 cyclone samplers were collected for 10 hours on each exposure day from 0800 to 1800 EDT and for 14 hours overnight from 1800 to 0800 EDT to determine the chemical and physical characterization of the urban ambient particulate matter. The volume of air drawn through each particulate sampling train was determined using a calibrated dry test meter (Schlumberger, Owenton KY). The dry test meters were calibrated against a spirometer (a primary calibration standard) before and after being deployed in the field. In addition, a calibrated rotameter (Matheson, Montgomeryville PA) was used to check the flow rate at the beginning and end of the both the 10-hour and 14-hour sampling periods.

Filter-based measurements of $PM_{2.5}$ from the cyclone samplers were compared to the $PM_{2.5}$ measured by both the MOI and TEOM. Regression of the MOI data against TEOM data yielded a slope of 1.14 with r^2 of 0.93 (n = 22); regression of the $PM_{2.5}$ cyclone data against TEOM data yielded a slope of 1.07 with r^2 of 0.91 (n = 22).

Aerodynamic Particle Sizer

An aerodynamic particle sizer (APS; TSI, model 3320, St Paul MN) was operated to monitor detailed information on the size-fractionated aerosol counts. This instrument provided real-time (5-minute intervals) aerodynamic measurements in the range from 0.5 μm to 20 μm.

Table 1. Particle Measurements and Analyses Performed for Southwest Detroit Rat Exposure Studies

Instrument or Sampler	PM Property	Type of Filter	Sample Duration (hours)	Number of Samples Collected	Analytic Method
Ambient Particles					
TEOM	Mass for $PM_{2.5}$	—	Continuous	30-min intervals	—
APS[a]	Size (0.5–20 µm)	—	Continuous	5-min intervals	—
SMPS[a]	Size (0.02–0.8 µm)	—	Continuous	5-min intervals	—
MOI	Size-segregated mass (6 stages) Trace elements	Teflon	10 and14	48 sets of 6	Gravimetry and ICPMS
$PM_{2.5}$ cyclone	Size-selective mass Trace elements	Teflon	10 and 14	48	Gravimetry and ICPMS
$PM_{2.5}$ cyclone	EC and OC	Quartz	10 and 14	48	Thermal-optical analyzer
$PM_{1.0}$ cyclone	Size-selective mass Trace elements	Teflon	10 and 14	48	Gravimetry and ICPMS
Annular denuder system	Acid gases, acid aerosols, major ions	Denuders, Teflon, glass backup filter	10 and 14	48	Ion chromatography and pH
Dichotomous sequential sampler	Soluble elements	Teflon	24	24	ICPMS
High-volume sampler[b]	$PM_{2.5}$	Teflon	24	24	ICPMS
CAPs					
TEOM (after stage 3[c])	Mass for $PM_{2.5}$	—	Continuous	—	—
MOI (after stage 2)	Size-segregated mass (6 stages) Trace elements	Teflon	10	22 sets of 6	Gravimetry and ICPMS
Filter pack (after stage 2)	Trace elements	Teflon	10	22	Gravimetry
Filter pack (after stage 3)	Mass Trace elements	Teflon	10	22	Gravimetry and ICPMS
Filter pack (chamber)	Major ions	Teflon, glass backup filter	10	22	Ion chromatography
Filter pack (chamber)	EC and OC	Quartz	10	22	Thermal-optical analyzer

[a] The APS and SMPS alternated sampling between ambient particles and chamber CAPs to compare particle characteristics as concurrently as possible.

[b] These particles were used for the instillation component.

[c] Stage numbers refer to stages of the concentrator.

Scanning Mobility Particle Sizer

The scanning mobility particle sizer (SMPS; TSI, 3936) system was operated to measure 5-minute average concentrations of submicrometer aerosols in the range from 20 nm to 800 nm in diameter. It consisted of an electrostatic classifier to determine particle size and a condensation particle counter to determine particle concentrations. Operation of the APS and SMPS together allowed measurements of the size distribution from about 0.01 µm to 20 µm in aerodynamic diameter.

Annular Denuder and Filter Pack Sampling System

Annular denuders and URG filter pack were employed to collect the acidic gaseous species (including nitrous acid [HONO], nitric acid [HNO_3], SO_2, and ammonia [NH_3]), and inorganic fine particulate ions (sulfate [SO_4^{2-}], nitrate

[NO_3^-], ammonium [NH_4^+], and aerosol strong acidity [H^+]). A detailed description of the annular denuder sampling system has previously been reported (Koutrakis et al 1988; Keeler et al 1991).

Dichotomous Sequential Sampler

Particles were also collected daily during each series of exposures on Teflon filters using a dichotomous sequential air sampler (Partisol-Plus Model 2025; Rupprecht and Patashnick, Inc). The dichotomous configuration permits the differentiated mass determination and chemical composition analysis of the fine (< 2.5 µm) and coarse (< 2.5–10 µm) particles contained in PM_{10}. During this project, only the fine particle samples were used for subsequent characterization of soluble elements.

Ozone

Ambient ozone was measured using a continuous UV photometric analyzer (TECO Teco 49; Teco Diagnostics, Anaheim CA), which was calibrated against an ozone calibrator (Teco 5009). Samples were collected at a flow rate of 2.0 L/min. Voltage from the analyzer that corresponded to the concentration was transmitted to a data logger. The average ozone concentration was recorded every 30 minutes. The instrument specifications listed the limit of detection (LOD) as 1 ppb and the precision as ± 1 ppb.

Carbon Monoxide

Continuous ambient carbon monoxide (CO) measurements were made using a nondispersive infrared analyzer (Teco 48S). The instrument specifications listed the LOD as 0.04 ppm and the precision as ± 0.1 ppm.

Oxides of Nitrogen

Ambient concentrations of the oxides of nitrogen (NO_x; ie, nitric oxide [NO] plus nitrogen dioxide [NO_2]) were measured using a commercial chemiluminescence detector (Teco 42S). The instrument has a single photomultiplier tube that automatically cycles between the NO and NO_x modes. Signals from the photomultiplier tube were conditioned and then sent to the microprocessor where a mathematical algorithm was utilized to calculate three independent outputs—NO, NO_2, and NO_x. The instrument specifications listed the LOD as 0.4 ppb and the precision as ± 0.4 ppb.

SO_2

Ambient SO_2 concentrations were measured using a pulsed fluorescence technique (Teco 43S). The instrument specifications listed the LOD as 0.6 ppm and the precision as ± 1 ppb.

CAPs MEASUREMENTS

CAPs were only collected only during animal inhalation exposure periods (0800–1800 hours). As previously described by Sioutas and colleagues (1997), the Harvard ambient fine particle concentrator consists of a series of three virtual impactors to concentrate ambient fine particles. The output flow from the third stage of the concentrator was ~50 L/min, of which 15 L/min was diverted to the various samplers used for characterization measurements (Table 1) and the remaining 35 L/min of flow was administered to the animal exposure chambers.

The mass of CAPs was determined by the TEOM and a 47-mm Teflon filter sample in a Teflon filter pack that was placed after stage 3 of the concentrator before the airflow entered the exposure chamber. The TEOM sampled CAPs at a flow rate of 3 L/min and the TEOM sample train including the filter was also heated to 40°C to match the ambient TEOM measurements.

The major ions of CAPs were determined by placing a 47-mm Teflon filter (Gelman Science) in a Teflon/Teflon-coated URG filter pack attached to the back of the animal exposure chamber at a flow rate of 2 L/min. A sodium carbonate–coated backup filter was placed behind the Teflon filter to correct for volatilization losses of NO_3^- from the Teflon filter during sampling.

A prebaked quartz filter (Gelman Science) for determination of EC and OC was placed in a URG filter pack mounted on the exposure chamber and sampled at a flow rate of 2 L/min. Because the MOI requires a sampling flow rate of 30 L/min to obtain size-fractionated chemical characteristics of CAPs, a six-stage MOI was placed after the concentrator's second stage (where a flow rate of about 200 L/min was available) instead of after the third stage (about 50 L/min) to provide enough CAPs to the exposure chamber. In addition, a Teflon filter sample in a Teflon filter pack was placed after stage 2 to compare the mass with the MOI.

As indicated in the section Methods / Urban Ambient Aerosol and Gaseous Pollutant Measurements, the APS and SMPS were utilized mainly for ambient particulate monitoring. However, experiments were performed using these continuous monitoring devices to compare size distributions of ambient particles and CAPs to examine the performance of the concentrator. Sample lines to the APS and SMPS were connected to sampling ports at the top of the CAPs exposure chamber to measure size distributions of CAPs for at least 15 to 30 minutes, from which we recorded multiple 5-minute average readings. The sampling lines were alternately switched between the ambient sampling lines and the CAPs chamber lines to compare particle characteristics as concurrently as possible.

Similar experiments were performed to compare the concentrations of ozone, CO, and NO_x. The only difference was ozone, for which we had two instruments that monitored both the ambient and chamber levels simultaneously.

ANALYTICAL METHODS FOR AMBIENT PARTICLES AND CAPs CHARACTERIZATION

All filters collected for characterizing ambient particles and CAPs were prepared and analyzed at the UMAQL. Sample handling, processing, and analysis took place in a Class 100 ultraclean laboratory designed for ultra–trace-element analysis with an emphasis on low-level environmental determinations. A brief overview of the analytical methods used at the UMAQL is provided below.

Gravimetry

All gravimetric determinations of Teflon filters were made using a microbalance (MT-5 Mettler Toledo, Columbus OH) in a temperature- and humidity-controlled environment (filter equilibrium within ± 5% for relative humidity between 30% and 40% and within ± 2°C for temperature between 20°C and 23°C) as described in Federal Reference Method (EPA Code of Federal Regulations 1997). Measurements of field blanks, filter-lot blanks, replicate analyses, and externally certified standard weights were incorporated into all gravimetric analyses for quality control purposes. Of the filters weighed before and after the sampling procedures, 10% were reweighed for the replicate analysis and all the values were within ± 15 µg. For 10 hours of sampling, the LOD for a flow rate of 16.7 L/min ($PM_{2.5}$ and PM_1 cyclone samplers) was 0.51 µg/m^3 and for a flow rate of 30 L/min (MOI) was 0.28 µg/m^3.

Carbon

Quartz filters were prebaked at 550°C for 2 hours before sampling. Particle samples collected on quartz filters were maintained at −40°C after sampling and were analyzed for carbonaceous aerosols by a thermal-optical analyzer (Sunset Labs, Forest Grove OR). The thermal-optical reflectance developed by Huntzicker and coworkers (1982) was used to measure the OC and EC by converting particles to gases under different temperature and oxidation conditions. First, OC was measured by the reflectance of a sample that was heated in helium up to 550°C. Then, oxygen was added to the helium at temperatures higher than 550°C; desorbed gases were oxidized to CO_2 by a catalytic conversion and reduced to methane, which was quantified by a flame ionization detector. EC was determined in a subsequent heating step in the presence of a gas mixture containing oxygen. In order to account for pyrolitic carbon, a laser monitored the sample reflectance. The estimated LODs for OC and EC were 0.38 µg/cm^2 and 0.17 µg/cm^2, respectively. Precision levels of the OC and EC data were 6.1% and 4.2%, respectively, based on replicate analyses.

Acid Aerosols and Ions

Filters for acid aerosol measurements were handled in acid-free environments and Petri dishes cleaned with Milli-Q (MQ) water (Millipore, Bedford MA). Particle-free gloves were worn when handling the samples in the field.

Annular denuder and filter pack samples were analyzed for gaseous species HNO_3, HONO, SO_2, and NH_3, and for fine particle ions SO_4^{2-}, NO_3^-, and NH_4^+ by ion chromatography (model DX-600; DIONEX, Sunnyvale CA). A detailed description of the annular denuder sampling system and the analytical method have been provided previously (Koutrakis et al 1988; Keeler et al 1991). One modification of the sampling system for this investigation was the use of phosphorous acid as denuder coating layers for the determination of both gaseous NH_3 and particulate NH_4^+. This choice of coatings was based upon the optimization by Perrino and Gherardi (1999) of the material for coating annular denuders for ambient sampling.

After sampling, filter packs were disassembled in an NH_3-free hood and Teflon filters were placed in a 6-mL solution of 10^{-4} N perchloric acid. The sodium carbonate–coated backup filters were placed in 5 mL of ultrapure MQ water (Millipore, Bedford MA). Denuders were extracted with 10 mL of ultrapure MQ water. Extracts were analyzed for anions and cations by ion chromatography. The Teflon filter samples were analyzed for the strong acidity (H^+) of ambient fine particle mass using a microelectrode (model 420A; ORION, Beverly MA) (Keeler et al 1991).

This analysis method incorporates routine daily quality control measures such as field blanks, MQ water blanks, replicate analyses, and external standards (Simulated Rainwater #2, ICA and ICB) (High-Purity Standards, Charleston SC). Accuracy for SO_4^{2-} and NO_3^- were determined to be 1.2% and 6.2%, respectively. Precision levels of the anion analysis was 2.6% for SO_4^{2-} and 9.0% for NO_3^-, which were calculated based on replicate analyses. The LODs for SO_4^{2-}, NO_3^-, and NH_4^+ ions for 10-hour samples at 10.0 L/min were estimated to be 0.15 µg/m^3, 0.33 µg/m^3, and 0.05 µg/cm^3, respectively.

Trace Elements

Special attention was given to the collection and analysis of trace constituents in the ambient environment. All equipment and supplies used in trace measurement sampling were rigorously acid-cleaned in a 5-step, 11-day procedure (Landis and Keeler 1997). Sample filters were

placed in 50-mm acid-cleaned Petri dishes, sealed with Teflon tape, and triple-bagged in the field.

After completion of gravimetric analysis, Teflon sample filters (Table 1) were placed in 15-mL centrifuge tubes and wetted with 150 µL of ethanol before extraction in 10 mL of 10% HNO_3 (made on the same day it was used) and dispensed using a repippetor calibrated for 10 mL. The extraction solution was then sonicated for 48 hours in an ultrasonic bath, and then the filters were passively acid-digested for a two-week duration. The extracts were analyzed for an array of elements by ICPMS similar to that previously described (Long and Martin 1992).

The extracts were analyzed for several trace elements (Rb, Sr, Y, Mo, Ag, Cd, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Pb, Na, Mg, Al, P, S, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, K, and As) using ICPMS (ELEMENT2; Thermo Finnigan, San Jose CA). The ELEMENT2 is a double-focusing magnetic sector field ICPMS. Samples are introduced to the instrument by ultrasonic nebulization into the argon plasma ion source where energy transfer processes cause desolvation, atomization, and ionization. The ions are extracted from the plasma and the magnetic field disperses ions according to their mass and energy. After passing through the magnetic field, the ions enter the electrostatic analyzer for energy focusing. The combination of the magnetic and electrostatic fields results in the double focusing.

Calibration curves were created using multi-element standards (SPEX CertiPrep, Metuchen NJ) in 10% HNO_3 solution and the matrix was matched with the samples. This analysis method incorporates daily quality control measures such as field blanks, acid blanks, laboratory blanks, and replicate analyses. The US National Institute of Standards and Technology (NIST, Gaithersburg MD) SRM 1643d was used as a quality control standard to check the calibration of the instrument. If the measured values were not within ± 20% of the expected values, the calibration procedure was repeated.

The ICPMS method's LOD and precision level for each trace element analyzed are summarized in Table 2. The method's LODs were calculated as 3 times the SD of seven consecutive measurements of a spiked blank. The corresponding LODs for air concentrations and the mean concentration for 45 samples taken from the last stage (< 0.18 µm) of the MOI are also listed. The samples from this stage ($PM_{0.18}$) were chosen because this stage usually collects the least amount of mass among the six stages of the MOI. As shown in Table 2, most elements (except some rare-earth elements) were significantly higher than the estimated method LODs. The $PM_{0.18}$ concentration of the select rare-earth elements (Sm, Nd, Pr, and Y) were very close to the method LODs. This was expected because, generally, rare-earth elements exist on larger particles in the atmosphere unless they are near a large point source. Therefore, these elements were examined closely to assure that concentrations of larger-size stages were above the method LODs.

Table 2. Limit of Detection and Precision for Each Trace Element Analyzed by ICPMS

Element	Isotope	Method LOD[a] (ppt)	Method LOD[b] (ng/m^3)	$PM_{0.18}$[c] (ng/m^3)	Precision[d] (%)
Na	23	894.5	0.398	5.004	2.6
Mg	24	194.6	0.086	5.207	3.5
Al	27	187.6	0.083	14.455	6.9
P	31	564.6	0.251	4.119	4.7
S	32	6528.9	2.902	159.161	3.8
K	39	549.4	0.244	6.853	3.1
Ca	44	1038.3	0.461	28.427	4.2
Ti	47	33.7	0.015	0.165	3.7
V	51	9.4	0.004	0.318	2.8
Cr	52	10.1	0.004	0.408	2.9
Mn	55	176.6	0.078	0.644	4.9
Fe	57	1047.4	0.466	9.854	6.6
Co	59	4.9	0.002	0.012	5.9
Ni	60	35.6	0.016	0.653	3.6
Cu	63	82.5	0.037	1.322	4.0
Zn	66	217.3	0.097	3.947	3.9
As	75	13.9	0.006	0.515	9.0
Rb	85	1.2	0.001	0.023	3.7
Sr	88	11.2	0.005	0.099	3.6
Y	89	6.2	0.003	0.003	2.4
Mo	95	6.9	0.003	0.337	4.3
Ag	107	1.0	0.0004	0.009	8.5
Cd	111	6.7	0.003	0.062	4.9
Sb	123	6.2	0.003	0.162	4.4
Cs	133	3.0	0.001	0.003	9.2
Ba	137	34.7	0.015	0.329	6.1
La	139	4.2	0.002	0.007	3.4
Ce	140	5.6	0.002	0.010	3.3
Pr	141	3.9	0.002	0.001	3.6
Nd	146	4.4	0.002	0.003	4.2
Sm	147	3.4	0.002	0.0004	12.0
Pb	208	37.9	0.017	1.088	3.2

[a] Three times the SD of seven consecutive measurements of a spiked blank.

[b] For air concentrations from one 10-hour sampling period from all stages of the MOI.

[c] Mean concentrations of the ultrafine fraction (< $PM_{0.18}$) from the MOI.

[d] Based on replicate analyses.

Water-Soluble Trace Elements

The determination of water-soluble trace elements was conducted using the 24-hour ambient $PM_{2.5}$ samples from the sequential sampler. Teflon filter samples were wetted with 200 µL of ethanol before extraction in 20 mL of MQ water and were sonicated for 2 hours, centrifuged for 20 minutes, and filtered through membrane filters. Then, 2.7 mL of the extract was transferred into an analysis vial and 300 µL of concentrated HNO_3 was added to the vial to make a 10%-HNO_3 sample. The extracts were analyzed using ICPMS as described above.

Retained Trace Elements

Extensive efforts were made to identify retained trace elements in the particulate matter deposited in the lungs of rats exposed in this investigation. A complete protocol for digestion of lung tissues and for analysis of trace elements by ICPMS was developed from biopsy mineral analysis (Braselton et al 1997). For quality control purposes, blank tissues and NIST Bovine Liver SRM 1577b were digested with all the exposure samples.

The right middle lung lobe from each animal was removed for trace element analysis. All the excised lung tissues were placed in acid-cleaned Teflon vials and were stored at −40°C. The tissue samples were transferred from original 15-mL vials to Teflon 4-mL vials (Savillex, Minnetonka MN) with Teflon-coated forceps and were weighed to determine the wet weight of the sample. In addition, 250 mg of NIST Bovine Liver was weighed and treated identically as the samples.

After the wet-weight determinations, samples were dried at 95°C in a muffle oven for 4 hours and the dry weights of lung tissues were recorded. Then, 1 mL (2 mL for NIST Bovine Liver) of concentrated HNO_3 was added to all the samples including blanks and the samples were digested in a 95°C muffle oven for 12 hours. The Teflon vials were weighed again to determine if any loss of extracts occurred during digestion.

Finally, the extracts were transferred to centrifuge tubing. MQ water was added to each tube to bring the sample to the appropriate volume so that the final acid concentration was 10%. Samples were then analyzed directly for trace elements using ICPMS.

ISOLATION OF $PM_{2.5}$ FOR THE INTRATRACHEAL INSTILLATION COMPONENT

On two days during the September 5-day exposure protocol, ambient particulate matter for intratracheal instillation of fine particles (< 2.5 µm) was collected on 8-inch × 10-inch Teflon-coated glass fiber filters (1 µm Zefluor, Gelman Sciences). Ambient air was drawn into an Anderson high-volume sampler with a $PM_{2.5}$-size inlet at a flow rate of 1.13 m^3/min. After sample collection, filters were removed, placed in plastic bags, and stored at −80°C until processed for particle extraction.

For ease of handling, filters were cut into two 8-inch × 5-inch pieces prior to extraction. After the surface of the filter was wetted with 70% ethanol, it was submersed face down in deionized water. Particles were dislodged by ultrasonication using a Tekmar Sonic Disruptor (model TMX40, 40% setting) equipped with a ¾-inch probe immersed directly above the filter. Water containing the extracted particles was collected into 50-mL polypropylene tubes, snap-frozen by immersion into liquid nitrogen, and placed on a lyophilizer for approximately 4 days to collect dry particles. Extracted $PM_{2.5}$ particle samples were kept at −20°C until prepared for intratracheal instillation.

Immediately before instillation in our laboratory at Michigan State University, $PM_{2.5}$ was warmed to room temperature and processed for separation into total, soluble, and insoluble fractions. $PM_{2.5}$ was diluted in saline at a concentration of 500 µg/mL (total $PM_{2.5}$), and vortexed for 2 minutes. Soluble and insoluble $PM_{2.5}$ were prepared as follows. Total $PM_{2.5}$ (500 µg/mL in saline) was centrifuged for 15 minutes at 1200 rpm. The supernatant (soluble $PM_{2.5}$) was collected, and the pellet containing insoluble material was resuspended in saline using the initial starting volume.

LABORATORY ANIMALS

In this project, all research protocols that involved laboratory animals were reviewed and approved by the All University Committee on Animal Use and Care at Michigan State University.

We used F344 and BN rats to examine the role of genetic background (strain) on the inflammatory and airway epithelial cell responses to $PM_{2.5}$. These two strains of rats differ in (1) the normal (baseline) amounts of stored mucosubstances in the surface epithelium that lines their pulmonary airways, and (2) their inflammatory and airway epithelial responses to inhaled antigen (ie, OVA for induction of eosinophilic inflammation) and bacterial endotoxin (for induction of neutrophilic inflammation).

F344 rats have been commonly used in studies examining the pathogenesis of toxicant-induced alterations to the upper and lower respiratory tract (Harkema et al 1989, 1994, 1997; Mauderly et al 1989; Gordon and Harkema 1995). Our laboratory has significant experience using F344 rats and we have characterized the inflammatory and epithelial cell responses of the upper or lower airways to

ozone (Harkema et al 1989, 1997; Hotchkiss et al 1991; Hotchkiss et al 1997), bacterial endotoxin (Harkema and Hotchkiss 1991, 1992, 1993; Steiger et al 1995), and tobacco smoke (Hotchkiss et al 1995; Tesfaigzi et al 1996).

BN rats have been used as an animal model of allergic airway disease (asthma) (Haczku et al 1996; Schneider et al 1997). This strain exhibited a response to allergic sensitization driven by type 2 helper T cells (T_H2) (Renzi et al 1996) with high levels of allergen-specific immunoglobulin E (Murphey et al 1974; Pauwels et al 1979). After aeroallergen challenge of sensitized animals, early- and late-phase bronchoconstrictions occurred (Renzi et al 1993), associated with pulmonary inflammation (eosinophils, T lymphocytes, and lesser numbers of neutrophils) (Elwood et al 1991; Renzi et al 1993; Haczku et al 1995). We have recently determined that BN rats sensitized and challenged with OVA rapidly develop mucous cell metaplasia with increased amounts of stored mucosubstances in the surface epithelium that lines their nasal and pulmonary airways (Wagner et al 2002a,b).

Male F344 and BN rats (Charles River, Portage MI) were used for all the studies described in this report. Male rats were chosen for these experiments to avoid hormonal changes during the estrous cycle that have been shown to influence epithelial cell populations and the mucous apparatus in the airways of rodents and may complicate the interpretation of results (Hayashi et al 1979). A total of 256 rats (128 F344 and 128 BN rats) were used in the inhalation component of this investigation that was conducted in AirCARE 1. Single and repeated inhalation exposures were conducted in July and September, 2000, using the experimental groups outlined in Table 3. For each exposure series in June and September, 64 rats of each strain were randomly assigned to one of eight experimental groups (n = 8 per group). The group assignments were adjusted to result in mean group body weights that were not significantly different from one another.

A total of 64 BN rats were used in the intratracheal instillation component of this investigation that was conducted at Michigan State University. As for the inhalation exposure component, rats were randomly assigned to one of eight experimental groups (n = 8 per group). The group assignments were also adjusted to result in mean group body weights that were not significantly different from one another.

Table 3. Summary of Animal Exposure Groups in the Inhalation Component and the $PM_{2.5}$ Instillation Component

Rat Strain	Airway Status	Number of Animals in Each Exposure Group							
		July 1 Day		July 4 or 5 Days		September 1 Day		September 4 or 5 Days	
Inhalation Component[a]		CAPs	Filtered Air	CAPs	Filtered Air	CAPs	Filtered Air	CAPs	Filtered Air
BN	Saline-sensitized and Saline-challenged	8	8	8	8	8	8	8	8
BN	OVA-sensitized and OVA-challenged	8	8	8	8	8	8	8	8
F344	Saline-pretreated	8	8	8	8	8	8	8	8
F344	Endotoxin-pretreated	8	8	8	8	8	8	8	8
$PM_{2.5}$ Instillation Component		Saline		Total $PM_{2.5}$		Soluble $PM_{2.5}$		Insoluble $PM_{2.5}$	
BN	Saline-sensitized and Saline-challenged	8		8		8		8	
BN	Ova-sensitized and Ova-challenged	8		8		8		8	

[a] For each 10-hour exposure period, four groups (32 animals) were exposed simultaneously: one strain, compromised and control groups, CAPs or filtered air.

Animal Models of Airway Inflammation

Pretreatment with Endotoxin to Induce Neutrophilic Airway Inflammation (A Model of Human Bronchitis)

Forty-eight hours prior to the start of the inhalation exposures, F344 rats (n = 128; 10–12 weeks of age) were deeply anesthetized with 4% halothane in oxygen and intranasally instilled (pretreated) with 100 µL of saline (50 µL per naris) containing 0 (control rats) or 20 µg of bacterial endotoxin (lipopolysaccharide from Pseudomonas aerugenosa, serotype 10; 3000 endotoxin units/µg; Sigma Chemical Co, St Louis MO).

Sensitization and Challenge with OVA to Induce Eosinophilic Inflammation (A Model of Human Asthma) BN rats (n = 128; 10–12 weeks of age) were divided into two groups. The rats in one group were intranasally instilled (sensitized) with 100 µL of saline once a day for 5 consecutive days; 14 days after the last intranasal saline sensitization, they were challenged intranasally with 100 µL of saline once a day for 3 consecutive days. The rats in the other group were sensitized with 100 µL of 0.5% OVA (albumen, chicken egg white, Grade V; Sigma Chemical Co) in saline once a day for 5 consecutive days; 14 days after the last intranasal OVA sensitization, they were challenged intranasally with 100 µL of 1% OVA once a day for 3 consecutive days. As with the F344 rats, inhalation exposures began 48 hours after the last intranasal challenge.

Additional OVA-sensitized and OVA-challenged BN rats were used in the component designed to determine the pulmonary effects of intratracheal instillations of soluble or insoluble fractions of $PM_{2.5}$ that had been collected on filters during 2 days of the 5-day inhalation protocol in September. (Other than the particle collection, this entire component was conducted in our laboratory at Michigan State University.) As in the inhalation component, BN rats (n = 32; 10–12 weeks old) were intranasally instilled (sensitized) with OVA (300 µL of 0.5% OVA in saline once a day for 3 consecutive days). Two weeks after the last intranasal sensitization, rats were intranasally challenged with OVA (1%, 300 µL) for 3 consecutive days to induce allergic airway disease. Forty-eight hours after the last intranasal challenge, rats were instilled intratracheally with either saline (n = 8), 100 µg of total $PM_{2.5}$ (n = 8), soluble $PM_{2.5}$ (n = 8), or insoluble $PM_{2.5}$ (n = 8). A second group of rats (n = 32) that had been both sensitized and challenged with saline (normal airways) were similarly instilled with the same $PM_{2.5}$ preparations.

Implantation of Osmotic Pumps

Approximately 24 hours before the start of the inhalation exposure, each rat was briefly anesthetized with 4% halothane in oxygen and then surgically implanted subcutaneously along the dorsal midline with an Alzet osmotic pump (ALZA, Palo Alto CA) containing bromodeoxyuridine (BrdU; 50 mg/kg body weight; Sigma Chemical). BrdU was osmotically delivered to the rats in vivo at a constant rate of 10 µL/hour during the 1-day, 4-day, or 5-day inhalation exposures. The osmotic pumps were removed at the time of necropsy, approximately 24 hours after the end of the last exposure period.

ANIMAL EXPOSURES BY INHALATION OR BY INTRATRACHEAL INSTILLATION

Inhalation Component

Each day approximately 1 hour before the start of an exposure, the rats were moved from the Hazelton chamber and loaded into individual cages in the CAPs and filtered-air chambers in the AirCARE 1 mobile laboratory. Immediately after the rats were loaded, the doors on the chambers were sealed and filtered room air (maintained at 21°C and 60% ± 10% relative humidity) was supplied at 50 L/min. At 0800, the CAPs exposure was started by turning on the main vacuum pump and closing the valve to the HEPA filter. All additional pumps used for filter and gas sampling were turned on and the exhaust flow adjusted so that the total flow through the CAPs chamber was 50 L/min. The pressure in the filtered-air chamber was then adjusted to the same level as the CAPs chamber.

The rats were exposed to the CAPs or filtered air for 10 hours. During the exposure time, chamber environmental conditions (temperature, humidity, airflow, and chamber pressure) were monitored continuously using a Campbell Scientific Recorder. A 5-minute average was logged on a computer for each exposure chamber. In addition, the major and minor pressures for each stage of the concentrator were manually recorded every 30 minutes during the exposure period.

After the 10-hour exposure, the CAPs chamber was switched back to filtered air and the pressure in both exposure chambers was reduced to atmospheric. The rats were then placed back into the Hazelton chamber where food and water were provided. A programmable timer was used to control the lighting system in the inhalation exposure laboratory. The system was set so that the rats were provided with 12 hours of daylight (0700–1900) and 12 hours of darkness (1900–0700).

Instillation Component

Rats were intratracheally instilled with 200-µL volumes of soluble or insoluble $PM_{2.5}$ (total dose/rat = 100 µg) by a procedure modified from Costa and coworkers (1986).

Briefly, animals were anesthetized with halothane (4% in O_2) and hung by the upper incisors from a clamp affixed to a vertically slanted Plexiglas board. The tongue was gently displaced with a cotton-tipped applicator, and the opening of the trachea was made visible with a small animal laryngoscope fitted with a Michaels blade. A short metal extension was affixed to the laryngoscope blade to better accommodate the dimension of the oral cavity of rodents. A catheter (18 gauge × 2 inches; Angiocath, modified with a blunt-end needle) was inserted into the trachea up to the hub, and a small ear-syringe bulb was used to gently inflate the lungs to confirm catheter placement in the airway. Animals were dosed with the $PM_{2.5}$ mixture via a 1-cc syringe affixed to the catheter. Intratracheal instillation procedures for each animal were accomplished in approximately 30 seconds.

ANIMAL NECROPSIES, AIRWAY LAVAGES, AND TISSUE SELECTION

For the inhalation component, rats were killed in the necropsy area within AirCARE 1 24 hours after the end of the last daily exposure to filtered air or CAPs. For the intratracheal instillation component, rats were killed in the laboratory at Michigan State University 24 hours after instillation. Figure 4 provides an illustrative summary of the pulmonary and nasal tissues used for the various morphometric, biochemical, and molecular analyses.

At the time of killing, rats were deeply anesthetized with sodium pentobarbital and exsanguinated by severing the abdominal aorta or renal arteries. Immediately after death, the thorax was opened, the trachea was canulated, and the heart/lung block was removed. The right extrapulmonary bronchus was ligated with suture and the right lung lobes were removed. The right cranial lobe was placed in 1 mL of TriReagent (Molecular Research Center, Cincinnati OH), homogenized, and immediately frozen and stored at −80°C until processed for isolation of total RNA for subsequent mucin-specific mRNA analysis. The right middle lobe was placed in a Teflon container, snap-frozen in liquid nitrogen, and stored at −80°C until processed for trace element analysis of retained CAPs. The right caudal lobe was removed and the main axial airway was microdissected, homogenized in 1 mL TriReagent, and frozen and stored until processed for isolation of total RNA. The right accessory lung lobe was removed, snap-frozen in liquid nitrogen, and stored at −80°C for potential analyses not identified at this time.

The left lung lobe was lavaged with two volumes (25 µL/g body wt × 0.4) of saline. The recovered BALF from each rat was placed in 15-mL centrifuge tubes and stored on ice until processed for total and differential cell counts, quantitation of secreted mucins, total protein, elastase, β-glucuronidase, and alkaline and acid phosphatase.

After bronchoalveolar lavage, the left lung lobe was perfusion-fixed with a solution of 1% paraformaldehyde and 0.1% glutaraldehyde via the trachea at a constant pressure of 30 cm of fixative. After 2 hours of perfusion-fixation, the trachea was ligated and the tissues were fixed in a large volume of the same fixative. Forty-eight hours later, the left lung lobe was processed for light microscopy and morphometric analysis (specific methods for tissue processing are described in the section Lung and Nasal Tissue Selection for Airway Epithelial Morphometry).

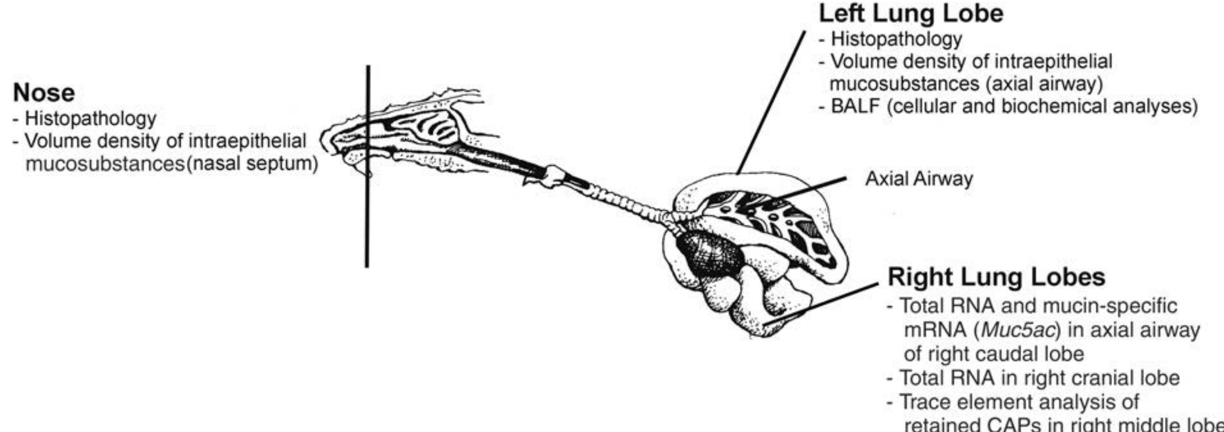


Figure 4. Sites of rat nasal and pulmonary tissues selected for analyses.

The head of each animal was also removed from the carcass. After the lower jaw and skin were removed, the head was fixed in a large volume of the 1% paraformaldehyde:0.1% glutaraldehyde solution until further processing.

ANALYSES OF THE BALF

Recovered Cells

Total cells recovered by bronchoalveolar lavage were determined manually using a hemacytometer. Cell smears were made with a cytocentrifuge and stained with Diff-Quick. Differential cell counts were determined by counting 200 cells per rat. The total number of each cell type recovered by lavage (eg, neutrophils, macrophages, eosinophils, lymphocytes, and epithelial cells) were estimated by multiplying the total recovered cells per animal by the percentage of occurrence of that cell type in the corresponding differential count. Cell smears and hemacytometer counts were prepared in the AirCARE 1 mobile laboratory. Other cytological analyses were performed in our laboratory at Michigan State University.

Secreted Mucins

An estimate of the amount of secreted mucins recovered in the BALF was determined by enzyme-linked immunosorbent assay (ELISA) for mucin glycoprotein 5AC using a mouse monoclonal antibody to the human MUC5AC protein (Mucin 5AC Ab-1; Neomarkers, Fremont CA) that has reactivity to the rat rMuc5ac core protein. Aliquots (50 µL) of BALF were applied to a 96-well microtiter plate (Microfluor 2 Black; Dynex Technologies, Chantilly VA) and dried overnight at 40°C. Plates were blocked with a solution of 1.5% horse serum in automation buffer solution (ABS; pH 7.5; Biomeda Corp, Foster City CA) for 30 minutes at 37°C. Plates were then incubated with anti-rMuc5ac antibody (1:400 in ABS containing 1.5% horse serum) for 1 hour at 37°C and then washed three times with ABS.

Bound primary antibody was detected with a biotinylated rabbit anti–mouse secondary antibody and quantitated using horseradish-peroxidase–conjugated avidin/biotin complex (ABC Reagent; Vector Laboratories, Burlingame CA) and a fluorescent substrate (QuantaBlue; Pierce Chemical, Rockford IL) using a fluorescence microplate reader (SpectraMax Gemini; Molecular Devices Corp, Sunnyvale CA; 318 nm excitation/410 nm emission). Readings were taken at 3-minute intervals for 24 minutes. Duplicate samples were averaged and the group data represented as mean maximal velocity (V_{max}) in units per second.

Total Protein

Protein concentration in BALF was determined by the bicinchonic acid method using a microplate assay (Pierce Kit #23225). Absorbance readings of protein standards and experimental samples were read at 550 nm using a BioTek ELx 808 plate reader.

Elastase

An estimate of the amount of elastase recovered in the BALF was determined by an ELISA using a rabbit monoclonal antibody to human elastase (Calbiochem). Aliquots (50 µL) of BALF were applied to a 96-well microtiter plate (Microfluor 2 Black; Dynex Technologies) and dried overnight at 40°C. Plates were blocked with a solution of 1.5% goat serum in ABS (pH 7.5; Biomeda Corp) for 30 minutes at 37°C. Plates were then incubated with antielastase antibody (1:400 in ABS containing 1.5% goat serum) for 1 hour at 37°C and then washed three times with ABS.

Bound primary antibody was detected with the procedure described for secreted mucins except that a biotinylated goat anti–rabbit secondary antibody was used.

β-Glucuronidase

Airway β-glucuronidase recovered in BALF was determined by an ELISA using a rabbit monoclonal antibody to β-glucuronidase (Molecular Probes, Eugene OR). Aliquots (50 µL) of BALF were applied to a 96-well microtiter plate (Microfluor 2 Black; Dynex Technologies) and dried overnight at 40°C. Plates were blocked with a solution of 1.5% goat serum in ABS (pH 7.5; Biomeda Corp) for 30 minutes at 37°C. Plates were then incubated with anti–β-glucuronidase antibody (1:400 in ABS containing 1.5% goat serum) for 1 hour at 37°C and then washed three times with ABS.

Bound primary antibody was detected with the procedure described for secreted mucins except that a biotinylated goat anti–rabbit secondary antibody was used.

Alkaline and Acid Phosphatase

Activities of alkaline and acid phosphatases in the BALF were determined in microplate assays using commercially available kits (# 104-LS and 104-AS from Sigma Chemical Co). *p*-Nitrophenol was liberated from *p*-nitrophenyl phosphate by phosphatase activity at either pH 10.3 (alkaline phosphatase) or pH 4.8 (acid phosphatase) and spectrophotometrically measured at 405 nm. Duplicate sample readings were compared to a standard curve made with *p*-nitrophenol.

Alkaline phosphatase activity in BALF is a marker of type II cell secretory activity or injury (Henderson et al 1995). The presence of the lysosomal enzymes acid phosphatase and β-glucuronidase may indicate phagocytic activity by alveolar macrophages (Henderson et al 1991).

LUNG AND NASAL TISSUE SELECTION FOR AIRWAY EPITHELIAL MORPHOMETRY

A fractionator technique (Cruz-Orive and Weibel 1990; Bolender et al 1993) was used to ensure uniformly random sampling of the left lung lobe from all animals. Twenty-four hours after the start of fixation, the fixed left lung lobe was sectioned perpendicularly to the long axis of the lobe (perpendicularly to the main axial airway), at a section thickness of 2 to 3 mm, to produce 15 to 20 lung sections per animal. The first cut was made at a randomly selected position within the first 3 mm of the apical portion of the lung. Selection of the first lung section for further processing was determined using a random number (1–6). This section and every sixth section after was embedded in Immuno-Bed plastic embedding medium (Polysciences, Warrington PA). The tissue blocks were cut at a thickness of 2 to 3 µm using a glass knife and sections were stained with the following: hematoxylin and eosin for general histology, immunohistochemically with an antibody to BrdU, or Alcian Blue (pH 2.5)/Periodic Acid Schiff Sequence (AB/PAS) for the detection of stored intraepithelial mucosubstances (IM) in the airway.

After fixation, the head from each animal was decalcified in 13% formic acid for 4 days and then rinsed in tap water for 4 hours. Two transverse tissue blocks were removed from the anterior nasal cavity at two locations: (1) immediately posterior to the upper incisors, and (2) at the level of incisive papilla. Nasal tissue blocks were embedded in paraffin, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin for general histology or with AB/PAS for the detection of stored IM.

LIGHT MICROSCOPIC ANALYSIS AND MORPHOMETRY OF THE SURFACE EPITHELIUM LINING THE PULMONARY AXIAL AIRWAY AND THE PROXIMAL NASAL SEPTUM

All nasal and pulmonary morphometric analyses were conducted in our laboratory at Michigan State University. All pulmonary morphometric analyses were performed on the largest-diameter, proximal, axial airway found in the lung sections of each rat; inner airway diameters were approximately 1.8 mm ± 0.2 (mean ± SEM). We morphometrically analyzed the surface epithelium lining this main axial airway from all the animals in our investigation (both the inhalation and instillation components).

Stored IM (Lung and Nose)

Morphometric measurements were made in each animal to estimate the amount of the IM in the respiratory epithelium that lines (1) the mid–nasal septum in the most proximal nasal section and (2) the most proximal section (largest cross-sectional diameter) of the main axial airway in the left lung lobe. The volume density of AB/PAS-stained mucosubstances was quantified using computerized image analysis and standard morphometric techniques. The area of AB/PAS-stained IM was calculated from the automatically circumscribed perimeter of stained material using a Dell computer and the public domain US National Institutes of Health Image program (written by Wayne Rasband, US National Institutes of Health; available at *http://rsb.info.nih.gov/nih-image/*). The length of the basal lamina underlying the surface epithelium was calculated from the contour length of the digitized image of the basal lamina. The volume of stored mucosubstances per unit of surface area of epithelial basal lamina was estimated using the method described in detail by Harkema and associates (1987). The volume density of IM is expressed as nanoliters of IM per square millimeter of basal lamina.

Epithelial Cell Numeric Densities (Lung)

The numeric epithelial cell density was determined by counting the number of epithelial cell nuclear profiles in the surface epithelium and dividing by the length of the underlying basal lamina. The length of the basal lamina was calculated from its contour length in a digitized image using the US National Institutes of Health Image system described above. Data are expressed as the mean (± SEM) number of epithelial cells present in the surface epithelium that lines the axial airway per millimeter of basal lamina (ie, cells/mm).

Epithelial Cell Labeling Index and the Systemic Delivery of BrdU (Lung)

The number of surface epithelial cells in the axial airway of the left lung lobe that were engaged in DNA synthesis was assessed by positive immunoreactivity with BrdU (Sigma Chemical Co). BrdU (50 mg/kg body weight) was delivered to the rats in vivo at a constant rate (10 µL/hr) during the 1-day, 4-day, or 5-day inhalation exposures via Alzet osmotic pumps (ALZA Corp, Palo Alto CA).

The cumulative epithelial cell labeling index (percentage of BrdU-labeled epithelial cells) in the axial airway was determined using lung sections immunohistochemically stained to label cells with BrdU incorporated into nuclear DNA (ie, cells that have undergone DNA synthesis) (Henderson et al 1993; Hotchkiss et al 1997). The cumulative labeling index for each airway was calculated by dividing the number of BrdU-labeled epithelial nuclei by the total number of nuclei (both labeled and unlabeled) and multiplying by 100. The data are expressed as the mean ± SEM cumulative labeling index (n = 8/group).

MOLECULAR ANALYSIS FOR MUCIN-SPECIFIC (*Muc5ac*) MESSENGER RNA

Quantifying *Muc5ac* mRNA Expression

Total RNA from the axial airway of the right caudal lobe from each rat was isolated using Tri-Reagent (Sigma Chemical Co), quantified spectrophotometrically at 260 nm, and adjusted to a concentration of 50 ng/μL. The RNA was amplified using one-step, real-time, multiplex reverse transcriptase–polymerase chain reaction (RT-PCR), which consisted of 4.0 μL (200 ng) of the RNA sample and 21 μL of the final reaction mixture (primers; probes; 2× MasterMix without UNG, 40× MultiScribe, RNase inhibitor mix [all from Applied Biosystems, Branchburg NJ]; and water). Samples were cycled 40 times at the appropriate annealing temperature (59°C) for the rat *Muc5ac* gene. In addition, the expression of 18S ribosomal RNA was employed as an RNA loading control in order to correct for minor variability in total RNA concentrations among samples. Fluorescently labeled primer and probe sequences for the rat *Muc5ac* gene were

Forward Primer: CCCTA CCCCG AGCGT AGTGT A;

Reverse Primer: ATGGT TAAGG TGGAG GCTGA AG;

Probe: TGTTG ACAGC CAGCA GCGTA TCGTC.

After the PCR, amplification plots (change in dye fluorescence vs cycle number) were examined and a dye fluorescence threshold within the exponential phase of the reaction was set separately for the target gene and the endogenous reference (18S ribosomal RNA). The cycle number at which each amplified product crossed the set threshold was identified as the C_T value. The amount of target gene normalized to its endogenous reference was calculated by subtracting the endogenous reference C_T from the target gene C_T (ΔC_T). Relative mRNA expression was calculated by subtracting the mean ΔC_T of the control samples from the ΔC_T of the treated samples ($\Delta\Delta C_T$). The amount of target mRNA, normalized to the endogenous reference and relative to the calibrator (ie, RNA from control), was calculated by using the formula $2^{-\Delta\Delta CT}$.

All RNA samples were also analyzed for the presence of contaminating genomic DNA using the same one-step multiplex RT-PCR but eliminating the MultiScribe (ie, no RT) from the reaction mixture. The ΔC_T produced in the absence of the MultiScribe RT was solely attributable to genomic DNA within the sample. For those samples possessing measurable DNA contamination, the ΔC_T for the target gene was adjusted by subtracting from that value the signal attributable to genomic DNA (ie, ΔC_T value in the absence of MultiScribe RT).

STATISTICAL ANALYSES

Data describing the type and magnitude of the pulmonary inflammatory response (ie, BALF cells) and the cellular markers of epithelial proliferation and mucous cell metaplasia were expressed as the mean group value ± SEM (n = 8/group). The data from all exposure protocols were subjected to two-way analysis of variance (ANOVA) for the type of intranasal challenge or pretreatment (saline, OVA, or endotoxin) and the type of inhalation exposure (CAPs or filtered air). Data from particle characterizations were expressed as the mean group value ± SD. Multiple comparisons were made by Student-Newman-Keuls post hoc test. Transformation of data (eg, usually log or $\arcsin^{-1}$) was performed if needed to render variances homogeneous. Group comparisons for inhaled particles were made by the Tukey-Kramer post hoc test. The criterion for statistical significance in all analyses was taken to be $P \leq 0.05$ as evaluated with two-way ANOVA and the Student-Newman-Keuls post hoc test.

RESULTS

CHARACTERIZATION OF URBAN AMBIENT PARTICLES

Mass

The daily average ambient $PM_{2.5}$ concentrations by means of TEOM (30-minute average interval) for each 10-hour exposure period (0800–1800) during July and September ranged from 5 to 36 μg/m^3 and from 7 to 34 μg/m^3, respectively. Table 4 shows the ambient conditions (including relative humidity, temperature, and wind direction) and average daily ambient and maximum $PM_{2.5}$ concentrations.

Short-term pollutant episodes, especially when contributions from local sources are predominant, can be examined by semicontinuous (30-minute average) fine-particle mass data from the TEOM. Figure 5 shows the temporal variations of ambient $PM_{2.5}$ concentrations as measured by the TEOM. For July measurements, the mean ± SD ambient $PM_{2.5}$ mass concentration measured by TEOM (30-minute average) was 18.5 ± 13.6 μg/m^3 and for September measurements was 14.2 ± 11.7 μg/m^3. These values were representative of particulate mass concentrations in southwest Detroit during summer months (Keeler et al 2002) and were below the National Ambient Air Quality Standard for $PM_{2.5}$ of 65 μg/m^3 for 24 hours. However, the Michigan Department of Environmental Quality Air Quality Division reported that the annual 2000 mean

Table 4. Ambient Conditions and $PM_{2.5}$ Measurements During 10-hour Exposure Periods

	$PM_{2.5}$ (µg/m^3) Mass from TEOM				
	Mean[a]	30-Minute Maximum	Relative Humidity[a] (%)	Temperature[a] (°C)	Wind Direction
July					
1 Day (F344)					
7/17	19.6 ± 11.2	36.4	60.6 ± 10.0	27.2 ± 3.2	244
5 Days (F344)					
7/18	7.1 ± 5.2	23.6	54.9 ± 7.2	19.7 ± 2.0	332
7/19	11.4 ± 6.7	27.2	69.8 ± 3.7	19.2 ± 1.2	50
7/20	13.0 ± 11.7	33.1	56.8 ± 23.3	22.6 ± 2.1	214
7/21	5.1 ± 5.4	16.7	56.3 ± 13.1	21.4 ± 1.4	283
7/22	7.1 ± 4.3	13.8	58.1 ± 11.3	20.3 ± 2.4	315
TWA	8.7 ± 6.7		59.2 ± 11.7	20.6 ± 1.8	
1 Day (BN)					
7/24	5.8 ± 4.7	16.5	48.9 ± 12.2	22.7 ± 1.8	40
4 Days (BN)					
7/25	25.7 ± 8.0	43.3	65.6 ± 7.8	24.0 ± 1.9	327
7/26	29.4 ± 8.1	40.9	67.7 ± 10.5	25.8 ± 1.8	148
7/27	34.7 ± 12.0	55.0	69.4 ± 14.3	26.5 ± 2.3	142
7/28	36.0 ± 25.7	61.9	77.6 ± 14.3	25.8 ± 2.5	167
TWA	31.3 ± 13.1		69.9 ± 11.7	25.5 ± 2.1	
September					
1 Day (F344)					
9/18	19.2 ± 11.3	53.2	66.7 ± 15.0	20.4 ± 2.8	167
4 Days (F344)					
9/19	31.7 ± 3.8	40.8	62.7 ± 11.0	22.9 ± 2.9	158
9/20	34.3 ± 2.7	39.7	60.3 ± 9.8	23.8 ± 2.4	172
9/21	6.5 ± 2.2	11.3	63.0 ± 8.0	14.0 ± 1.4	238
9/22	9.1 ± 3.4	18.1	66.0 ± 7.8	15.3 ± 2.1	267
TWA	20.4 ± 3.0		63.0 ± 12.5	19.0 ± 2.2	
1 Day (BN)					
9/25	6.0 ± 3.1	11.6	66.3 ± 7.1	11.4 ± 1.1	9
5 Days (BN)					
9/26	10.9 ± 5.7	24.8	58.9 ± 15.0	14.7 ± 3.1	245
9/27	14.3 ± 4.9	22.9	58.0 ± 13.9	18.4 ± 4.2	221
9/28	8.8 ± 2.4	12.4	60.0 ± 8.6	10.5 ± 1.5	22
9/29	10.6 ± 6.3	24.6	65.8 ± 12.2	13.3 ± 2.9	166
9/30	20.8 ± 2.1	25.1	61.7 ± 7.2	17.3 ± 3.2	163
TWA	13.2 ± 4.2		61.0 ± 13.3	14.8 ± 3.0	

[a] Values are presented as means ± SD.

$PM_{2.5}$ concentration at West Fort site in southwest Detroit was 18.1 µg/m^3. Michigan Department of Environmental Quality (2001) also concluded that, although more monitoring data would be required to determine future designations of nonattainment areas, there was an ample chance that Detroit would not meet the new annual US National Ambient Air Quality Standard for $PM_{2.5}$ of 15 µg/m^3.

Particle Size Distribution

The size distribution of urban ambient particles was monitored throughout the series of exposures using the continuous instruments (SMPS and APS) and the MOI. For each 10-hour exposure period, Table 5 shows size fractions and number concentrations of ambient particles including an average fraction of PM_1/$PM_{2.5}$ mass and $PM_{0.18}$/$PM_{2.5}$

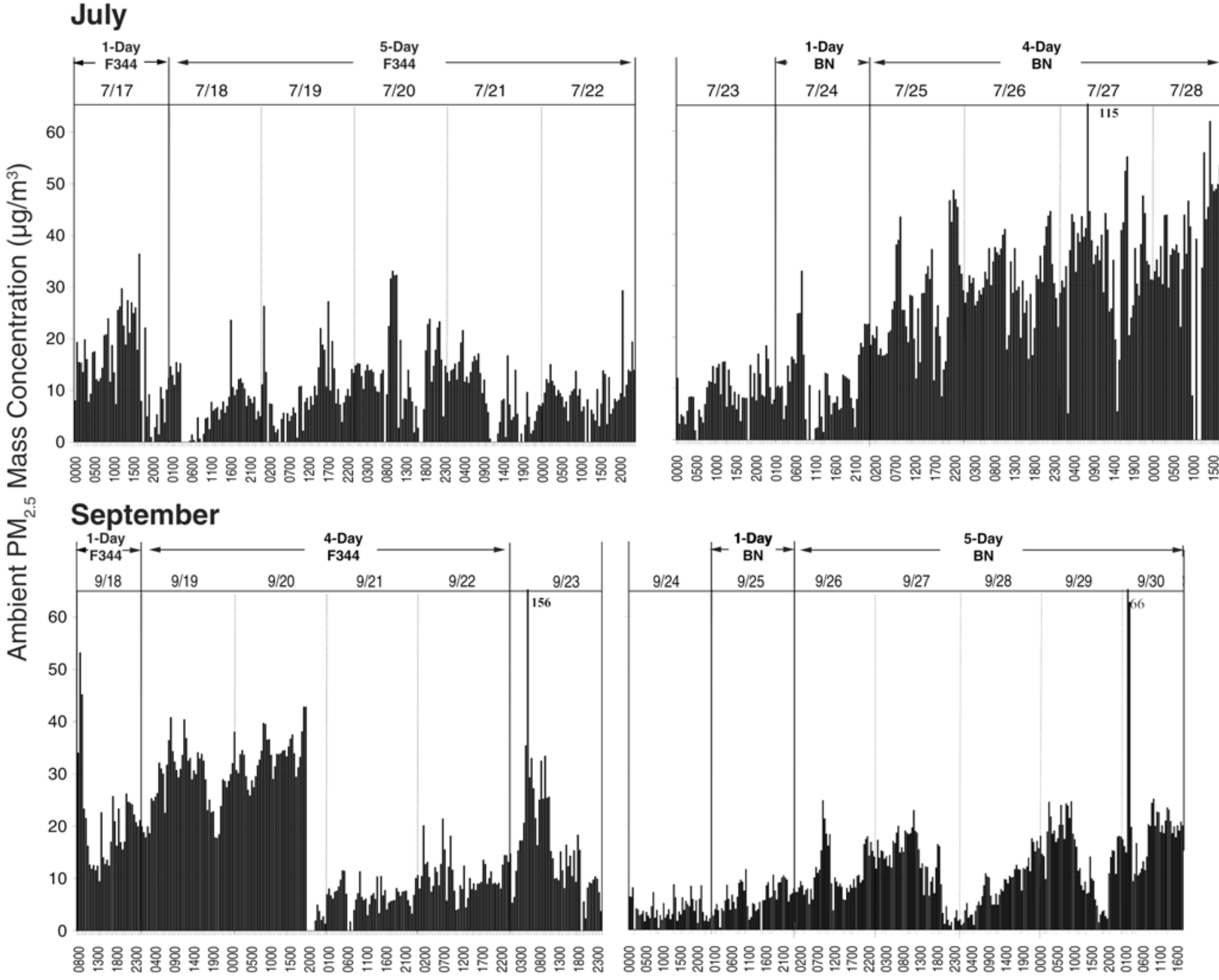


Figure 5. Temporal variations of ambient $PM_{2.5}$ concentrations measured by the TEOM in southwest Detroit during atmospheric measurements in July and September.

mass from the MOI; average number concentration for $PM_{0.1}$, average and maximum number concentrations for $PM_{0.18}$, and an average fraction of $PM_{0.18}/PM_1$ numbers of particles measured by SMPS.

Based on the MOI measurements during the 10-hour exposure periods, we found that, on average, 81.8% ± 9.4% in July and 79.1% ± 5.8% in September of the ambient $PM_{2.5}$ mass was actually from submicron (< PM_1) particles. It is interesting to note that a study by Lippman and associates (2000) reported that, on average, $PM_{2.5}$ in Detroit accounted for 60% of PM_{10} (up to 80% on some days) and that the temporal correlation between $PM_{2.5}$ and PM_{10} mass was $r = 0.90$, which suggests that many of the effects of larger particles may well be driven by smaller $PM_{2.5}$ particles that covary with the larger particles over time. Combined results from the Lippman study and ours imply that the inhalable fraction of particulate matter (PM_{10}) in Detroit is actually driven by much smaller particles (PM_1), and local sources are responsible for the frequent pollution events in this area.

As expected, the contribution of ultrafine ($PM_{0.18}$) particles to $PM_{2.5}$ mass concentration was not significant (15.3% ± 9.0% in July and 12.3% ± 6.1% in September).

During the series of exposures, meteorological and thermodynamic conditions of the atmosphere as well as emissions from local sources influenced the size distribution of particles. On some days, the size distribution of ambient particles displayed a relatively low $PM_{0.18}/PM_1$ number concentration ratio (< 0.7), and was observed when (a) atmospheric conditions were relatively humid, especially

Table 5. Sizes and Number Concentrations of Ambient Particles During 10-Hour Exposure Periods

	MOI			SMPS			
	Ambient $PM_{2.5}$ Mass ($\mu g/m^3$)	Fraction of $PM_1/PM_{2.5}$ Mass (%)	Fraction of $PM_{0.18}/PM_{2.5}$ Mass (%)	Average $PM_{0.1}$ Number Concentration[a] (number × $10^3/cm^3$)	Average $PM_{0.18}$ Number Concentration[a] (number × $10^3/cm^3$)	Maximum $PM_{0.18}$ Number Concentration (number × $10^3/cm^3$)	Fraction of $PM_{0.18}/PM_1$ Numbers of Particles[a] (%)
July							
1 Day (F344)							
7/17	20	75	14	14.158 ± 17.283	16.856 ± 18.998	110.106	92
5 Days (F344)							
7/18	8	63	21	14.578 ± 6.573	15.523 ± 6.991	25.171	99
7/19	7	79	12	7.577 ± 4.499	8.922 ± 5.406	17.539	94
7/20	17	77	15	12.982 ± 5.026	15.390 ± 5.360	44.508	88
7/21	5	91	22	6.362 ± 1.342	7.391 ± 1.452	11.202	94
7/22	7	73	35	9.439 ± 3.047	10.624 ± 2.468	18.340	91
TWA	9	77	21	10.188 ± 4.097	11.570 ± 4.335		93 ± 4
1 Day (BN)							
7/24	7	79	35	16.225 ± 6.720	17.538 ± 6.950	35.024	98
4 Days (BN)							
7/25	21	89	8	7.631 ± 2.610	11.266 ± 2.383	20.136	83
7/26	24	92	6	7.000 ± 4.380	10.868 ± 5.065	34.616	79
7/27	27	91	6	4.498 ± 1.622	7.761 ± 1.933	12.909	58
7/28	34	89	7	3.675 ± 1.549	5.426 ± 1.846	13.194	62
TWA	26	90	8	5.753 ± 2.566	8.918 ± 2.831		71 ± 12
September							
1 Day (F344)							
9/18	21	75	15	12.941 ± 11.756	16.741 ± 13.617	73.954	93
4 Days (F344)							
9/19	27	83	14	18.751 ± 6.925	24.425 ± 7.350	53.300	90
9/20	25	85	15	9.467 ± 9.023	12.096 ± 9.905	46.205	77
9/21	4	71	6	4.192 ± 1.582	4.613 ± 1.631	9.631	95
9/22	7	77	11	10.560 ± 7.389	10.694 ± 4.538	27.645	92
TWA	16	79	11	10.743 ± 6.230	12.964 ± 5.856		89 ± 8
1 Day (BN)							
9/25	4	84	11	6.024 ± 2.870	6.644 ± 3.082	16.993	93
5 Days (BN)							
9/26	6	77	15	10.144 ± 6.594	11.466 ± 7.595	30.773	98
9/27	12	73	17	13.006 ± 4.406	14.911 ± 4.795	29.393	95
9/28	6	85	22	9.040 ± 5.081	9.940 ± 5.491	19.694	97
9/29	10	73	9	10.595 ± 6.267	12.029 ± 6.705	32.811	91
9/30	20	87	10	11.428 ± 3.650	14.107 ± 3.879	24.678	93
TWA	11	79	15	10.879 ± 5.126	12.545 ± 5.460		95 ± 3

[a] Values are presented as means ± SD.

during the July exposure period (relative humidity > 70%); or (b) the level of secondary SO_4^{2-} was elevated; or (c) both conditions were observed. At high relative humidity, hygroscopic fine particles such as SO_4^{2-} increase in size due to accumulation of particle-bound water. During the period of July 25 through 28, the predominant southerly wind was associated with a high-pressure system over Ohio. Increased concentration of secondary or aged aerosol in the accumulation mode (0.1–1 µm) was also associated with the low $PM_{0.18}/PM_1$ ratio. This situation usually arises because the monitoring site is influenced strongly by the regional transport of particles (secondary particles) rather than by particles from local sources. More details on chemical composition are discussed later.

In contrast, sampling periods with a high $PM_{0.18}/PM_1$ number concentration ratio (> 0.8) were frequently observed during the series of exposures. Although the contribution of ultrafine particles to particle mass concentration is relatively small in urban environments, particles smaller than 0.1 µm dominate the number distributions of urban aerosols due to new particle formation by nucleation near sources (Hinds 1999). In fact, it has been reported that ultrafine particles comprised over 80% of particles in terms of number concentration in an urban environment (Morawska et al 1998) although particles of the accumulation mode (0.1–1 µm) usually dominated mass concentration measurements. During our series of exposures, 5-minute average size distributions of submicron particles were also monitored by the SMPS to examine how ultrafine particles and the $PM_{0.18}/PM_1$ number concentration ratio altered throughout a day. Figure 6 displays the time series of the 30-minute average number concentrations of ambient PM_1 and $PM_{0.18}$ during animal exposure sessions in September. This figure shows frequent spikes from local sources including morning and evening rush hours in southwest Detroit, indicating the formation of ultrafine particles emitted from local sources including motor vehicle and other combustion sources. Furthermore, well-correlated time series of $PM_{0.18}$ and PM_1 number concentrations implies that most of ambient PM_1 particles were ultrafine particles during this series of particular exposures in September.

Several studies have investigated size distributions of transportation-related particles and reported that the particle number distribution from vehicle exhaust peaked in the size range 20 to 130 nm for diesel engines (Morawska et al 1998) and 20 to 60 nm for gasoline engines. Kittelson (1998) also has described that the nuclei mode (0.005–0.05 µm range) contains more than 90% of the particle number from diesel exhaust emissions. These studies support the finding that

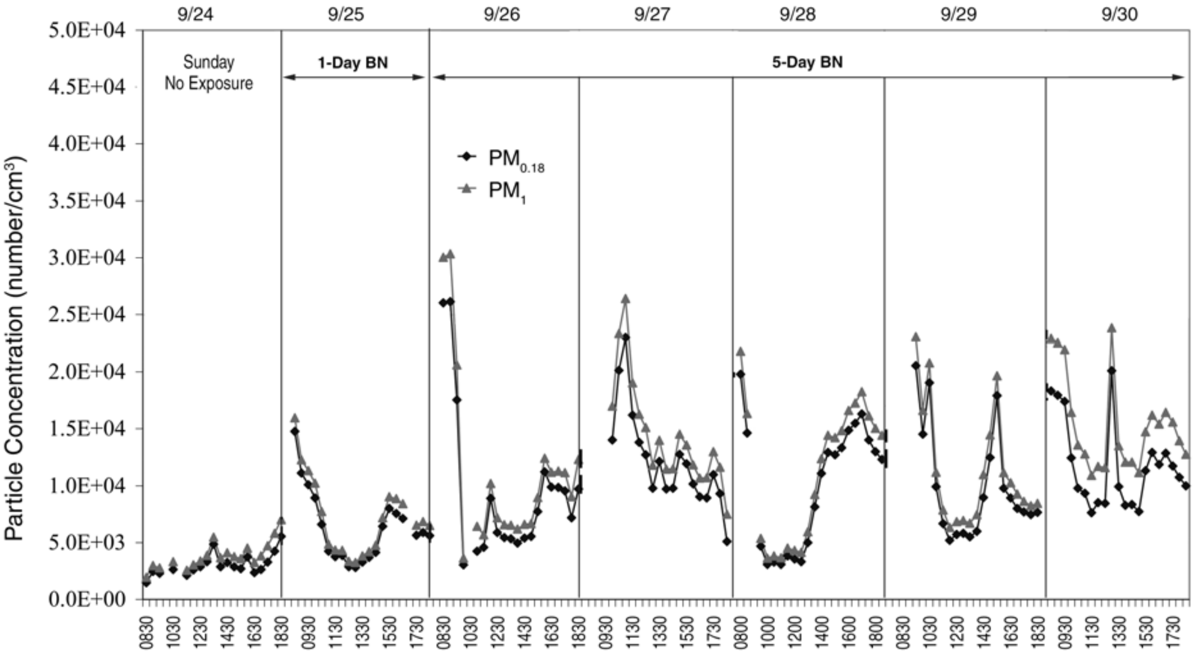


Figure 6. Time series of 30-minute average number concentrations of ambient PM_1 and $PM_{0.18}$ measured by the SMPS during animal exposure periods in September.

most of the particles emitted by motor vehicles are in the ultrafine particle size range.

It is interesting to note that the number concentrations were relatively low on Sunday, September 24th due to the lack of morning and evening rush hours, local source activity, and a northerly wind that brought a relatively clean air mass from Canada (Figure 6).

The continuous monitoring also helped identify frequent ultrafine particle events and short-term pollutant episodes from local sources or photochemical activities. The 30-minute average size distributions of submicron particles as measured by the SMPS focusing on the ultrafine mode ($PM_{0.18}$) were examined closely. The highest average number concentration was observed during the 10-hour sampling period on September 19. Measurements of the aerosol number distributions as a function of aerosol aerodynamic diameter are shown in Figure 7. Large variations in the number concentration on this day are evident because several distinct ultrafine particle events took place. Particles smaller than 100 nm became noticeable in the morning and in the late afternoon through the evening hours. In the morning of the 19th (~0800), the typical morning rush-hour traffic and an impact of other local sources increased the number concentration of ultrafine particles. Until mid-morning, ultrafine particles grow into the accumulation mode (0.1–1 µm) by coagulation and gas-to-particle conversion and the bimodal distributions are seen in the figure. Mid-day peaks are likely the result of photochemical processes as solar radiation reaches its maximum in the afternoon. During this sampling period, the sampling site was downwind of the Ambassador Bridge and the major interstate highways.

Gaseous Pollutants

Gaseous air pollutants including ozone, SO_2, NO_x (NO + NO_2), and CO were also measured continuously. $PM_{0.18}$ number concentrations were plotted with the gaseous pollutants including ozone, SO_2, and NO_x concentrations to examine temporal variations. Figure 8 illustrates the temporal variations in gaseous pollutants and the aerosol number concentrations for $PM_{0.18}$ on July 17–18 and September 28–29, 2000. The plots revealed several short-term peaks in pollutant levels at the sampling site. The morning increase in NO and ultrafine particle number concentrations were likely the result of motor vehicle emissions during morning rush hour as well as other local emissions sources that emitted particles and their precursors into a relatively shallow atmospheric layer. These figures clearly show impacts from local urban emission sources of air pollution in southwest Detroit.

As shown in this figure, the average concentrations of primary gaseous pollutants such as NO and SO_2 in September were higher than the averages observed in July 2000. This was likely the result of high photochemical activity during July that enhanced the rates of gas-to-particle conversion of primary pollutants—SO_2, NO, and volatile organic compounds. The formation, accumulation, and removal processes of $PM_{2.5}$ were closely related to these gaseous pollutants.

Chemical Characteristics of Particles

Major Elements Ambient $PM_{2.5}$ mass concentrations during this series of exposures were dominated by OC (27% ± 12%; mean ± SD), EC (15% ± 9%), SO_4^{2-} (24% ± 13%), NO_3^- (10% ± 6%), and NH_4^+ (9% ± 4%) (Table 6). The OC value was multiplied by 1.4 to account for unmeasured oxygen and hydrogen. Crustal elements (Fe, Al, Si, and Ca) constituted 8% ± 5% of fine particles. Si was estimated as K/0.15 (Keeler 1987).

OC and EC together composed a large portion of ambient $PM_{2.5}$ mass concentrations in Detroit. The ratio OC to total carbon during this series of exposures varied from 0.5 to 0.8 and the mean average ratio was 0.61 ± 0.10. This is similar to ratios in vehicle exhaust profiles that previous studies have reported (Watson et al 1994, 2001; Chow et al 2002). Watson and colleagues (1994) showed that the ratio of OC to total carbon was 0.63 in $PM_{2.5}$ for gasoline-fueled vehicle exhaust and 0.55 for diesel-fueled vehicle exhaust in Phoenix AZ. Thus, our results suggest that the sampling site we used was impacted by local combustion sources such as motor vehicles. Because the Maybury School sampling site was in close proximity to major interstate highways (US 75 and US 94) and to the entrance to the Ambassador Bridge leading to Windsor, Canada, we expected that mobile sources would contribute significantly to the $PM_{2.5}$ concentrations.

Previous research has provided strong evidence that secondary organic aerosols were being formed in the afternoons when photochemical reactions dominate the chemistry of the urban atmosphere, especially during photochemical smog episodes (Turpin et al 1994). Several studies have estimated the amount of secondary organic aerosols by measuring ambient OC/EC ratios and comparing those ratios to a primary OC/EC ratio (estimated as $1.8 < OC/EC < 2.3$) (eg, Turpin and Huntzicker 1995; Strader et al 1999). During the series of exposures we conducted, OC/EC ratios ranged from 0.8 to 3.0. Assuming that an OC/EC ratio exceeding 2.0 indicates the presence of secondary organic aerosols, those aerosols predominated during about half of the exposure periods. However, more investigation is required to distinguish between primary and secondary OC

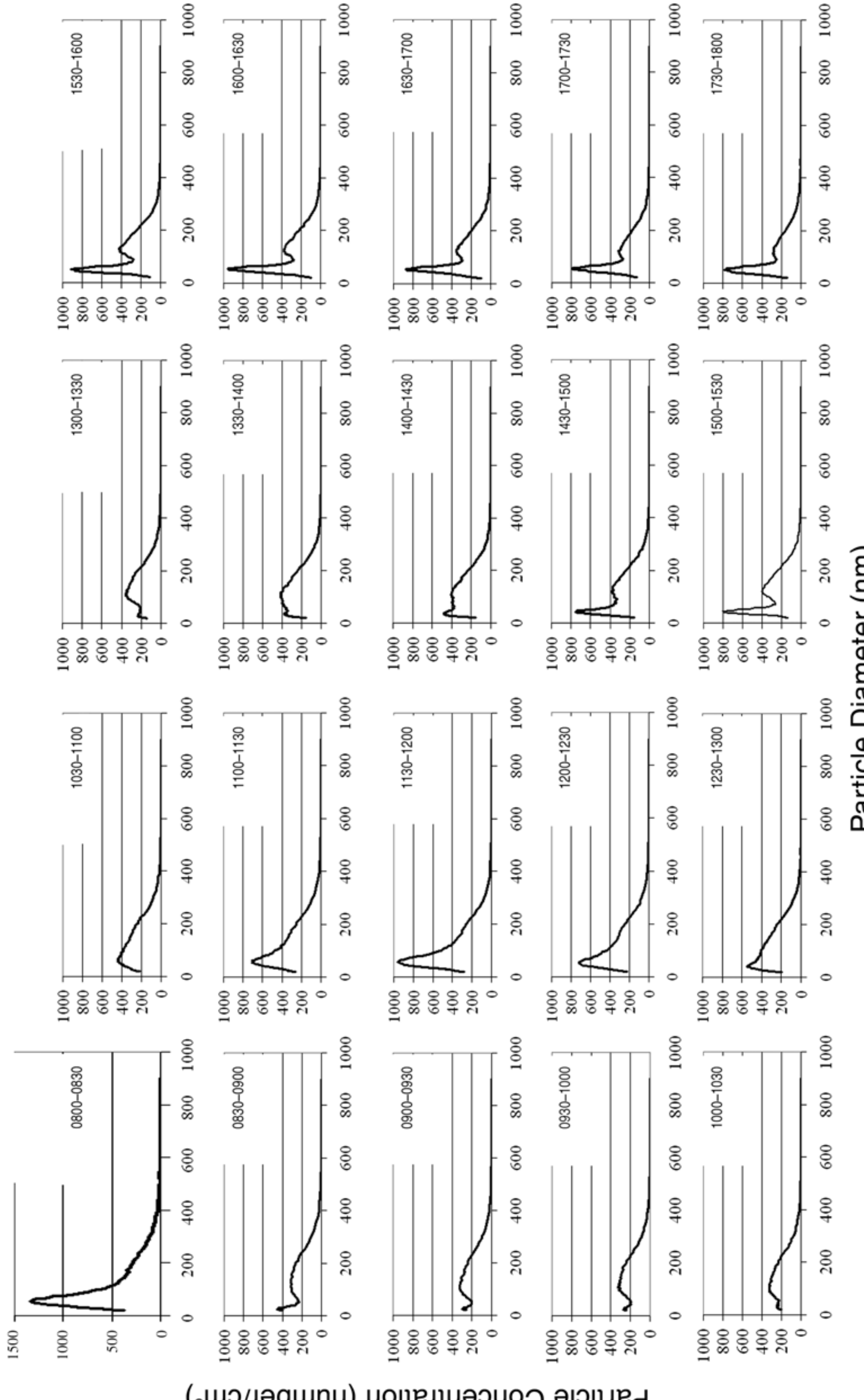


Figure 7. Number concentrations from the SMPS by ambient particle sizes in half-hour segments on September 19. Note the additional length of the y axis in the uppermost left panel.

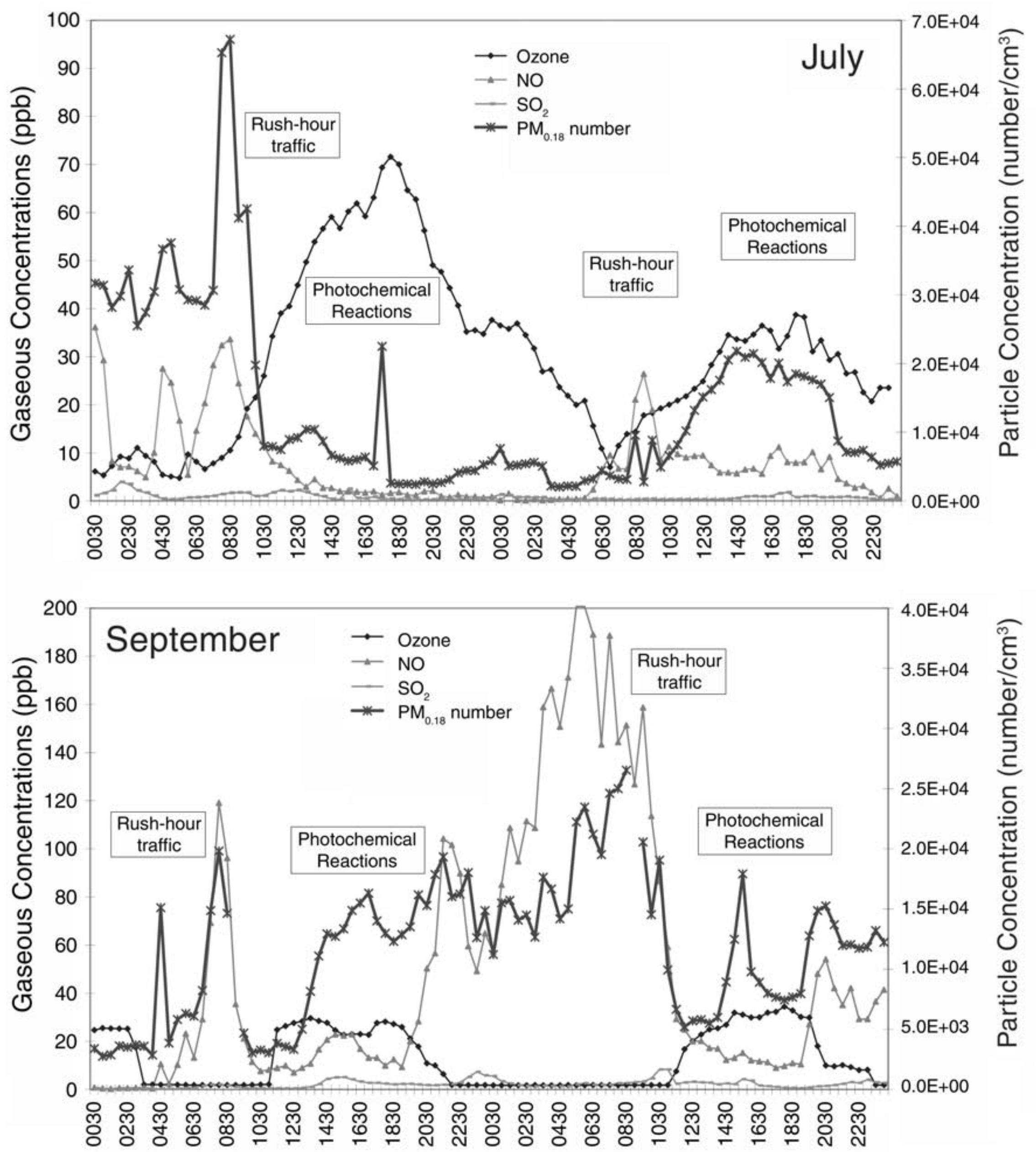


Figure 8. Ambient concentrations of gaseous pollutants and number concentration of $PM_{0.18}$ measured by the SMPS on July 17 and 18 (top) and on September 28 and 29 (bottom). Note the different scales used on the y and z axes.

contributions because OC/EC ratios can be influenced by weather patterns, diurnal and seasonal fluctuations in emissions, and local sources (Strader et al 1999).

The concentration of aerosol strong acidity (H^+) was observed to be quite low in both July and September, with mean levels of 0.9 ± 0.7 µg/m^3 and 0.3 ± 0.2 µg/m^3, respectively. This was not surprising because the amount of NH_3 emissions is copious in urban areas and the potential for local neutralization is high.

Trace Elements Table 7 shows the concentration of each ambient trace element during the animal inhalation studies. Contributions of elements such as S, V, Ni, Cu, Zn, and Pb that are related to combustion and anthropogenic sources were substantial. The data clearly indicated day-to-day variations of aerosol compositions and the impact of local sources on $PM_{2.5}$ compositions. These trace element concentrations were used to examine how the composition of trace elements in CAPs reflected that in ambient particles.

Table 6. Composition of Ambient $PM_{2.5}$ During 10-Hour Exposure Periods[a]

	Mass[b]	OC	EC	OC/TC	SO_4^{2-}	NO_3^-	NH_4^+	Crustal Elements[c]	Unidentified
July									
1 Day (F344)									
7/17	19.6	4.3	1.9	0.7	3.1	0.4	0.6	1.4	7.9
% of Total		22	9		16	2	3	7	40
5 Days (F344)									
7/18	7.7	1.9	2.2	0.5	0.4	0.5	0.1	0.9	1.7
7/19	7.3	1.8	0.8	0.7	2.9	0.2	0.1	0.7	0.8
7/20	17.2	3.7	1.2	0.8	2.3	1.5	1.2	1.1	6.1
7/21	5.2	3.1	1.7	0.6	0.9	0.3	0.4	1.3	0.0
7/22	7.3	1.8	2.1	0.5	0.4	0.4	0.2	0.6	1.8
TWA	8.9	2.5	1.6		1.4	0.6	0.4	0.9	2.1
% of Total		28	18		15	7	4	10	23
1 Day (BN)									
7/24	6.5	2.7	0.9	0.8	1.3	0.9	0.5	0.8	0.0
% of Total		42	13		19	14	8	1	0
4 Days (BN)									
7/25	20.9	3.3	1.5	0.7	8.5	1.5	2.5	1.1	2.5
7/26	23.5	2.9	2.0	0.6	10.7	1.2	3.1	1.1	2.5
7/27	27.1	3.2	1.7	0.7	11.5	0.7	3.5	0.8	5.7
7/28	34.2	2.8	2.0	0.6	16.4	1.8	4.0	1.6	5.5
TWA	26.2	3.1	1.8		11.7	1.3	3.3	1.1	4.0
% of Total		12	7		44	5	12	4	15
September									
1 Day (F344)									
9/18	20.5	5.7	2.2	0.7	2.7	3.4	1.1	1.7	3.7
% of Total		28	11		13	17	5	8	18
4 Days (F344)									
9/19	26.8	4.7	2.1	0.7	9.9	3.3	3.7	1.5	1.5
9/20	25.0	7.3	2.2	0.8	6.3	1.9	2.6	2.7	1.9
9/21	4.2	1.4	1.0	0.6	0.9	0.3	0.4	0.3	0.0
9/22	7.1	1.7	1.7	0.5	1.5	1.3	0.7	0.3	0.0
TWA	15.8	3.8	1.8		4.6	1.7	1.9	1.2	0.8
% of Total		24	11		29	11	12	8	5
1 Day (BN)									
9/25	3.7	1.3	0.5	0.7	0.8	0.5	0.4	0.4	0.0
% of Total		36	15		21	13	10	11	0
5 Days (BN)									
9/26	5.6	3.0	1.5	0.7	1.0	1.2	0.5	0.6	0.0
9/27	11.7	3.9	1.6	0.7	1.9	2.1	0.9	1.3	0.0
9/28	5.5	1.5	0.7	0.7	1.6	0.3	0.8	0.2	0.3
9/29	10.2	2.4	1.5	0.6	1.4	2.2	0.8	1.5	0.4
9/30	20.1	4.5	1.4	0.8	5.9	2.7	2.5	1.1	2.0
TWA	10.9	3.1	1.3		2.4	1.7	1.1	1.0	0.6
% of Total		28	12		22	16	10	9	5

[a] All values are expressed as $\mu g/m^3$ except for % of Total (as noted). TC = total carbon.

[b] From MOI measurements.

[c] Ca + Fe + Al + Si (where Si was estimated as K/0.15).

Table 7. Trace Element Concentrations in Ambient $PM_{2.5}$ During 10-Hour Exposure Periods[a]

	Na	Mg	Al	P	S	K	Ca	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Y	Cd	Sb	Ba	La	Sm	Pb
July																										
1 Day (F344)																										
7/17	55	125	119	41	1684	72	576	6.0	0.7	1.1	16	257	0.06	1.6	7	179	2.0	0.36	3.5	0.09	1.5	3.5	8	0.28	0.02	16
5 Days (F344)																										
7/18	52	72	76	9	485	59	301	2.6	0.3	1.1	7	156	0.05	2.3	9	31	0.9	0.13	1.5	0.04	0.1	2.6	5	0.08	0.01	4
7/19	29	47	56	6	359	45	200	1.8	2.4	3.0	7	147	0.06	3.9	8	43	1.9	0.11	1.0	0.02	0.1	0.5	6	0.09	0.01	3
7/20	63	95	94	36	806	55	450	2.6	2.1	1.6	10	236	0.07	2.8	7	38	0.9	0.24	1.9	0.05	0.2	0.3	4	0.20	0.01	4
7/21	55	64	255	29	1567	78	310	2.1	0.3	1.3	7	192	0.06	4.1	10	28	2.0	0.12	1.5	0.03	0.2	0.7	4	0.09	0.01	5
7/22	20	38	41	55	472	37	208	1.7	10.9	0.0	4	95	0.05	5.3	8	8	1.8	0.08	1.0	0.02	0.1	0.6	6	0.33	0.01	3
TWA	44	63	104	27	738	55	294	2.1	3.2	1.4	7	165	0.06	3.7	9	29	1.5	0.14	1.4	0.03	0.1	0.9	5	0.16	0.01	4
1 Day (BN)																										
7/24	36	69	113	1	604	20	453	3.9	4.9	0.0	9	140	0.04	2.1	4	13	0.7	0.11	1.6	0.05	0.1	0.4	8	0.18	0.01	5
4 Days (BN)																										
7/25	27	77	97	37	4137	53	375	5.1	1.6	1.0	16	268	0.05	0.8	6	37	3.1	0.19	1.7	0.05	0.3	0.8	9	0.13	0.01	9
7/26	67	83	74	14	5050	70	377	2.7	3.0	1.4	9	154	0.06	7.0	9	18	2.7	0.16	1.4	0.03	0.2	1.0	7	0.09	0.01	5
7/27	36	45	55	17	5962	60	220	3.0	2.3	0.0	6	114	0.04	0.8	25	24	1.4	0.16	1.2	0.04	0.2	1.1	7	0.12	0.01	6
7/28	93	102	89	22	9432	113	517	6.1	2.7	1.5	17	250	0.44	9.2	12	39	3.5	0.24	2.1	0.07	0.4	1.2	11	0.16	0.01	10
TWA	55	76	79	23	6061	73	368	4.2	2.4	1.0	12	195	0.14	4.4	13	29	2.7	0.19	1.6	0.05	0.3	1.0	9	0.12	0.01	8
September																										
1 Day (F344)																										
9/18	40	127	116	37	778	50	634	3.8	2.9	0.2	21	570	0.13	2.1	40	77	2.7	0.23	2.8	0.07	0.6	6.5	15	0.35	0.02	26
4 Days (F344)																										
9/19	56	98	148	48	1891	78	502	5.1	3.3	1.1	10	301	0.06	5.6	6	74	5.5	0.41	2.9	0.07	0.5	0.9	9	0.32	0.01	8
9/20	124	117	122	49	2348	245	620	4.3	2.4	1.7	13	338	0.13	2.4	5	76	3.1	0.71	3.4	0.07	0.4	0.9	8	0.20	0.02	8
9/21	0	36	7	0	194	3	157	0.8	0.1	0.0	5	97	0.01	0.3	0	18	0.3	0.08	0.9	0.02	0.1	5.5	3	0.06	0.00	12
9/22	7	33	91	2	327	6	119	1.2	1.3	0.0	5	81	0.00	2.0	4	28	1.7	0.09	0.7	0.02	0.1	0.6	6	0.08	0.00	4
TWA	47	71	92	25	1190	83	349	2.8	1.8	0.7	8	204	0.05	2.6	4	49	2.6	0.32	2.0	0.05	0.3	2.0	6	0.17	0.01	8
1 Day (BN)																										
9/25	14	33	60	38	305	13	176	1.0	5.2	4.7	2	82	0.00	1.8	0	45	2.3	0.08	0.7	0.02	0.1	0.4	5	0.17	0.00	4
5 Days (BN)																										
9/26	8	54	34	11	256	19	235	1.4	0.2	0.1	7	174	0.03	0.1	13	22	0.6	0.12	1.3	0.03	0.1	0.9	10	0.07	0.01	6
9/27	12	86	76	16	391	50	666	2.6	1.1	0.1	13	214	0.05	0.3	0	99	0.7	0.44	2.3	0.06	0.2	0.5	6	1.12	0.01	6
9/28	5	27	18	13	323	8	102	0.7	2.5	0.0	3	54	0.02	0.6	0	17	1.5	0.09	0.7	0.02	0.1	0.3	5	0.09	0.01	4
9/29	51	75	58	16	622	112	452	2.6	3.2	3.5	9	224	0.12	1.5	2	24	4.0	0.28	2.5	0.04	0.4	0.6	6	0.16	0.01	6
9/30	95	66	84	79	2374	75	372	2.7	1.9	1.9	8	171	0.11	3.0	6	55	7.1	0.27	2.3	0.05	2.9	1.0	7	0.23	0.01	10
TWA	35	61	53	28	810	53	353	2.0	1.8	1.2	8	165	0.07	1.1	4	41	2.9	0.23	1.8	0.04	0.8	0.7	7	0.30	0.01	7

[a] All values are from MOI samples and are given in ng/m^3.

(The data were also used as "fingerprints" of possible sources for source apportionment analyses to improve our understanding of the source-receptor pathway of anthropogenic particles [Morishita 2003]. However, source identification was beyond the scope of this investigation.)

Water-Soluble Trace Elements The soluble fractions of the trace elements were determined to investigate their potential role in the observed CAPs-induced pulmonary alterations in some of the BN rats. The most water-soluble elements (more than 50% of each element in ambient $PM_{2.5}$) during this investigation were Pb, Zn, Cd, Sb, and Na. Water-soluble concentrations of these trace elements accounted for 71.5% ± 13.0% (Pb), 66.1% ± 15.5% (Zn), 61.6% ± 18.1%(Cd), 57.4% ± 12.2% (Sb), and 52.1% ± 45.1% (Na) of the concentration of each metal in ambient $PM_{2.5}$. These metals likely existed as soluble sulfates and ammonium sulfates. The least–water-soluble trace metals (less than 10% of ambient $PM_{2.5}$) were Ti (5.8% ± 5.3%), Al (6.0% ± 2.8%), and La (8.0% ± 4.8%).

CHARACTERIZATION OF CAPs AND CONCENTRATOR PERFORMANCE

In our inhalation studies, rats were exposed to real-world urban aerosol by utilizing a Harvard ambient fine particle concentrator that can increase the concentration of ambient air particles.

One of the specific aims of this series of exposures was to understand how the CAPs to which laboratory animals were exposed in the inhalation chamber reflected the complicated mixture of ambient $PM_{2.5}$. A complete chemical and physical characterization of the chamber CAPs enabled us to relate adverse health responses of animals to $PM_{2.5}$ and to test our guiding hypotheses.

Because it has previously been shown that the Harvard ambient fine particle concentrator concentrates aerosols in the 0.15–1 µm size range most effectively, size distributions of ambient particles and CAPs were examined closely to understand how the size distributions of CAPs differed from those of ambient $PM_{2.5}$; to do this, we used MOIs and SMPS. Measuring the concentrations of SO_4^{2-}, NO_3^{-}, NH_4^{+}, EC, OC, and trace elements allowed a comparison of the average chemical composition of the ambient $PM_{2.5}$ and CAPs during animal inhalation exposure periods.

In addition, ambient ozone and CO concentrations were compared with concentrations in animal exposure chambers. For CO concentrations, no difference was observed between ambient and chamber air. For ozone, however, only 36% ± 14% of the ambient ozone level was conserved in the animal exposure chambers. Ozone was assumed to be stripped out of the incoming air flow by the stainless-steel concentrator.

CAPs Mass and Concentration Efficiency Factors

Daily CAPs mass concentrations during the exposure periods (0800–1800) in July ranged from 16 µg/m^3 to 895 µg/m^3 and in September ranged from 81 µg/m^3 to 755 µg/m^3 (Tables 8 and 9). Levels of CAPs using filter-based and TEOM measurements were examined to assure accuracy of measurements. Just as several studies have shown good agreement between these methods during the summer in urban areas (Allen et al 1997; Dvonch et al 2000), our results also revealed a relatively good correlation. This level of correlation assured that CAPs had been monitored properly (Figure 9). The difference between the two measurements may have been caused by changes in temperature, relative humidity, or composition of the aerosol. Furthermore, the sampling locations from which the two measurements were calculated were slightly different: The TEOM sampling port was located right after stage 3 within the concentrator; the filter samples were collected both after stage 3 and from within the exposure chamber.

Based on levels of $PM_{2.5}$ mass concentration in both the ambient air and the chamber, a concentration efficiency factor (CEF), that is, the ratio of CAPs mass concentration to ambient particle mass concentration, was calculated for each inhalation exposure period to evaluate the concentrator's performance. Tables 8 and 9 also show a summary of CEFs and conditions of the animal exposure chambers during the 10-hour exposure periods. The overall average CEF for CAPs mass concentrations during this investigation (based on regression analysis) was 20.5 (R^2 = 0.71, n = 22).

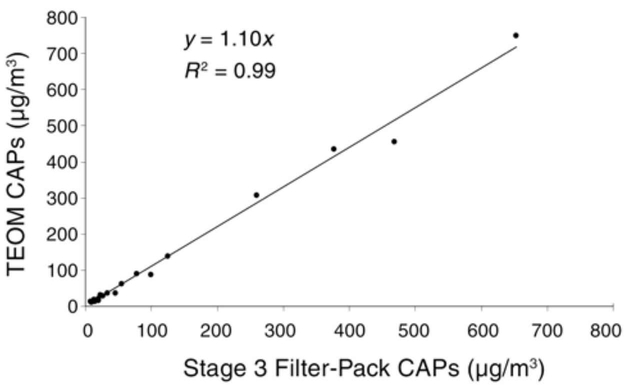


Figure 9. Comparison of CAPs measurements from the TEOM and from the stage 3 filter pack.

Table 8. BN Rats: CAPs Composition and Chamber Conditions During 10-Hour Exposure Periods[a]

	CAPs										Exposure Chamber[b]	
	CEF	Mass[c]	30-Minute Maximum Mass[d]	OC	EC	SO_4^{2-}	NO_3^-	NH_4^+	Crustal Elements[e]	Unidentified	Relative Humidity (%)	Temperature (°C)
July, BN Rats												
1 Day												
7/24	9	59.5	129.8	17.8	4.9	5.0	4.2	5.3	7.6	14.7	56.7 ± 3.8	25.7 ± 0.2
% of Total				30	8	8	7	9	13	25		
4 Days												
7/25	19	405.8	656.1	95.0	11.8	119.8	11.4	40.1	15.4	112.2	72.2 ± 3.6	25.3 ± 0.3
7/26	21	481.9	681.3	97.0	13.2	155.2	15.4	75.4	6.2	119.6	75.7 ± 6.3	25.3 ± 0.3
7/27	26	707.8	1284.3	124.2	13.8	146.5	15.6	106.5	8.4	292.8	76.7 ± 5.9	25.1 ± 0.3
7/28	26	895.1	2109.1	146.4	15.6	329.2	47.9	176.3	9.5	170.4	77.6 ± 5.9	24.9 ± 0.5
TWA	23	615.7		114.8	13.5	184.0	21.9	97.6	9.9	173.8	75.5 ± 5.4	25.2 ± 0.3
% of Total				19	2	30	4	16	2	28		
September, BN Rats												
1 Day												
9/25	22	79.7	146.8	58.3	20.6	8.2	16.9	6.0	6.6	0.0	59.3 ± 5.2	25.3 ± 0.2
% of Total				73	26	10	21	8	8	0		
5 Days												
9/26	14	81.1	272.9	55.5	3.7	9.6	9.5	4.5	8.6	0.0	59.6 ± 5.7	25.9 ± 0.7
9/27	36	417.8	641.5	159.3	14.8	29.6	24.1	15.6	46.2	128.2	55.3 ± 3.5	26.1 ± 0.6
9/28	25	136.7	243.6	61.6	14.0	24.6	11.0	10.1	12.5	3.0	52.6 ± 3.9	25.3 ± 0.6
9/29	27	273.3	713.4	133.9	15.7	33.3	48.7	22.7	40.2	0.0	53.9 ± 3.2	25.1 ± 0.8
9/30	25	493.7	760.4	252.5	17.3	110.6	53.1	44.5	32.7	0.0	55.8 ± 3.2	25.2 ± 0.4
TWA	26	291.0		136.6	13.6	43.2	30.3	20.3	29.1	27.6	55.2 ± 3.8	25.4 ± 0.6
% of Total				47	5	15	10	7	10	9		

[a] Other than CEFs and "% of Total", all values related to the elements of CAPs composition are given as $\mu g/m^3$.

[b] Values are presented as means ± SD.

[c] From the Teflon filterpack placed after stage 3 of the concentrator.

[d] From the TEOM.

[e] Ca + Fe + Al + Si (where Si was estimated as K/0.15).

Table 9. F344 Rats: CAPs Composition and Chamber Conditions During 10-Hour Exposure Periods[a]

	CAPs										Exposure Chamber[b]	
	CEF	Mass[c]	30-Minute Maximum Mass[d]	OC	EC	SO_4^{2-}	NO_3^-	NH_4^+	Crustal Elements[e]	Unidentified	Relative Humidity (%)	Temperature (°C)
July, F344 Rats												
1 Day												
7/17	14	266.3	625.6	86.3	18.3	49.6	9.2	24.1	13.7	65.2	66.5 ± 2.5	26.0 ± 2.5
% of Total				32	7	19	3	9	5	24		
5 Days												
7/18	2	16.0	103.0	0.0	9.6	1.0	2.9	0.0	5.5	0.0	57.4 ± 3.0	25.7 ± 0.2
7/19	17	124.9	458.9	46.9	7.4	35.1	4.7	16.5	3.2	11.2	62.5 ± 2.9	25.2 ± 0.2
7/20	5	80.8	121.0	45.0	6.1	13.6	10.6	8.3	4.9	0.0	61.7 ± 6.6	25.6 ± 0.3
7/21	15	77.5	245.0	23.5	1.9	10.1	11.7	6.2	5.8	18.4	60.9 ± 6.0	25.6 ± 0.2
7/22	8	55.6	101.5	8.7	3.0	1.5	2.8	0.0	2.0	37.6	58.7 ± 3.5	25.4 ± 0.1
TWA	9	71.0		24.8	5.6	12.3	6.5	6.2	4.3	13.4	60.2 ± 4.4	25.5 ± 0.2
% of Total				35	8	17	9	9	6	19		
September, F344 Rats												
1 Day												
9/18	9	175.8	600.4	74.2	7.8	21.2	25.6	7.9	28.7	10.3	63.9 ± 4.4	24.6 ± 0.3
% of Total				42	4	12	15	5	16	6		
4 Days												
9/19	13	345.6	556.5	83.0	49.1	66.7	50.9	29.2	33.9	32.8	68.2 ± 5.7	24.5 ± 0.2
9/20	30	754.7	984.0	211.1	26.9	132.8	41.4	53.1	56.1	233.3	67.6 ± 5.8	24.9 ± 0.3
9/21	19	81.3	187.1	35.1	12.2	11.3	16.4	5.2	13.0	0.0	56.2 ± 3.5	25.1 ± 0.4
9/22	18	128.9	219.8	51.9	13.6	21.1	20.4	11.8	10.6	0.0	57.0 ± 1.8	25.1 ± 0.5
TWA	20	327.7		95.3	25.5	58.0	32.3	24.8	28.4	66.5	62.3 ± 4.2	24.9 ± 0.4
% of Total				29	8	18	10	8	9	20		

[a] Other than CEFs and "% of Total", all values related to the elements of CAPs composition are given as μg/m^3.

[b] Values are presented as means ± SD.

[c] From the Teflon filterpack placed after stage 3 of the concentrator.

[d] From the TEOM.

[e] Ca + Fe + Al + Si (where Si was estimated as K/0.15).

We noted that the CEFs were influenced by meteorological conditions, the alignment of the slits of the virtual impactors within the concentrator, and low ambient concentrations. During this investigation, an unusually clean dry air mass was brought by northerly winds for several days during the summer months. As expected, the CEFs and the ambient particle levels were low and the concentration of the CAPs delivered to the exposure chamber increased by only a factor of less than 10 (see Tables 8 and 9 for July 18, 20, 22, and 24 and September 18). On the other hand, when a relatively humid air mass brought by southerly winds resulted in increased levels of pollutants (especially during the 4-day exposure period in July), the Harvard ambient fine particle concentrator generated CAPs in the size range of 0.18 to 2.5 µm effectively and the CEFs reached as high as 26-fold. The Harvard group previously characterized the concentrator and found that the CEFs were likely to be influenced by environmental factors (such as levels of ambient particle concentration and size distribution) and meteorological conditions (such as humidity) (Sioutas et al 1997; Godleski et al 2000). They also observed that the operation of the concentrator (including the minor pressure drop), the ratio of minor flow to total flow, and the alignment of the slits of the virtual impactors had a significant impact on the CEFs observed.

Figure 10 shows the temporal variation in the observed 30-minute averaged CAPs concentrations measured by the TEOM, which sampled from just after the third stage of the concentrator before the airflow entered the animal exposure chamber, during 10-hour exposures periods in July and September. Comparing the ambient TEOM measurements of $PM_{2.5}$ to those measured from the concentrator before the air entered the exposure chamber revealed the same temporal variations during the exposure periods when the concentrator was performing properly.

CAPs Size Distribution

As described previously, the concentration enrichment of the Harvard ambient fine particle concentrator depends on particle size; the 0.15 µm to 1 µm particles of the accumulation mode are concentrated far more effectively than particles smaller than 0.15 µm (Sioutas et al 1997).

Figure 11 illustrates the number enrichment factor for morning (0910–0930) and afternoon (1740–1750) exposure periods on September 20. The number enrichment factor is defined here as the ratio of the number of particles measured by SMPS after stage 3 of the concentrator to the number of particles in the ambient air as a function of particle aerodynamic diameter. During these two time periods, the concentrator was effectively concentrating the particles in the submicron size range that the SMPS can detect; the enrichment factor was more than 5 for particles with aerodynamic diameters larger than 225 nm, and more than 20 for particles with aerodynamic diameters larger than 350 nm.

The comparison of particle mass categorized by size distributions of average ambient particles and CAPs as measured by the two MOIs (> 5 µm, 5–2.5, 2.5–1, 1–0.6, 0.6–0.18, < 0.18) is illustrated in Figure 12. The mass size distribution revealed that the Harvard concentrator effectively concentrated the particle mass in the 0.15–2.5 µm size range during the exposure period and that the mass of particles in the 0.18–1.0 µm size range dominated the mass distribution during our exposure studies. The average CEF of ultrafine particles (< 0.18 µm) from the 22 exposure periods was 2.2. This implies that ultrafine particles were not concentrated as effectively as particles in the 0.6–1.0 µm size range and that the concentration of ultrafine particles in the chamber was equal to or slightly higher than their concentration in ambient air.

Overall, these results revealed that during the July and September time periods of our inhalation exposures, the particle concentrator was effectively concentrating the particles in the submicron size range. However, the concentrator did alter the size distribution of CAPs from that of ambient $PM_{2.5}$ even within the 0.18–1.0 µm size range.

CAPs Chemical Composition

The average chemical composition of ambient $PM_{2.5}$ and CAPs during the 10-hour exposure periods in July and September are shown in Table 6 for ambient particles and Tables 8 and 9 for CAPs. As was observed with ambient $PM_{2.5}$ constituents, the identifiable CAPs constituents were also dominated by OC (36% ± 18%; mean ± SD), EC (9% ± 13%), SO_4^{2-} (17% ± 9%), NO_3^- (10% ± 6%), and NH_4^+ (8% ± 5%). Tables 8 and 9 summarize the major chemical constituents of CAPs for each exposure period.

As discussed in previous sections, the overall average CEF for mass during this investigation was calculated to be about 20.5 (R^2 = 0.71) based on concentrations of ambient $PM_{2.5}$ and CAPs. Similar regression analyses were performed on particulate SO_4^{2-} and NO_3^-; the average CEFs during this investigation for SO_4^{2-} and NO_3^- were found to be 16.5 (R^2 = 0.93) and 16.9 (R^2 = 0.65), respectively.

In order to examine concentration enrichment processes more closely, we calculated the percentage of major chemicals composing CAPs and ambient $PM_{2.5}$ for each exposure period (Table 10). Although some variability was observed, generally the concentrations of SO_4^{2-}, NO_3^-, NH_4^+, and crustal elements in the exposure chamber reflected the chemical composition of ambient particles.

However, OC and EC did not show good correlations between CAPs and ambient $PM_{2.5}$. As shown in Table 10, the OC fraction in total CAPs was often higher than the ambient measurements; sampling artifacts of OC mass may be a major culprit of this situation. In fact, the average CEF of

Figure 10. Temporal variation in the observed 30-minute averaged CAPs concentrations measured by the TEOM after the third stage of the concentrator during 10-hour exposure periods in July and September.

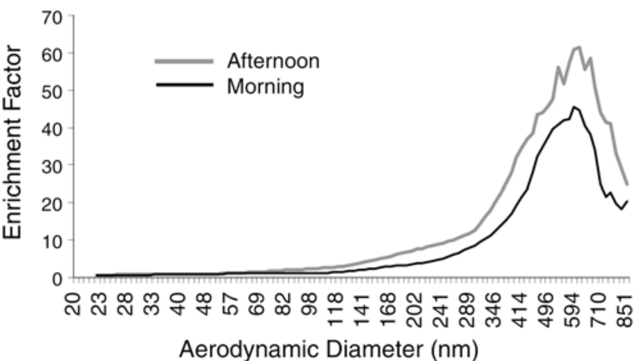


Figure 11. Number enrichment factors for the Harvard ambient fine particle concentrator during the morning and afternoon on September 20.

OC was calculated to be 24.6 (R^2 = 0.31), which is about 32% higher than the average mass CEF during this series of exposures. As previously reported, organic gas adsorption (a positive artifact) and particle volatilization (a negative artifact) are two major issues when measuring concentrations of OC particulates (Turpin et al 1994). Because temperature, relative humidity, precursor gas concentrations, and the face velocity of the sampling device all influence these artifacts, more investigation into how best to measure particulate OC in exposure chambers is required. In fact, Turpin and coworkers (2000) reported a significant dependence on face velocity and showed that OC concentrations were 22% greater, on average, when measured at a face velocity of 20 cm/sec than when measured at 40 cm/sec. Because CAPs

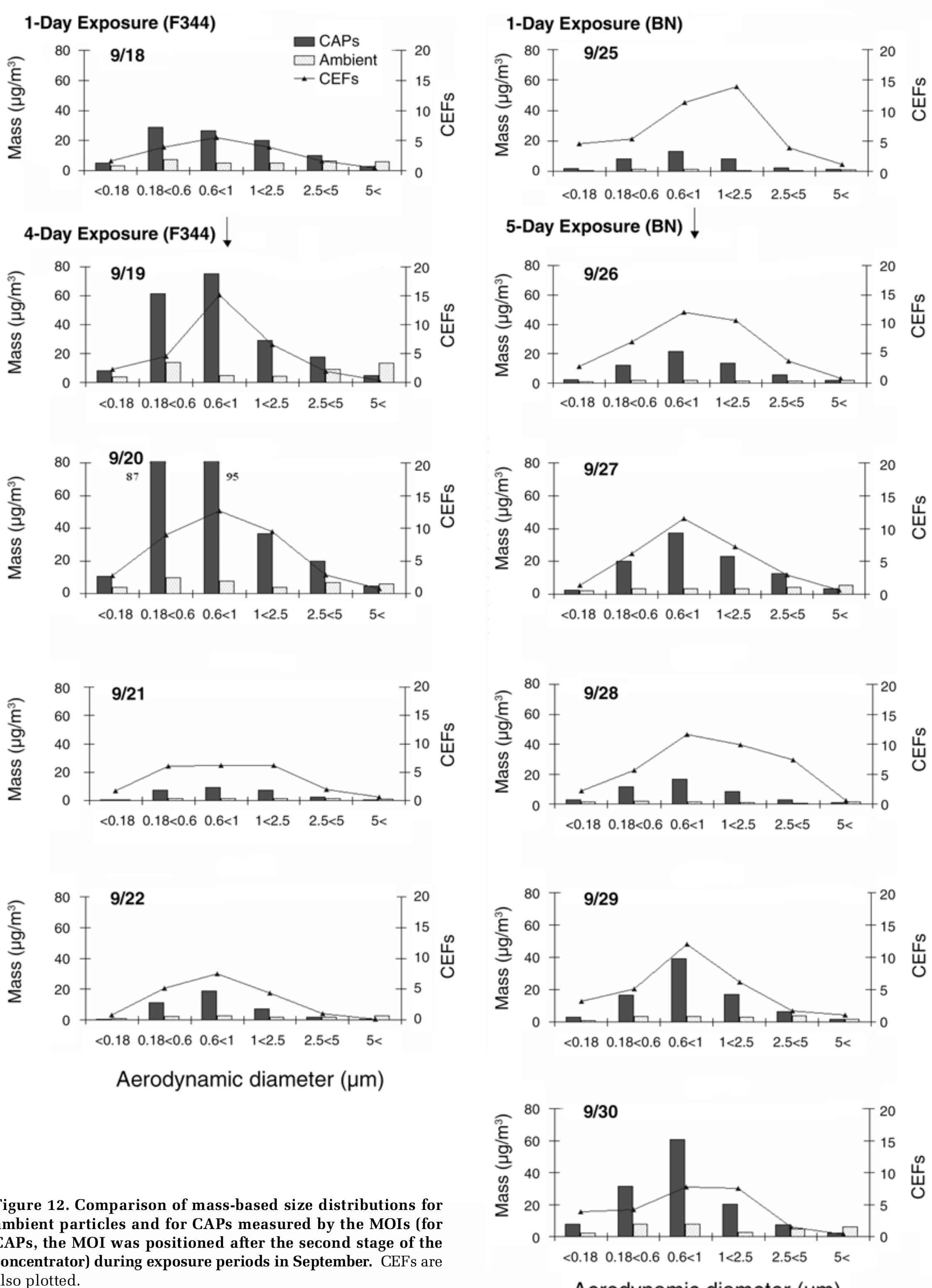


Figure 12. Comparison of mass-based size distributions for ambient particles and for CAPs measured by the MOIs (for CAPs, the MOI was positioned after the second stage of the concentrator) during exposure periods in September. CEFs are also plotted.

Table 10. Comparison of Average Chemical Composition of Ambient $PM_{2.5}$ and CAPs[a]

	OC		EC		SO_4^{2-}		NO_3^-		NH_4^+		Crustal Elements		Unidentified	
	CAPs	Ambient	CAPs	Ambient	CAPs	Ambient	CAPs	Ambient	CAPs	Ambient	CAPs	Ambient	CAPs	Ambient
July														
1 Day (7/17)	32	22	7	9	19	16	3	2	9	3	5	7	24	40
5 Days (7/17–7/22)	35 ± 21	28 ± 16	8 ± 25	18 ± 12	17 ± 10	15 ± 14	9 ± 6	7 ± 2	9 ± 6	4 ± 3	6 ± 13	10 ± 7	19 ± 28	23 ± 14
1 Day (7/24)	30	42	8	13	8	20	7	14	9	8	13	1	25	0
4 Days (7/25–7/28)	19 ± 3	12 ± 3	2 ± 1	7 ± 1	30 ± 7	44 ± 3	4 ± 1	5 ± 2	16 ± 4	12 ± 1	2 ± 1	4 ± 1	28 ± 9	15 ± 5
September														
1 Day (9/18)	42	28	4	11	12	13	15	17	5	5	16	8	6	18
4 Days (9/19–9/22)	29 ± 9	24 ± 7	8 ± 5	11 ± 9	18 ± 2	29 ± 8	10 ± 6	11 ± 5	8 ± 1	12 ± 2	9 ± 4	8 ± 3	20 ± 15	6 ± 4
1 Day (9/25)	73	35	26	15	10	21	21	13	8	10	8	11	0	0
5 Days (9/26–9/30)	47 ± 11	28 ± 13	5 ± 3	12 ± 7	15 ± 6	22 ± 7	10 ± 5	16 ± 7	7 ± 2	10 ± 3	10 ± 3	9 ± 4	9 ± 14	5 ± 4

[a] Values are percentages; for multiple-day exposures, values are means ± SD.

was sampled at a significantly lower flow rate (2 L/min) than ambient samples (16.7 L/min), the face velocity of the sampling device might have affected these results.

For EC, we also did not observe a correlation between ambient levels and CAPs. EC particles are known to be formed primarily by condensation of C_2 molecules generated during the combustion process and have low vapor pressures to form particles smaller than 0.1 µm (EPA 1996a,b). Furthermore, Venkataraman and associates (1994) reported that measurements inside tunnels have confirmed that over 85% of the emitted EC mass is in particles smaller than 0.2 µm and that, in polluted urban areas, particles in the size range of 0.05 to 0.12 µm usually dominate, comprising almost 75% of the total EC. Thus, we assumed that EC, which was dominated by ultrafine particles, was not concentrated because they just passed through the concentrator; the Harvard ambient fine particle concentrator did not increase the concentration of particles smaller than 0.15 µm (Sioutas et al 1997).

Some of the unexplained fraction of the total mass may be explained by the water content of the atmosphere. A previous study has shown that the water content of particles is strongly dependent on deliquescent points of chemical composition and concluded that liquid water could represent a significant mass fraction of aerosol concentration at a relative humidity above 60% (Pilinis et al 1989). The relative humidity of the exposure chamber during July exceeded 60% and a major portion of the unexplained fraction of the total mass may be explained by particle-bound water.

Figure 13 illustrates the day-to-day temporal variations of Sb, V, and Fe for ambient $PM_{2.5}$ and CAPs. Although some variability was observed due to fluctuations of day-to-day concentrator performance, the overall composition of these trace elements in CAPs reflected that in ambient particles. The variation in the concentration of the trace elements from day-to-day was strongly related to meteorological conditions and source influences.

ELEMENTS FROM PARTICLES RETAINED IN RAT LUNGS

As described in the Methods section, rats were killed 24 hours after the end of the last daily exposure to filtered air or CAPs; thus, the results reflect element retention at only one time point. The right middle lobe was placed in a Teflon container, snap-frozen in liquid nitrogen, and stored at −80°C until processed for trace element analysis of retained CAPs.

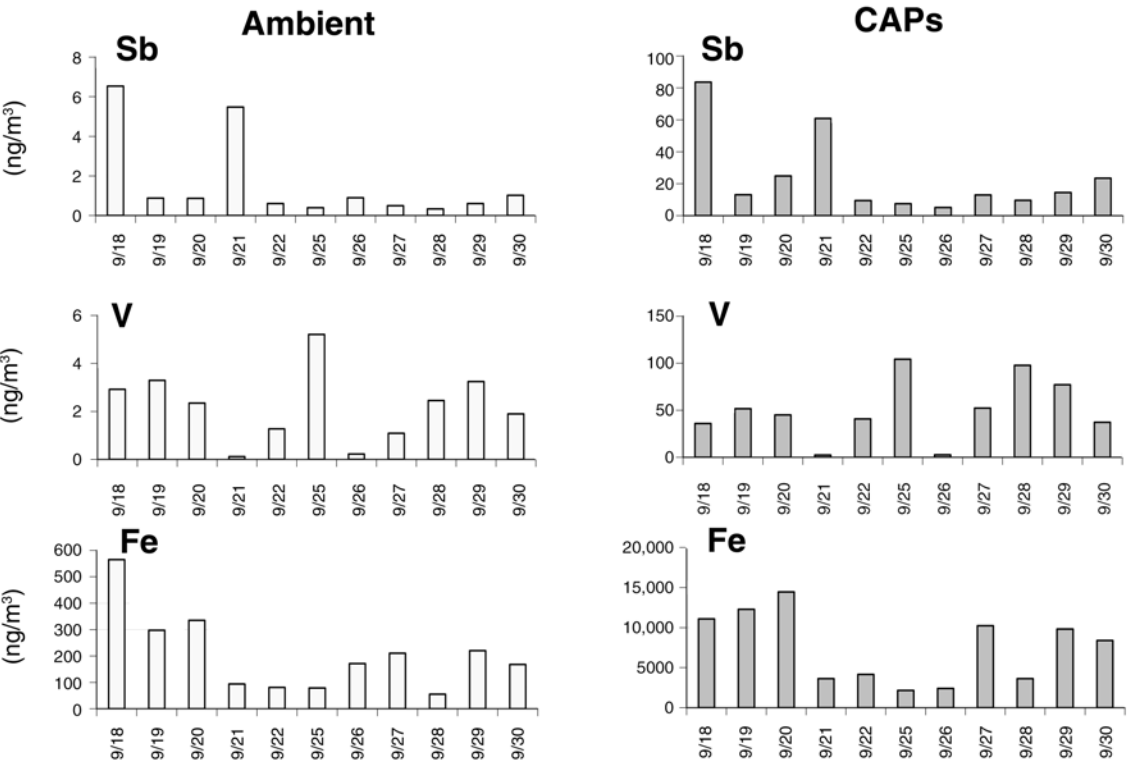


Figure 13. Temporal variations of three trace metals in ambient $PM_{2.5}$ measured by the MOI and in CAPs measured by the stage 3 filter pack during 10-hour exposure periods in September. Note that the scales on the y axes differ between the ambient and CAPs bar graphs and for each of the metals.

For groups of BN and F344 rats in July, F344 rats in September, and BN rats exposed for 1 day in September—all exposed to CAPs—anthropogenic trace elements in pulmonary tissues were below the limits of detection.

For the CAPs-exposed BN rats at the end of the 5-day exposure protocol in September, several trace elements were recovered from the lung tissues successfully. Figure 14 shows elevated concentrations of La, V, and S. For example, for the two air-exposed groups, mean (± SEM) concentrations of La were 0.00169 ± 0.00085 µg/g of dry lung tissue for the saline-challenged group, and 0.00191 ± 0.00161 µg/g for the OVA-challenged group. In contrast, for the two CAPs-exposed groups, mean (± SEM) concentrations were elevated: 0.00545 ± 0.00303 µg/g for the saline-challenged group and 0.00736 ± 0.00083 µg/g for the OVA-challenged group. The differences between air-exposed and CAP-exposed groups were statistically significant based upon the results of the Tukey-Kramer comparison. It is also interesting to note that the OVA-challenged CAP-exposed rats (with preexisting hypersecretory airway disease) appeared to have retained more particles than the saline-challenged CAPs-exposed rats did.

Similar results were observed for V and S; S, however, had a relatively weak statistical result (see Figure 14). We speculated that the results with S levels were influenced by background levels because S is a major biological element found in lung tissues. Furthermore, because S is water-soluble, it was likely absorbed into the blood or cleared through the lymphatic system relatively quickly.

Because normal lung tissues contain many trace elements that are also found in ambient particles, profiles of element concentrations in normal and nonexposed lung tissues must be examined carefully to distinguish elements in CAPs from biological elements that occur normally in lung tissues. In order to assure that the trends we recorded in Figure 14 were not caused by essential elements or other systematic errors, several nutrient elements were plotted in box charts. As examples, amounts of the essential elements P and Fe in lung tissues are shown in Figure 15. Clearly no significant differences were observed among groups for these elements.

In addition, we estimated the amount of each trace element that would have been transported into the rat lungs during CAPs exposure. The estimate was based on the mass of each element found in lung tissues (after subtracting levels found in tissues from air-exposed rats), estimated breathing volume of the rats, and the average concentration of trace elements measured in the CAPs during the exposure periods. We concluded that approximately 3% to 4% of the trace elements in the CAPs to which the animals were exposed penetrated to the lung lobes.

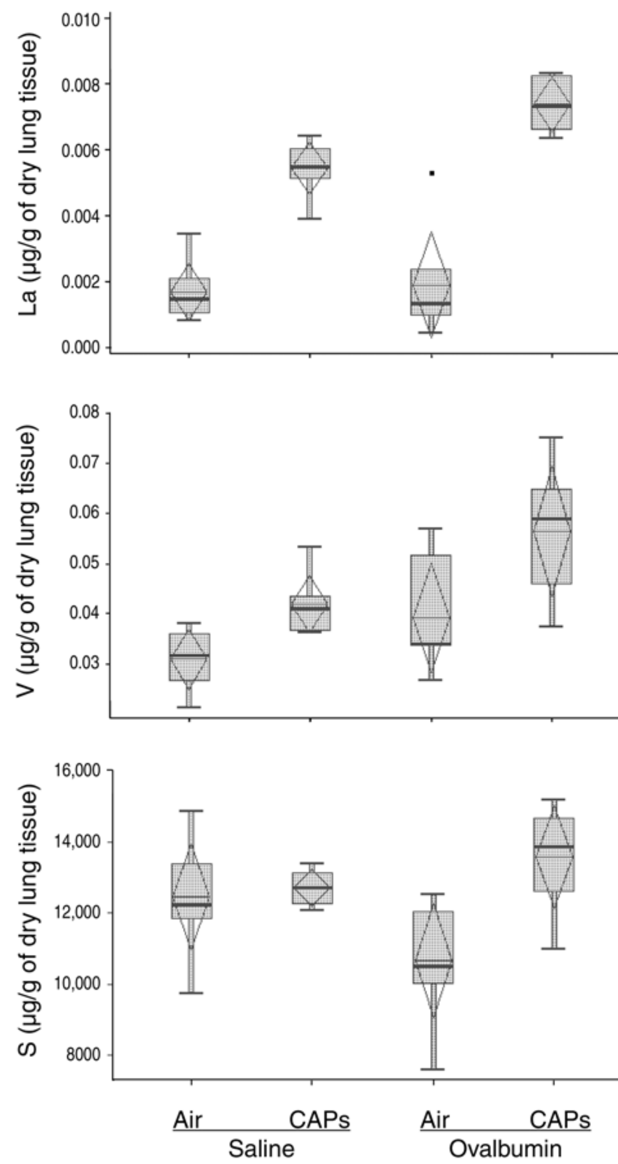


Figure 14. Three trace elements retained in BN rat lung tissues from the September 26–30 exposure protocol. The central line in each box marks the median value; the edges of the box mark the 25th and 75th percentiles; the diamond in each block shows the mean; and the bars that extend from the top and bottom show the highest and lowest values (the dot in the La panel is an outlier).

Previously, Brauer and colleagues (2001) showed that a significant portion of small particles could be retained in the lungs. Measured at autopsy, the lungs from residents of Mexico City contained over 7.4 times the concentration of these particles than the lungs from residents of Vancouver. Our investigation showed direct evidence of real-world urban particulates retained within animal tissues. To date, however, no data are available to show the detailed chemical composition of urban particles retained in both human

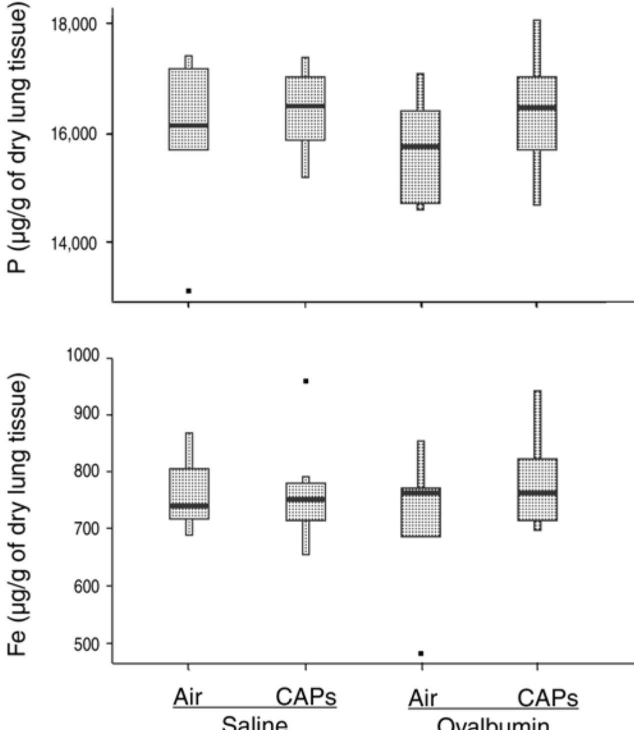


Figure 15. Two nutrient elements found in BN rat lung tissues from the September 26–30 exposure protocol. The central line in each box marks the median value; the edges of the box mark the 25th and 75th percentiles; the bars that extend from the top and bottom show the highest and lowest values; and the dots are outliers.

and animal lung tissues (such as recovered trace element particles); if those data were gathered, one could assess any relations between ambient particles and retained particles to understand the short-term impacts of air pollution. Our investigation assessed real-world particle levels in lung tissues to improve our understanding of a relation between what is in the air and what is, in fact, deposited in respiratory system. These findings and further studies of the chemical and physical characteristics of the inhaled particles are important because the tissue dose of toxic components is not always proportional to the ambient exposure of the compound and because the response is more likely related to the tissue dose.

INHALATION COMPONENT: BN RATS

The next step in this investigation attempted to find if any physical properties or chemical components of $PM_{2.5}$ had any association with airway inflammation and epithelial alterations that might be observed in the animal models that were exposed to CAPs. The CAPs composition and inhalation chamber conditions during each of the inhalation series are summarized in Tables 8 and 9. Meteorological conditions for each 10-hour animal exposure period are also presented below. The following sections describe each exposure period and highlight the key observations and interesting findings.

Tables 11 through 14 provide a complete data summary of the biochemical (Table 14) and cellular analyses of the BALF (Table 13), the morphometric and molecular analyses for airway IM and mucin gene expression (Table 11), and the morphometric analyses of airway epithelial cell density and cell proliferation (Table 12) for each animal exposure group.

Urban Atmospheric Conditions and CAPs Characteristics (BN Rats)

BN Rats, 1-Day Exposure (July 24) Northerly winds were dominant during this 1-day exposure period, and the average ambient $PM_{2.5}$ concentration only reached 6.5 µg/m^3 (Table 6). The CEF on this day was only 9 (Table 8), which produced a mass concentration of CAPs of only 59.5 µg/m^3. Although the concentration of $PM_{2.5}$ was relatively low, the fraction of $PM_{0.18}/PM_{2.5}$ reached the highest number of 0.35, and the average number of $PM_{0.1}$ was 16,225 ± 6,720 particles/cm^3 (Table 5). The relative humidity was low to moderate during the day (48.9% ± 12.2%; see Table 4), which minimized the growth of hygroscopic particles smaller than 0.1 µm into particles in the size range of the accumulation mode (0.1–1 µm).

BN Rats, 4-Day Exposure (July 25–28) The average ambient $PM_{2.5}$ and CAPs concentrations during this period were 26.2 µg/m^3 (Table 6) and 615.7 µg/m^3 (Table 8), respectively. This 4-day exposure period recorded the highest mass concentration of ambient $PM_{2.5}$ and CAPs among eight series of inhalation exposures. The temporal variation in the mass of the CAPs measured by the TEOM is shown in Figure 10 (top panel for July). Note that the CAPs mass concentrations were recorded only while the concentrator was in operation for the 10 hours each day. The maximum 30-minute mass concentrations on July 27 and 28 exceeded 1 mg/m^3, with mean concentrations of 707.8 and 895.1 µg/m^3, respectively, on these days (Table 8).

During this exposure period, 31% of CAPs mass was SO_4^{2-}, and the time-weighted average concentration of SO_4^{2-} was 184 µg/m^3 (Table 8). A large amount of SO_4^{2-} particles is typically observed in the Midwest; it has been well established that the ambient fine particle mass in the Midwest and Northeastern US is dominated by regional transport of secondary particles composed largely of SO_4^{2-} and OC, especially during the summer (Burton et al 1996). The air mass history and the observed meteorological conditions during this investigation also supported those previous studies.

First, we used the HYbrid Single Particle Lagrangian Integrated Trajectories model (HYSPLIT) and projected backward 72 hours to understand the transport of particles that impacted communities in southwest Detroit during this exposure period (Figure 16). The upper air meteorological data were obtained from the Air Resources Laboratory of the US National Oceanic and Atmospheric Administration (NOAA 2000). As can be seen in Figure 16, during the 4-day exposure period of July 25–28, the dominant wind direction was southerly and a high-pressure system dominated the weather as it migrated from west to east with regional transport from the south. The probable scenario from Figure 16 and the chemical composition we identified in $PM_{2.5}$ is that an upwind stagnation over the high-density SO_2-emission areas in the Ohio River Valley resulted in slow transport into Detroit, which resulted in aged aerosols composed primarily of coal combustion products with SO_4^{2-} dominating the fine particle mass. Anderson and associates (2002) also reported episodes of high concentrations of $PM_{2.5}$ with transport of emissions from the Ohio River Valley to the National Energy Technology Laboratory Office sited in Pittsburgh on July 27 and 29, 2000.

Furthermore, local ambient NH_3 concentrations were high enough during this period (average NH_3 levels were 4 ppb [data not shown]) that all of the acidic SO_4^{2-} was neutralized by the time it reached the monitoring site, again supporting the observation that the $PM_{2.5}$ was the result of long-range transport.

Although the highest $PM_{2.5}$ mass concentrations were observed during this exposure period (July 25–28), the contribution of $PM_{0.18}$/$PM_{2.5}$ mass was lowest, as evidenced by

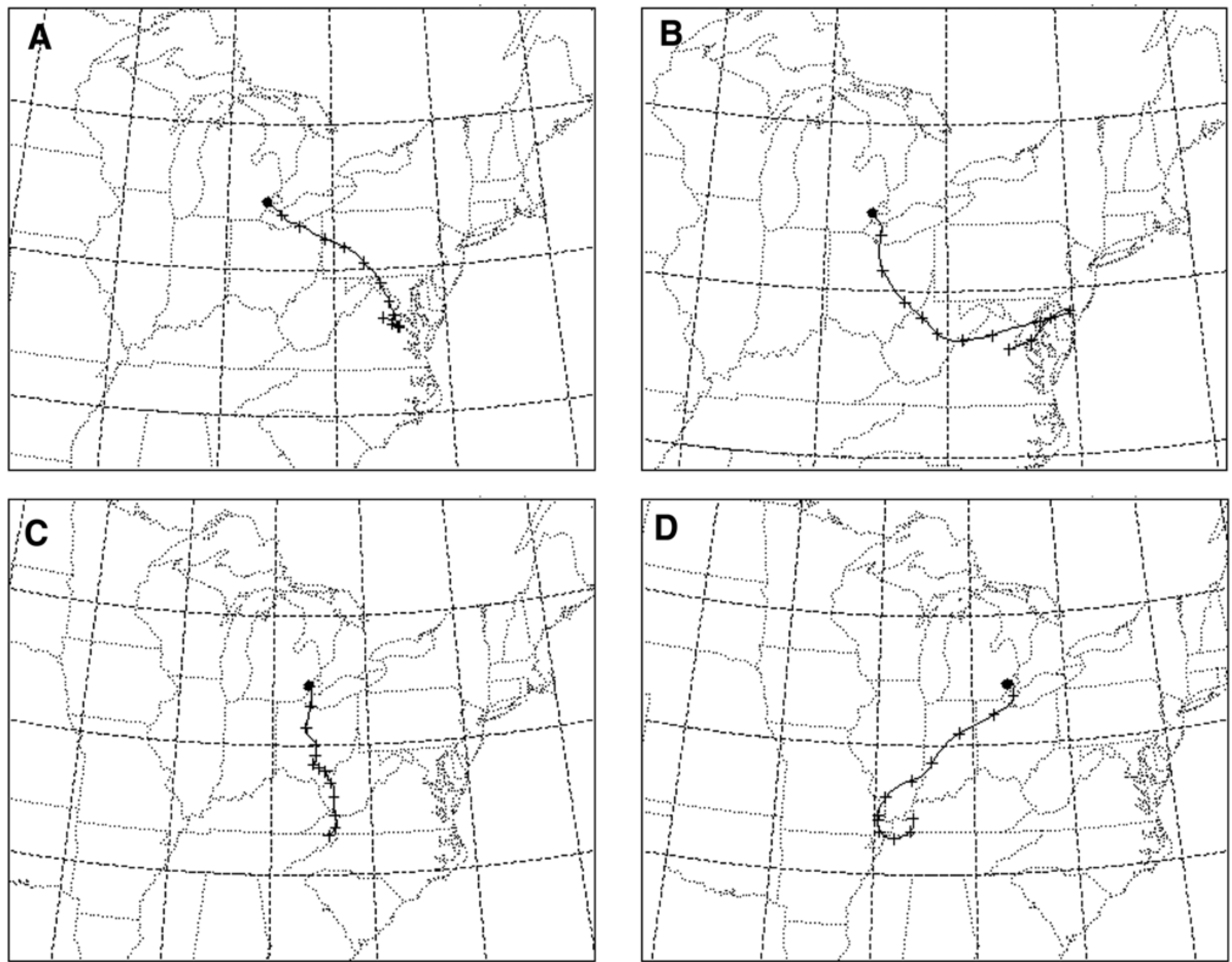


Figure 16. HYSPLIT-generated 72-hour backward trajectories showing the history of air masses arriving in Detroit at 1800 hours EST on (A) July 25, (B) July 26, (C) July 27, and (D) July 28. (Data are from NOAA 2000.)

the lowest average ratio of $PM_{0.18}$ to $PM_{2.5}$ of any exposure period. This is partially the result of a relatively faster gas-to-particle conversion rate in July (than in September) and the high average relative humidity (69.9% ± 11.7%; see Table 4) observed during this exposure period. At high relative humidity, hygroscopic ultrafine and fine particles increase in size due to the accumulation of particle-bound water. This also leads to high formation of fine secondary particles during hot and humid summer periods.

BN Rats, 1-Day Exposure (September 25) Northerly winds brought cool air masses to southwest Detroit communities and the lowest ambient $PM_{2.5}$ mass concentration in the entire investigation: 3.7 µg/m^3 (Table 6) during this 10-hour exposure period. The CAPs concentration was 79.7 µg/m^3 (Table 8). As expected, most of the trace element levels were below the average concentrations calculated from all exposure periods (Table 7). Comparatively cold (11.4 ± 1.1°C) temperatures were recorded (Table 4) and the mean number concentration of $PM_{0.1}$ was relatively low at 6024 ± 2870 particles/cm^3 (Table 5). However, increased levels of Ni, V (Table 7), and EC (Table 8) were observed, which implies that emissions from oil combustion sources near the sampling site were substantial.

BN Rats, 5-Day Exposure (September 26–30) The average ambient $PM_{2.5}$ and CAPs concentrations during this period were 10.9 µg/m^3 (Table 6) and 291.0 µg/m^3 (Table 8), respectively. Figure 10 (bottom panel for September) shows the temporal variation of CAPs concentration during this exposure period. In contrast to the other multiple-day exposure period with BN rats in July, the HYSPLIT trajectories (Figure 17) in the last week of September show that moderate synoptic flows from the north and northwest were dominant, which were associated with a high-pressure system over the Midwest. A relatively clean background air mass was transported from Canada and the impact of local sources of ambient $PM_{2.5}$ emissions was strong during this exposure period in September. On the last exposure day, however, flow from southerly direction was observed as the high-pressure system migrated to the east; the concentration of ambient SO_4^{2-} was 5.9 µg/m^3, the highest sulfate concentration for this 5-day exposure period.

During this 5-day exposure period, elevated levels of La (Table 7) and HNO_3 (data not shown) were also observed. As described in Results / Elements from Particles Retained in Rat Lungs, only the rats exposed to CAPs during this 5-day period in September showed anthropogenic trace elements in the lung tissues. One of the recovered trace elements was La; the elevated concentrations of La in ambient $PM_{2.5}$ and in CAPs during this exposure period were consistent with the recovery of La from the lung tissues.

La or La-rich lanthanide compositions have been used extensively in oil refinery processes for cracking reactions in fluid catalytic crackers (Kitto et al 1992). Olmez and Gordon (1985) reported that the concentration pattern of rare earth elements such as La was distorted in areas influenced by emissions from oil-fired plants and refineries and that refineries and oil-fired plants became major sources of rare earth elements. Concentration ratios of La to other rare earth elements such as Sm were used to determine the influences of oil-fired and oil-refining plants; the ratio of La/Sm has often been found to be in the range of 10 to 20 compared with the ratio of crustal elements at lower than 6. During our exposure period of September 26–30, the La/Sm ratio reached over 20 (data not shown).

Moreover, several large oil refinery industries are located on and around Zug Island, which is only 1 or 2 miles south of the monitoring site. The EPA facilities emissions report (EPA 2000) shows that Marathon Oil Company, which is one of the major petroleum refining industries in southwest Detroit, emitted approximately 5% of the total $PM_{2.5}$ emissions in Wayne County in 1999. The dominant wind direction when the La/Sm ratio reached over 20 was south to southwest. A relatively clean and dry air mass came from the north, which provided a relatively clean "background" pollution level compared with the air mass in July.

Pulmonary and Nasal Histopathology and Airway IM (BN Rats)

Histopathology and Changes in BALF Caused by OVA Challenge Alone in BN Rats The principal morphologic lesion in the lungs of BN rats intranasally challenged with OVA was an allergic bronchiolitis of the conducting airways involving both the large-diameter, proximal, axial airways and the small-diameter, distal, preterminal and terminal airways. The OVA-induced inflammatory and epithelial changes were usually more severe in the more proximal axial bronchioles compared to those in the more distal preterminal and terminal bronchioles. OVA-induced bronchiolitis was characterized by peribronchiolar edema associated with a mixed inflammatory cell influx of eosinophils, lymphocytes, plasma cells, and occasional neutrophils (Figure 18). Peribronchiolar inflammation was principally located in the subepithelial interstitial tissues (eg, lamina propria and submucosa) with markedly fewer inflammatory cells in the surface epithelium that lines these airways. Bronchiole-associated lymphoid tissues in these OVA-challenged airways were also enlarged due to lymphoid hyperplasia. Perivascular interstitial (ie, the surrounding pulmonary arteries adjacent to bronchioles and pulmonary veins scattered throughout the alveolar parenchyma) accumulation of a similar mixture of eosinophils

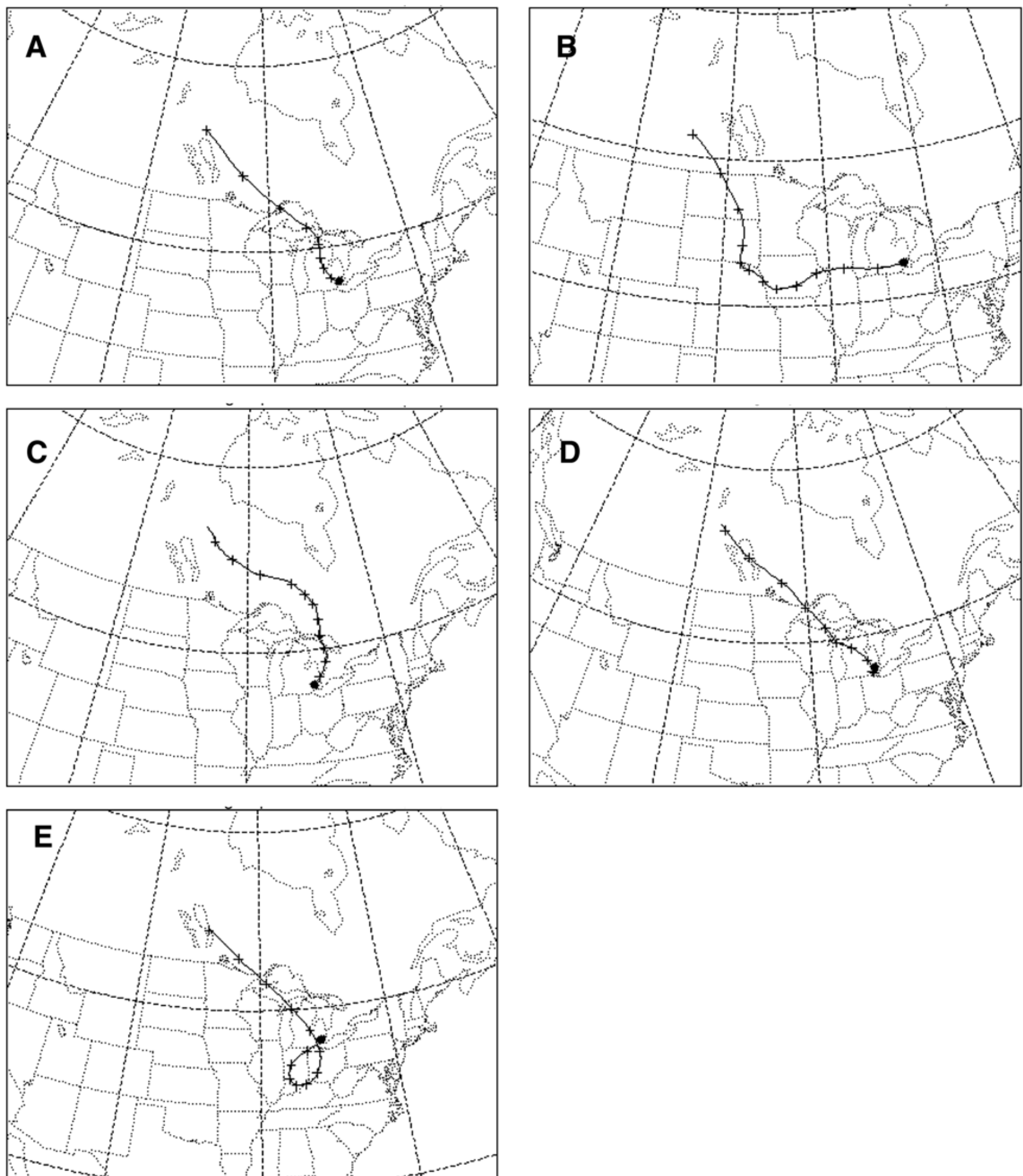


Figure 17. HYSPLIT-generated 72-hour backward trajectories showing the history of air masses arriving in Detroit at 1800 hours EST on (A) September 26, (B) September 27, (C) September 28, (D) September 29, and (E) September 30. (Data are from NOAA 2000.)

and mononuclear cells, along with perivascular edema, were also present in the lungs of OVA-challenged rats.

In addition to the perivascular and peribronchiolar lesions, there were various sizes of focal areas of allergic alveolitis in the parenchyma surrounding or adjacent to the affected large and small conducting airways. These alveolar lesions were characterized by accumulations of large numbers of alveolar macrophages, epithelioid cells, and eosinophils, and lesser numbers of lymphocytes, monocytes, and plasma cells in the alveolar airspace. Often the alveolar septa in these areas of alveolitis were thickened due to mild-to-moderate type II pneumocyte

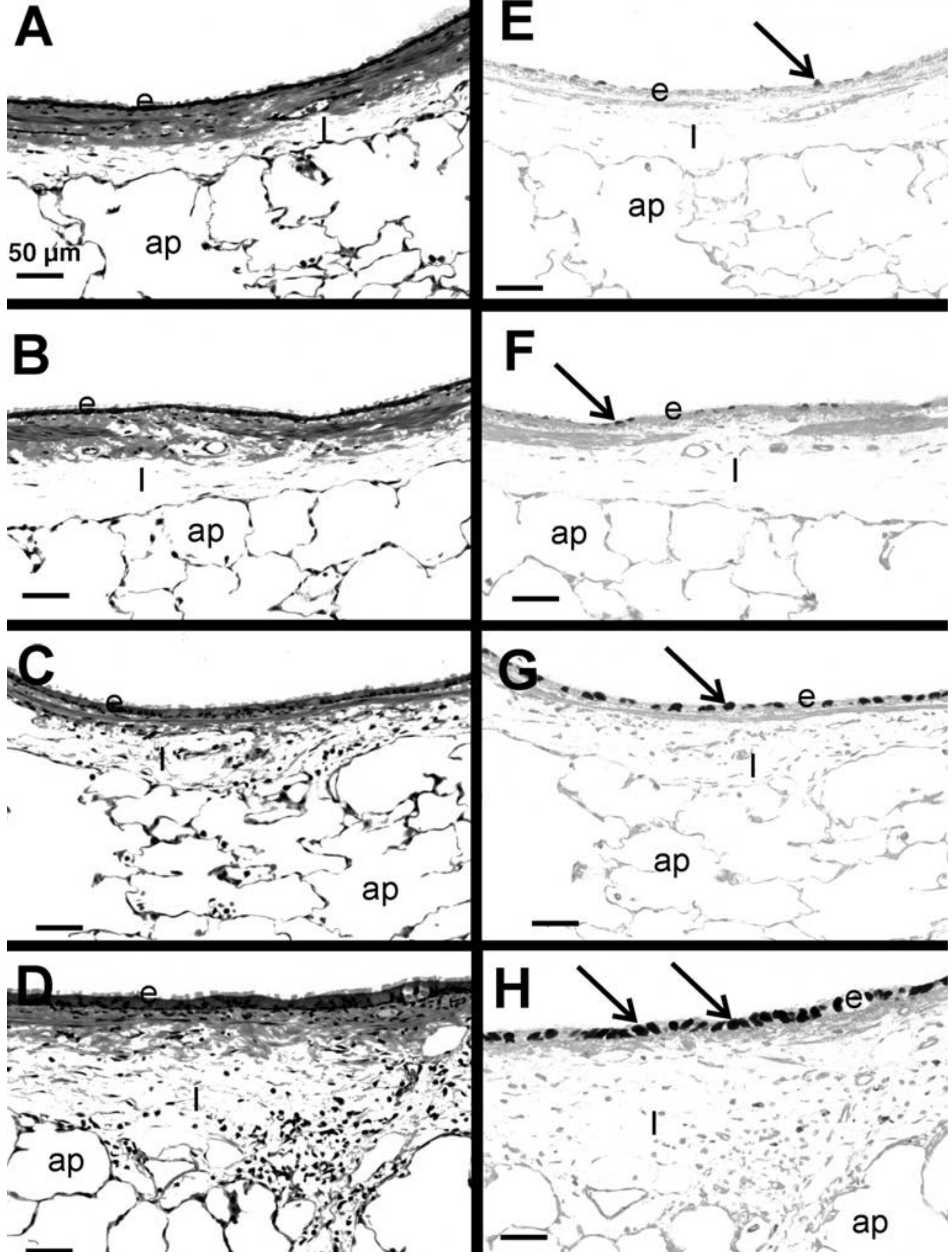


Figure 18. Light photomicrographs of pulmonary axial airways from the left lung lobe of BN rats exposed to CAPs or filtered air for 5 days in September 2000. *A and E*: saline-challenged/air-exposed rat; *B and F*: saline-challenged/CAPs-exposed rat; *C and G*: OVA-challenged/air-exposed rat; *D and H*: OVA-challenged/CAPs-exposed rat. Airway tissues stained with hematoxylin and eosin (*A–D*) or AB/PAS (*E–H*). e = surface epithelium; I = peribronchiolar interstitium; ap = alveolar parenchyma. Arrows indicate AB/PAS-stained IM in surface epithelium. Inflammatory cell influx in peribronchiolar interstitium is apparent in panels C and D. Bars = 50 µm.

hyperplasia and hypertrophy, intracapillary accumulation of inflammatory cells, and capillary congestion.

OVA-challenged rats exposed to either air or CAPs had a mild to marked mucous cell metaplasia/hyperplasia with increased amounts of AB/PAS-stained IM lining the affected large-diameter bronchioles, including the proximal axial airway (Figure 18). Mucous cell metaplasia/hyperplasia was less evident in the more distal, small diameter bronchioles of these OVA-challenged rats. Saline-challenged/air-exposed or saline-challenged/CAPs-exposed rats had significantly fewer mucous cells and IM compared to the OVA-challenged animals (Figure 18).

OVA challenge also caused mild-to-moderate rhinitis characterized by a mixed inflammatory cell influx consisting of eosinophils, lymphocytes, and plasma cells. The allergen-induced inflammatory influx in the nose was conspicuous in the lamina propria of the mucous membranes lining the middle septum and the lateral meatus (lateral wall, nasoturbinates, and maxilloturbinates) in the proximal half of the nasal airways.

In addition, there were no statistically significant differences in the BrdU-labeling index (estimate of induced DNA synthesis) in the surface epithelium lining the axial airways of any of the BN rats in this exposure protocol or in any of the other three inhalation protocols conducted in July or September (Table 12).

BN Rats, 1-Day Exposure (July 24) We found no apparent differences in the number of mucous cells or in the morphometrically measured amount of IM in the large- or small-diameter bronchioles (pulmonary axial airways) in the OVA-challenged rats, whether exposed to filtered air or CAPs, compared to those in the saline-challenged/air-exposed animals (Table 11). We found no histologic or morphometric evidence for a CAPs-induced alteration to these OVA-induced nasal lesions after a 1-day inhalation exposure.

BN Rats, 4-Day Exposure (July 25–28) The severity of the OVA-induced pulmonary lesions was minimal to mild in these OVA-challenged animals. OVA-challenge caused a significant increase in the amount of IM in the axial airways of both filtered-air– and CAPs-exposed rats compared to the respective control rats challenged with saline (Table 11). However, no CAPs-related changes in the amount of the IM in the pulmonary axial airways were found in OVA- or saline-challenged animals. Interestingly, there was a significant increase of IM (1.7 × saline-challenged/air-exposed controls) in the respiratory epithelium lining the nasal septum of rats challenged with OVA and exposed to CAPs (Table 11). This increase in nasal IM was also associated with a mild-moderate allergic rhinitis characterized by an influx of eosinophils and mononuclear cells (lymphocytes and plasma cells) in the nasal mucous membranes lining the middle septum and lateral meatus in the proximal half of the nose. The amount of nasal IM of OVA-challenged/CAPs-exposed rats was significantly greater than that in either OVA-challenged/air-exposed rats or in saline-challenged/CAPs-exposed rats, indicating both an OVA- and CAPs-induced effect (Table 11). Interestingly, the amount of nasal IM was not different between OVA-challenged/air-exposed rats and saline-challenged/air-exposed rats in this exposure protocol.

BN Rats, 1-Day Exposure (September 25) Although no significant OVA-related increases of IM were found in the pulmonary airways examined, we noted a marked increase in the amount of IM in the respiratory epithelium lining the proximal nasal septum (approximately 75%–80% increase compared to saline-challenged/air-exposed controls; Table 11). No CAPs-specific changes in the histopathology or airway morphometry for IM were found in this exposure protocol.

Compared to control rats, neither OVA challenge nor CAPs exposure caused changes in the gene expression of *Muc5ac* (Table 11) or in the BrdU-labeling index (Table 12) on the surface epithelium in the pulmonary axial airways of these animals.

BN Rats, 5-Day Exposure (September 26–30) There was no significant difference in the amounts of IM between saline-challenged rats exposed to CAPs and saline-challenged rats exposed to only filtered air.

However, OVA-challenged rats exposed for 5 days to CAPs had a slightly more severe mucous cell metaplasia and hyperplasia in the epithelium lining the proximal axial airway compared to OVA-challenged rats exposed to filtered air. This was reflected in the mean volume densities of IM in the proximal axial airways that are graphically illustrated in Figure 19. Also in the proximal axial airways, OVA-challenged/CAPs-exposed animals had significantly greater amounts of IM compared to those in both OVA-challenged/air-exposed rats (43% more) and saline-challenged/CAPs-exposed rats (173% more) (Table 11). In contrast, there were no significant differences in the amount of IM among the rats in the other exposure groups.

The amount of *Muc5ac* mRNA in OVA-challenged/air-exposed rats was a significantly greater than that in saline-challenged/air-exposed rats (Table 11). Interestingly, OVA-challenged/CAPs-exposed rats had significantly less expression of *Muc5ac* mRNA than OVA-challenged/air-exposed rats (Table 11). These were the only groups of OVA-challenged or CAPs-exposed rats from any of the exposure protocols to have significant differences in mucin-specific gene expression.

Table 11. Intraepithelial Mucosubstances and Mucin Gene Expression in the Pulmonary Axial Airways from Rats Exposed via Inhalation[a]

	Airway Status and Exposure Atmosphere[b]	IM (nL/mm^2) Nasal Proximal Septum	IM (nL/mm^2) Pulmonary Axial Airways	Gene Expression[c] *Muc5ac* mRNA in Axial Airways
BN Rats, July				
1 Day	Sal/Air	8.2 ± 0.7	1.9 ± 0.2	1.00 (0.77–1.30)
	Sal/CAPs	7.5 ± 0.9	2.4 ± 0.4	0.82 (0.51–1.32)
	OVA/Air	16.0 ± 1.8[d]	1.9 ± 0.3	0.71 (0.54–1.32)
	OVA/CAPs	14.8 ± 1.5[e]	1.2 ± 0.3[e]	0.52 (0.27–0.98)
4 Days	Sal/Air	5.8 ± 0.6	1.1 ± 0.3	1.00 (0.79–1.24)
	Sal/CAPs	4.6 ± 0.7	0.8 ± 0.1	0.92 (0.78–1.08)
	OVA/Air	7.0 ± 0.6	2.3 ± 0.6[d]	0.57 (0.38–0.84)
	OVA/CAPs	9.8 ± 1.2[e,f]	1.8 ± 0.3[e]	0.68 (0.43–1.07)
BN Rats, September				
1 Day	Sal/Air	8.6 ± 0.7	1.3 ± 0.3	1.00 (0.76–1.32)
	Sal/CAPs	6.7 ± 1.0	2.3 ± 1.0	0.92 (0.45–1.89)
	OVA/Air	15.0 ± 1.2[d]	2.0 ± 0.4	0.21 (0.08–0.57)
	OVA/CAPs	14.6 ± 0.7[e]	1.1 ± 0.3	0.32 (0.13–0.73)
5 Days	Sal/Air	8.1 ± 1.0	1.7 ± 0.3	1.00 (0.71–1.40)
	Sal/CAPs	7.9 ± 0.6	1.1 ± 0.2	2.56 (1.44–4.56)
	OVA/Air	12.1 ± 1.0[d]	2.1 ± 0.3	4.10 (3.39–4.96)[d]
	OVA/CAPs	11.3 ± 1.5	3.0 ± 0.3[e,f]	0.93 (0.60–1.44)[f]
F344 Rats, July				
1 Day	S/Air	2.7 ± 0.5	0.2 ± 0.1	1.00 (0.58–1.74)
	S/CAPs	3.4 ± 1.2	0.2 ± 0.0	0.33 (0.13–0.83)
	E/Air	6.9 ± 1.5[g]	0.5 ± 0.1[g]	0.51 (0.35–0.73)
	E/CAPs	9.7 ± 1.3[h]	0.5 ± 0.1[h]	0.58 (0.41–0.82)
5 Days	S/Air	3.8 ± 0.4	0.1 ± 0.0	1.00 (0.63–1.61)
	S/CAPs	3.2 ± 0.8	0.1 ± 0.0	0.93 (0.60–1.44)
	E/Air	2.0 ± 0.6	0.2 ± 0.1	1.17 (1.62–2.22)
	E/CAPs	1.8 ± 0.5	0.2 ± 0.1	1.05 (0.78–1.41)
F344 Rats, September				
1 Day	S/Air	6.2 ± 1.1	0.1 ± 0.0	1.00 (0.73–1.36)
	S/CAPs	2.6 ± 0.6[g]	0.1 ± 0.0	1.03 (0.76–1.39)
	E/Air	10.0 ± 1.2[g]	0.1 ± 0.0	0.27 (0.19–0.38)
	E/CAPs	10.5 ± 1.0[h]	0.1 ± 0.1	0.38 (0.23–0.62)[h,i]
4 Days	S/Air	4.7 ± 0.7	0.0 ± 0.0	1.00 (0.77–1.29)
	S/CAPs	4.9 ± 1.0	0.0 ± 0.0	0.98 (0.80–1.19)
	E/Air	6.0 ± 0.7	0.0 ± 0.0	0.82 (0.70–0.96)
	E/CAPs	4.1 ± 0.8	0.1 ± 0.0	0.99 (0.79–1.24)

[a] Data are group means ± SEM.

[b] Sal = saline-challenged rats; OVA = OVA-challenged rats; S = saline-pretreated rats; E = endotoxin-pretreated rats.

[c] Relative fold in expression as compared with OVA + S/Air or S/Air control group. In parantheses is the range of the relative expression as determined by evaluating the expression $2^{-\Delta\Delta CT}$ with $\Delta\Delta C_T$ + s and $\Delta\Delta C_T$ −s, where s = the SD of the $\Delta\Delta C_T$ value.

[d] Significantly different from Sal/Air group ($P \leq 0.05$).

[e] Significantly different from Sal/CAPs group ($P \leq 0.05$).

[f] Significantly different from OVA/Air group ($P \leq 0.05$).

[g] Significantly different from S/Air group ($P \leq 0.05$).

[h] Significantly different from S/CAPs group ($P \leq 0.05$).

[i] Significantly different from E/Air group ($P \leq 0.05$).

Table 12. Numeric Cell Density and Cell Proliferation in the Pulmonary Axial Airway Epithelium from Rats Exposed via Inhalation[a]

	Airway Status and Exposure Atmosphere[b]	Cell Density (cells/mm of basal lamina)	BrdU-Labeling Index (% BrdU-labeled cells)
BN Rats, July			
1Day	Sal/Air	95.7 ± 9.8	0.3 ± 0.6
	Sal/CAPs	96.9 ± 19.9	0.3 ± 1.0
	OVA/Air	81.3 ± 9.0	0.4 ± 1.9
	OVA/CAPs	105.7 ± 15.9	0.4 ± 1.0
4 Days	Sal/Air	121.8 ± 11.1	1.5 ± 0.3
	Sal/CAPs	125.6 ± 10.2	2.2 ± 0.9
	OVA/Air	109.4 ± 9.5	1.5 ± 0.5
	OVA/CAPs	137.1 ± 16.6	2.0 ± 0.4
BN Rats, September			
1 Day	Sal/Air	621.2 ± 233.6	1.2 ± 0.5
	Sal/CAPs	524.8 ± 279.2	1.1 ± 0.4
	OVA/Air	737.2 ± 388.0	1.3 ± 0.9
	OVA/CAPs	554.1 ± 193.6	1.9 ± 0.7
5 Days	Sal/Air	100.6 ± 9.8	2.3 ± 0.6
	Sal/CAPs	91.9 ± 7.4	2.2 ± 0.5
	OVA/Air	86.5 ± 9.5	5.5 ± 1.8
	OVA/CAPs	112.9 ± 5.3 [c]	4.7 ± 0.9
F344 Rats, July			
1 Day	S/Air	76.4 ± 2.5	1.5 ± 0.5
	S/CAPs	81.8 ± 2.3	1.0 ± 0.2
	E/Air	89.2 ± 4.0	2.6 ± 0.4
	E/CAPs	90.4 ± 16.0	2.9 ± 0.5[d]
5 Days	S/Air	76.3 ± 10.4	3.4 ± 1.0
	S/CAPs	77.3 ± 2.9	1.7 ± 0.3
	E/Air	92.2 ± 3.4	3.0 ± 0.5
	E/CAPs	76.8 ± 9.8	4.6 ± 0.9[d]
F344 Rats, September			
1 Day	S/Air	68.0 ± 2.0	0.2 ± 0.1
	S/CAPs	71.6 ± 3.3	0.4 ± 0.1
	E/Air	70.6 ± 3.9	0.4 ± 0.2
	E/CAPs	91.0 ± 11.1[d,e]	0.7 ± 0.2
4 Days	S/Air	53.5 ± 6.2	1.1 ± 0.4
	S/CAPs	64.0 ± 3.8	1.5 ± 0.4
	E/Air	58.7 ± 2.3	1.7 ± 0.4
	E/CAPs	60.6 ± 3.0	2.7 ± 0.7

[a] Data are group means ± SEM.

[b] Sal = saline-challenged rats; OVA = OVA-challenged rats; S = saline-pretreated rats; E = endotoxin-pretreated rats.

[c] Significantly different from OVA/Air group ($P \leq 0.05$).

[d] Significantly different from S/CAPs group ($P \leq 0.05$).

[e] Significantly different from E/Air group ($P \leq 0.05$).

Interestingly, OVA-challenged/CAPs-exposed rats did have significantly greater numbers of total epithelial cells (numeric cell density) compared to those in OVA-challenged/air-exposed rats (Table 12). This epithelial hyperplastic response was not evident in any of the other groups of rats in this investigation.

Analyses of BALF (BN Rats)

BN Rats, 1-Day Exposure (July 24) OVA-challenged rats, exposed to CAPs or filtered air, had significantly more total leukocytes in the BALF than saline-challenged/air-exposed rats (approximately 3.5 times for OVA-challenged/CAPs-exposed and 2.5 times for OVA-challenged/air-exposed; Table 13). Both OVA-challenged/air-exposed rats and OVA-challenged/CAPs-exposed rats had significantly more eosinophils in the BALF than the respective saline-challenged controls. However, only the OVA-challenged/CAPs-exposed rats had significantly more lymphocytes and alveolar macrophages in the BALF compared saline-challenged/air-exposed rats (Table 13). We found no significant differences in the number of neutrophils in the BALF among the exposure groups. CAPs exposure did not alter the cellularity of the BALF in saline-challenged rats (without allergen-induced airway disease).

The only significant biochemical differences in the BALF among the exposure groups were significantly greater amounts of elastase and Muc5ac protein in the BALF of OVA-challenged/CAPs-exposed rats compared to saline-challenged/CAPs-exposed or OVA-challenged/air-exposed rats (Table 14). The amount of β-glucuronidase in the BALF of OVA-challenged/ CAPs-exposed rats was also a significantly greater than in saline-challenged/CAPs-exposed rats. Interestingly, neither CAPs exposure nor OVA challenge alone induced any significant changes in the biochemical parameters that were analyzed.

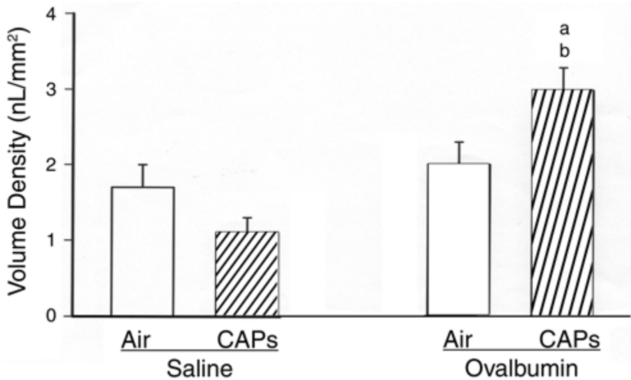


Figure 19. Morphometric estimates of the amount of AB/PAS-stained IM in the surface epithelium of the pulmonary axial airway of saline-challenged or OVA-challenged BN rats exposed to CAPs or filtered air for 5 days in September. a = significantly different from saline-challenged/CAPs-exposed rats; and b = significantly different from OVA-challenged/air-exposed rats; $P \leq 0.05$.

Table 13. BALF Analyses: Cells Recovered from Rats Exposed via Inhalation[a]

	Airway Status and Exposure Atmosphere[b]	Total Leukocytes	Macrophages	Neutrophils	Eosinophils	Lymphocytes
BN Rats, July						
1 Day	Sal/Air	31.8 ± 3.2	23.8 ± 3.0	1.4 ± 0.3	5.2 ± 1.0	1.3 ± 0.4
	Sal/CAPs	27.6 ± 3.6	20.4 ± 3.6	1.6 ± 0.5	6.6 ± 2.7	0.9 ± 0.3
	OVA/Air	81.8 ± 12.7[c]	49.3 ± 8.8	1.1 ± 0.5	23.9 ± 7.4[c]	5.6 ± 1.8
	OVA/CAPs	112.4 ± 45.5[d]	73.9 ± 33.8[d]	0.3 ± 0.1	26.9 ± 9.7[d]	10.3 ± 4.2[d]
4 Days	Sal/Air	30.8 ± 5.5	22.4 ± 2.3	1.1 ± 0.4	6.9 ± 2.9	1.2 ± 0.5
	Sal/CAPs	32.2 ± 4.9	25.1 ± 4.1	0.8 ± 0.3	5.8 ± 2.3	1.2 ± 0.4
	OVA/Air	52.2 ± 8.4[c]	38.9 ± 5.0[c]	0.4 ± 0.1	8.5 ± 2.2	5.0 ± 1.9[c]
	OVA/CAPs	43.6 ± 3.8	33.6 ± 3.6	0.1 ± 0.1	5.4 ± 1.1	2.9 ± 0.5
BN Rats, September						
1 Day	Sal/Air	25.2 ± 3.1	18.5 ± 2.0	1.9 ± 0.9	3.8 ± 2.8	1.1 ± 0.8
	Sal/CAPs	29.0 ± 4.3	24.4 ± 4.0	1.2 ± 0.3	2.4 ± 1.8	0.9 ± 0.6
	OVA/Air	105.6 ± 41.1[c]	41.5 ± 7.9[c]	5.0 ± 4.0	50.6 ± 29.4[c]	8.6 ± 3.7[c]
	OVA/CAPs	57.0 ± 10.3	35.4 ± 3.9	1.1 ± 0.3	17.0 ± 7.0[d]	3.5 ± 1.1[d]
5 Days	Sal/Air	21.0 ± 8.5	19.1 ± 2.8	0.4 ± 0.2	1.4 ± 1.0	0.2 ± 0.1
	Sal/CAPs	22.1 ± 12.2	20.6 ± 5.2	0.1 ± 0.04	1.3 ± 0.5	0.1 ± 0.1
	OVA/Air	34.3 ± 13.0[c]	28.9 ± 3.7	0.3 ± 0.04	3.9 ± 1.7[c]	1.3 ± 0.6[c]
	OVA/CAPs	43.0 ± 10.6[d]	36.1 ± 2.9 [d]	0.4 ± 0.1	5.4 ± 1.7[d]	1.2 ± 0.4[d]
F344 Rats, July						
1 Day	S/Air	44.8 ± 11.2	43.3 ± 11.1	0.4 ± 0.3	0.0	1.1 ± 0.4
	S/CAPs	36.3 ± 2.8	35.3 ± 2.6	0.2 ± 0.1	0.0	0.8 ± 0.4
	E/Air	69.0 ± 9.3[e]	52.8 ± 7.8	5.7 ± 0.9[e]	0.0	9.6 ± 1.3[e]
	E/CAPs	67.5 ± 3.6[f]	50.9 ± 2.6[f]	6.2 ± 2.2[f]	0.0	10.3 ± 1.2[f]
5 Days	S/Air	33.8 ± 4.8	32.1 ± 4.1	0.2 ± 0.1	0.0	1.4 ± 0.8
	S/CAPs	28.0 ± 2.7	27.5 ± 2.8	0.0 ± 0.0	0.0	0.2 ± 0.1
	E/Air	39.0 ± 3.4	37.6 ± 3.1	0.0 ± 0.0	0.0	1.3 ± 0.5
	E/CAPs	41.7 ± 4.1[f]	40.6 ± 4.1[f]	0.0 ± 0.0	0.0	1.0 ± 0.3
F344 Rats, September						
1 Day	S/Air	25.4 ± 1.3	24.9 ± 1.4	0.4 ± 0.1	0.0	0.4 ± 0.1
	S/CAPs	28.0 ± 2.5	27.3 ± 2.7	0.5 ± 0.2	0.0	0.3 ± 0.1
	E/Air	33.2 ± 3.1	30.6 ± 2.3	1.8 ± 1.6	0.0	0.9 ± 0.3
	E/CAPs	32.5 ± 4.7	30.2 ± 4.0	0.9 ± 0.5	0.0	1.8 ± 0.9
4 Days	S/Air	23.8 ± 1.8	23.8 ± 1.8	0.1 ± 0.04	0.0	0.1 ± 0.04
	S/CAPs	27.6 ± 5.2	27.7 ± 6.3	0.2 ± 0.1	0.0	0.1 ± 0.1
	E/Air	24.1 ± 2.1	23.8 ± 2.3	0.1 ± 0.04	0.0	0.4 ± 0.1
	E/CAPs	23.3 ± 3.3	22.7 ± 2.7	0.1 ± 0.04	0.0	0.5 ± 0.1[f]

[a] Data are group means ± SEM (cells × 10^4/mL).

[b] Sal = saline-challenged rats; OVA = OVA-challenged rats; S = saline-pretreated rats; E = endotoxin-pretreated rats.

[c] Significantly different from Sal/Air group ($P \leq 0.05$).

[d] Significantly different from Sal/CAPs group ($P \leq 0.05$).

[e] Significantly different from S/Air group ($P \leq 0.05$).

[f] Significantly different from S/CAPs group ($P \leq 0.05$).

Table 14. BALF Analyses: Biochemical Data for Rats Exposed via Inhalation[a]

	Airway Status and Exposure Atmosphere[b]	Muc5ac (V_{max})	Total Protein (µg/mL)	Elastase (V_{max})	Alkaline Phosphatase (units/mL)	Acid Phosphatase (units/mL)	β-Glucuronidase (V_{max})
BN Rats, July							
1 Day	Sal/Air	34.2 ± 2.8	144.2 ± 15.1	11.5 ± 0.7	4.4 ± 0.3	0.5 ± 0.02	10.6 ± 0.7
	Sal/CAPs	29.9 ± 3.2	129.4 ± 15.9	11.7 ± 1.0	4.3 ± 0.4	0.5 ± 0.04	9.6 ± 0.7
	OVA/Air	46.2 ± 3.9	145.0 ± 12.4	14.2 ± 0.9	4.3 ± 0.5	0.5 ± 0.02	11.3 ± 0.8
	OVA/CAPs	58.4 ± 6.0[c,d]	173.8 ± 31.7	17.5 ± 1.0[c,d]	5.5 ± 0.8	0.5 ± 0.02	12.8 ± 0.6[c]
4 Days	Sal/Air	25.2 ± 2.0	270.7 ± 18.0	15.7 ± 1.1	6.4 ± 0.4	0.5 ± 0.01	9.5 ± 0.7
	Sal/CAPs	25.0 ± 2.1	251.0 ± 9.0	13.3 ± 0.8	5.6 ± 0.5	0.5 ± 0.01	7.7 ± 0.4
	OVA/Air	37.0 ± 2.8[e]	319.3 ± 20.4[e]	15.8 ± 1.0	6.4 ± 0.4	0.5 ± 0.02	8.7 ± 0.6
	OVA/CAPs	34.0 ± 2.3[c]	291.6 ± 15.5	15.3 ± 0.7	6.4 ± 0.5	0.5 ± 0.02	8.5 ± 0.3
BN Rats, September							
1 Day	Sal/Air	4.4 ± 0.2	114.6 ± 7.7	3.7 ± 0.3	3.2 ± 0.5	0.3 ± 0.02	3.8 ± 0.2
	Sal/CAPs	3.8 ± 0.3	129.4 ± 9.3	4.0 ± 0.2	3.5 ± 0.6	0.3 ± 0.02	4.2 ± 0.1
	OVA/Air	6.0 ± 0.7[e]	206.6 ± 30.5[e]	5.3 ± 0.6[e]	5.9 ± 1.4	0.4 ± 0.03	5.8 ± 0.7[e]
	OVA/CAPs	5.9 ± 0.5[c]	159.2 ± 27.1	5.1 ± 0.5	3.8 ± 0.8	0.4 ± 0.04	5.7 ± 0.8
5 Days	Sal/Air	3.6 ± 0.3	151.1 ± 13.5	4.7 ± 0.4	2.0 ± 0.6	0.4 ± 0.02	4.9 ± 0.4
	Sal/CAPs	3.1 ± 0.3	164.8 ± 10.0	4.7 ± 0.2	2.3 ± 0.6	0.4 ± 0.02	4.7 ± 0.4
	OVA/Air	4.1 ± 0.2	163.6 ± 8.8	6.1 ± 0.5[e]	3.0 ± 0.7	0.4 ± 0.02	6.0 ± 0.6
	OVA/CAPs	4.3 ± 0.4	196.7 ± 16.7	6.4 ± 0.6[c]	3.5 ± 0.5	0.5 ± 0.03[c,d,e]	5.7 ± 0.3
F344 Rats, July							
1 Day	S/Air	23.8 ± 3.5	103.8 ± 9.6	5.3 ± 0.3	3.4 ± 0.5	0.3 ± 0.02	6.8 ± 0.8[f]
	S/CAPs	21.8 ± 2.7	113.2 ± 18.0	5.3 ± 0.2	3.2 ± 0.5	0.3 ± 0.005	5.2 ± 0.3
	E/Air	47.2 ± 3.3[g]	156.0 ± 18.2	7.4 ± 0.3[g]	5.3 ± 0.5[g]	0.4 ± 0.06	6.5 ± 0.4
	E/CAPs	41.0 ± 4.5[f]	148.7 ± 21.7	7.0 ± 0.5[f]	4.7 ± 0.8	0.4 ± 0.09	6.9 ± 0.4[f]
5 Days	S/Air	24.6 ± 3.4	219.6 ± 6.4	6.3 ± 0.6	9.2 ± 0.5	0.7 ± 0.04	5.1 ± 0.4
	S/CAPs	25.0 ± 4.7	279.8 ± 47.9	8.0 ± 1.5	9.8 ± 0.5	0.9 ± 0.13	5.0 ± 0.3
	E/Air	28.2 ± 3.1	195.6 ± 7.8	6.5 ± 0.3	7.8 ± 0.4	0.7 ± 0.03	5.0 ± 0.1
	E/CAPs	38.0 ± 6.0	243.7 ± 24.0	7.0 ± 0.5	8.3 ± 0.8	0.8 ± 0.06	5.8 ± 0.6
F344 Rats, September							
1 Day	S/Air	11.8 ± 1.1	170.3 ± 7.4	0.04 ± 0.01	11.7 ± 0.5	0.6 ± 0.03	6.1 ± 0.6
	S/CAPs	12.5 ± 1.0	136.3 ± 9.7[g]	0.009 ± 0.005	10.9 ± 0.7	0.5 ± 0.02	5.9 ± 0.4
	E/Air	14.6 ± 1.6	156.9 ± 6.4	0.2 ± 0.2	10.7 ± 0.5	0.6 ± 0.02	5.5 ± 0.3
	E/CAPs	16.3 ± 1.6	153.5 ± 5.3	0.03 ± 0.007	10.5 ± 0.5	0.5 ± 0.01	6.5 ± 0.6
4 Days	S/Air	8.3 ± 0.5	112.2 ± 7.7	10.3 ± 0.5	13.0 ± 2.0	0.5 ± 0.06	8.0 ± 0.6
	S/CAPs	10.5 ± 1.5	113.6 ± 7.0	12.2 ± 1.8	12.5 ± 1.7	0.5 ± 0.05	7.7 ± 0.5
	E/Air	10.2 ± 1.0	100.7 ± 4.9	11.6 ± 0.6	12.9 ± 1.4	0.7 ± 0.03[f]	9.3 ± 0.4
	E/CAPs	12.6 ± 1.5	100.5 ± 5.9	10.0 ± 0.6	13.1 ± 1.2	0.6 ± 0.04	8.0 ± 0.5

[a] Data are group means ± SEM.

[b] Sal = saline-challenged rats; OVA = OVA-challenged rats; S = saline-pretreated rats; E = endotoxin-pretreated rats.

[c] Significantly different from Sal/CAPs group ($P \leq 0.05$).

[d] Significantly different from OVA/Air group ($P \leq 0.05$).

[e] Significantly different from Sal/Air group ($P \leq 0.05$).

[f] Significantly different from S/CAPs group ($P \leq 0.05$).

[g] Significantly different from S/Air group ($P \leq 0.05$).

BN Rats, 4-Day Exposure (July 25–28) Compared to the saline-challenged/air-exposed controls, OVA-related changes in the cellularity or biochemistry of the BALF were primarily seen as increases in the numbers of total leukocytes, lymphocytes, and macrophages (Table 13) and in the amount of total protein and Muc5ac protein (Table 14).

No changes related to CAPs exposure were detected in BALF from rats challenged with either saline or OVA.

Compared to control rats, neither OVA challenge nor CAPs exposure caused changes in the gene expression of *Muc5ac* (Table 11) or in the BrdU-labeling index of the surface epithelium (Table 12) in the pulmonary axial airways.

BN Rats, 1-Day Exposure (September 25) OVA-challenged rats had statistically significant increases in the numbers of total leukocytes, lymphocytes, eosinophils, and alveolar macrophages compared to saline-challenged control rats (Table 13). This was accompanied by significant biochemical changes that included elevations in total protein, Muc5ac protein, elastase, and β-glucuronidase (Table 14).

No CAPs-related changes in the cellular or biochemical profiles of BALF were detected when compared with either group of air-exposed rats.

BN Rats, 5-Day Exposure (September 26–30) OVA-challenged rats exposed to either filtered air or CAPs had greater numbers of total leukocytes, eosinophils, and lymphocytes in BALF compared with their respective saline-challenged control groups (Table 13; Figure 20). OVA-challenged/CAPs-exposed rats also had greater numbers of macrophages in BALF compared to saline-challenged/CAPs-exposed rats.

No significant differences in the cellularity of the BALF could be attributed to the CAPs exposures in either the saline- or OVA-challenged animals.

Differences in the biochemical content of BALF in this exposure protocol were restricted to elastase and acid phosphatase (Table 14, Figure 21). OVA challenge induced a significant increase in elastase for both CAPs- and air-exposed rats. There was no significant difference in the

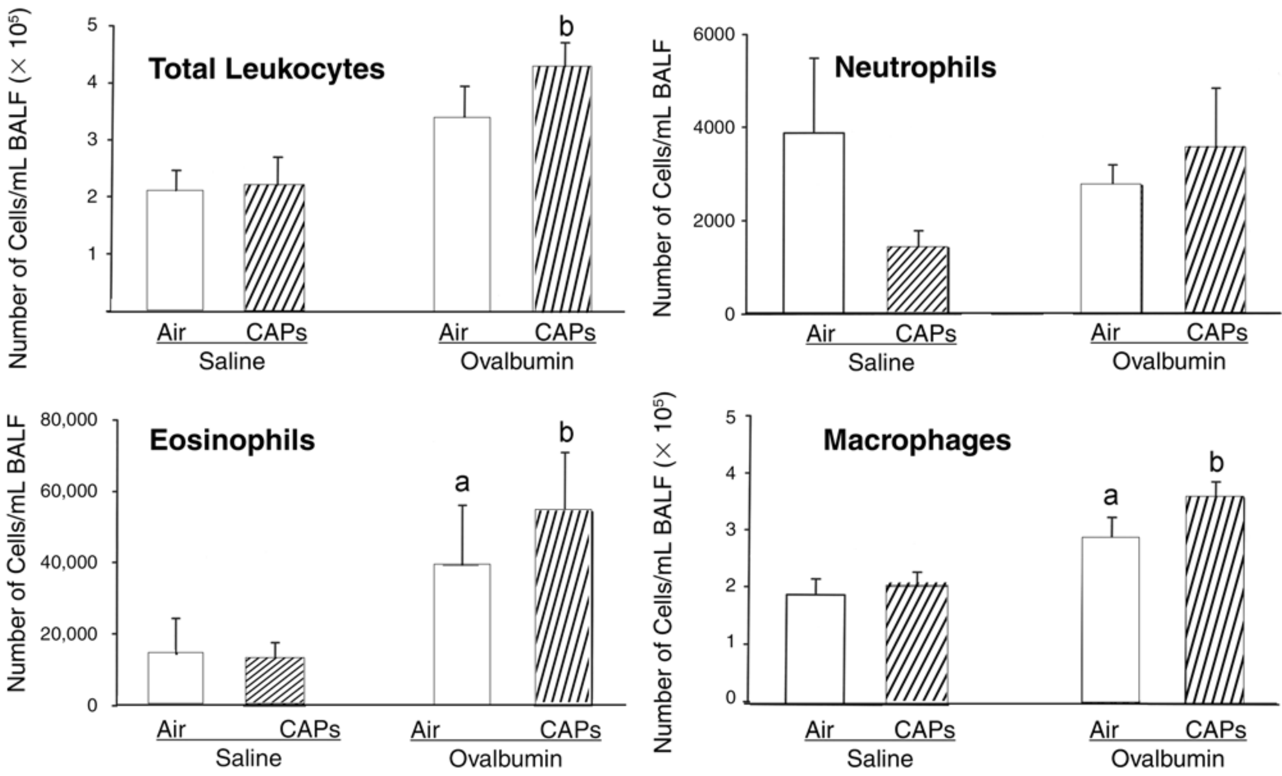
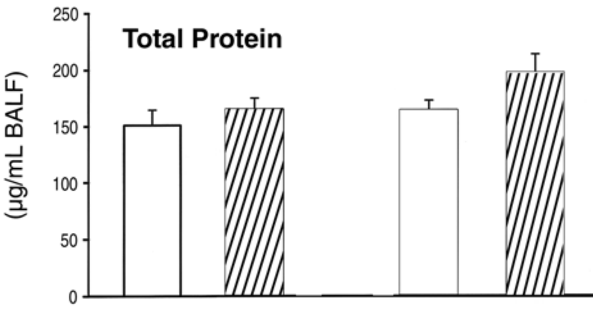
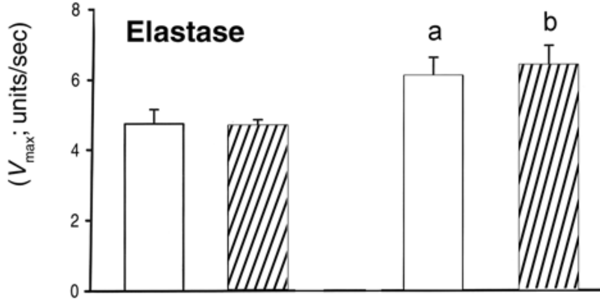


Figure 20. Recovered cells in the BALF from saline-challenged or OVA-challenged BN rats exposed to CAPs or filtered air for 5 days in September. a = significantly different from saline-challenged/air-exposed rats; and b = significantly different from saline-challenged/CAPs-exposed rats; $P \leq 0.05$. Note the different scales on the y axes.

amounts of elastase between OVA-challenged/air-exposed rats and OVA-challenged/CAPs-exposed rats. The amount of acid phosphatase was significantly greater in OVA-challenged/CAPs-exposed rats compared all three other exposure groups (Table 14, Figure 21). No differences in these parameters of BALF were detected among the other exposure groups. In addition, no differences in the amount of β-glucuronidase were found among any of the four exposure groups.





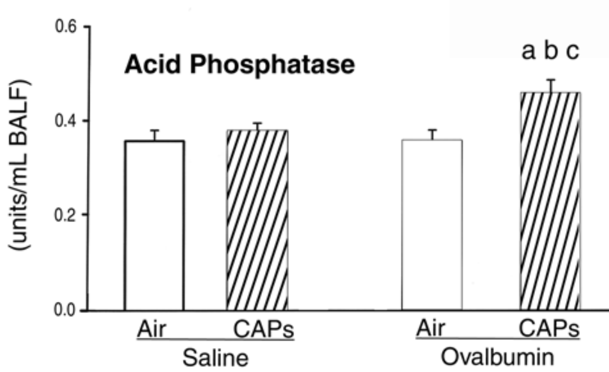


Figure 21. Biochemical determinations of total protein, elastase, and acid phosphatase in the BALF from saline-challenged or OVA-challenged BN rats exposed to CAPs or filtered air for 5 days in September. a = significantly different from saline-challenged/air-exposed rats; b = significantly different from saline-challenged/CAPs-exposed rats; and c = significantly different from OVA-challenged/air-exposed rats; $P \leq 0.05$. Note the different scales on the y axes.

Interestingly, saline-challenged/CAPs-exposed rats had no biochemical or cellular differences from saline-challenged/air-exposed rats, which suggests that CAPs does not have an adverse effect on normal airways that have no preexisting allergic airway disease. In addition, no increases in Muc5ac protein (another indicator of airway hypersecretion of mucosubstances) were found in any of the CAPs-exposed or OVA-challenged rats compared to saline-challenged/air-exposed control rats (Table 14).

Comparison of the July and September Inhalation Exposure Protocols for BN Rats

Histopathologic analysis of lung tissues revealed increased levels of IM in the epithelium that lines the pulmonary conducting airways in OVA-challenged/CAPs-exposed rats during the 5-day exposure protocol in September (Figure 19). Elevated numbers of alveolar macrophages, leukocytes, and eosinophils in BALF were also observed in the these OVA-challenged/CAPs-exposed animals compared with the numbers in the saline-challenged/CAPs-exposed BN rats (Table 13).

The next step in our investigation attempted to ascertain whether any physical properties or chemical components of $PM_{2.5}$ were associated with the observed airway inflammation and epithelial alterations. To identify $PM_{2.5}$ properties that were likely to be responsible for the observed adverse respiratory responses, we compared the major constituents of ambient $PM_{2.5}$ and CAPs from the two time periods during which BN rats were exposed: July 25–28 (4 days) and September 26–30 (5 days). These two periods were chosen because significant adverse health effects had been found in September and minimal adverse effects had been observed in July.

Distinct differences in particle characteristics were found between these BN rat exposure protocols. Both ambient (Tables 4, 5, and 6) and CAPs (Table 8) mass concentrations in July were at least twice the concentrations in September, reaching the highest concentrations measured during our entire investigation. Other major differences: (1) The fraction of $PM_{0.18}/PM_1$ mass (Table 5) in September was twice as high as in July, although the average number concentrations of PM_1 were similar. Well-correlated increases of both primary pollutants (NO and SO_2 concentrations) and the number concentration of ultrafine particles in September implicate local sources (see the sections Results / Characteristics of Urban Ambient Particles / "Particle Size Distribution" and "Gaseous Pollutants"). (2) The fractions of SO_4^{2-} in ambient $PM_{2.5}$ and in CAPs in July reached over 30%. Southwest winds, which brought significant amounts of secondary particles, were dominant in July. (3) In September, metal components (eg, V) that could undergo the

Fenton reaction and relatively insoluble elements (eg, La) that are typically associated with local combustion sources (eg, oil refinery and oil combustion) were recovered from CAPs-exposed rat lung tissues. In contrast, anthropogenic trace elements were not recovered from lung tissues of rats exposed to CAPs in July.

These findings suggest that adverse biological responses to ambient $PM_{2.5}$ may be associated more closely with an impact of local sources and weather patterns than with particle mass.

INHALATION COMPONENT: F344 RATS

Urban Atmospheric Conditions and CAPs Characteristics (F344 Rats)

F344 Rats, 1-Day Exposure (July 17) Ambient $PM_{2.5}$ was 19.6 g/m^3 and CAPs reached 266.3 g/m^3 (Table 9). The wind direction was predominantly out of the southwest on this day. On average, the fraction of $PM_{0.18}$ /PM_1 number concentration was over 0.9 (Table 5), which implies strong impacts from local sources. In fact, the ultrafine number concentration during morning rush hour reached its maximum of 110,106 particles/cm^3 during this 10-hour exposure period (Table 5). The morning increases in NO_x and in the number of ultrafine particles was observed during the morning rush hours when local motor vehicle emissions and other local-source emissions were released into a relatively shallow atmospheric boundary layer.

F344 Rats, 5-Day Exposure (July 18–22) During this exposure period, northerly winds brought relatively clean air masses into the city and ambient $PM_{2.5}$ during the 5 days reached only 8.9 g/m^3 (Table 6). The average concentration of OC was only 1.8 g/m^3, SO_4^{2-} only 1.4 g/m^3, and NO_3^- only 0.6 g/m^3 (Table 6). The average concentration of CAPs was only 71.0 g/m^3 (Figure 10 and Table 9).

F344 Rats, 1-Day Exposure (September 18) The average ambient $PM_{2.5}$ and CAPs concentrations during this period were 20.5 µg/m^3 (Table 6) and 175.8 µg/m^3 (Table 9), respectively. The impact of local emissions was pronounced on this exposure day. Increased levels of many trace metals were observed. Concentrations of Pb, La, Ba, Sb, Cu, Co, Fe, Mn, Zn, Y, Cd, and Sr were approximately twice as high as average concentrations of each element during all the exposure periods in July and September (Table 7). Particularly, levels of Sb and Cu were 5 times higher than their average concentrations (Table 7). In addition, one of the highest average $PM_{0.1}$ number concentrations measured by SMPS (12,941 ± 11,756 particles/cm^3; Table 5) was observed on this day. Concentrations of ambient OC and NO_3^- particles on this day reached the second highest and highest levels, respectively, during all the exposure periods (Table 6).

F344 Rats, 4-Day Exposure (September 19–22) Some variability was observed during this exposure period. The average ambient $PM_{2.5}$ concentrations were approximately 30 µg/m^3 for the first two days (Table 6). Our data revealed strong impacts from local sources, particularly on the September 19. First, the average $PM_{0.1}$ number concentration reached one of the highest levels during this entire series of exposures: 18,751 ± 6925 particles/cm^3 (Table 5). Next, the predominant wind direction was from the southeast and the levels of NO_3^-, SO_4^{2-}, and OC were elevated (Table 6). In addition, both Zn and Rb concentrations were twice as high as the average concentrations of each element during all the exposure periods in July and September.

A previous study in Detroit had determined that Rb and Zn are associated with emissions from sludge incinerators that are located close to our sampling site (Gildemeister 2001). This is not surprising because several municipal incinerators are located in southwest Detroit. In fact, the City of Detroit Water and Sewerage Department operates one of the largest sewage sludge incinerators in the United States and it is located between our sampling site and Zug Island on Jefferson Avenue. The EPA (2000) AirData NET facility emissions report estimated that approximately seven tons of $PM_{2.5}$ were emitted from this facility in 2000.

In contrast, for the last 2 days of this 4-day exposure period, the average ambient $PM_{2.5}$ concentration was below 10 µg/m^3 (Figure 5). The wind direction gradually shifted away from the southeast and was more variable on the last day of this exposure period.

Pulmonary and Nasal Histopathology, Airway IM, and Analyses of BALF (F344 Rats)

Histopathology and Changes in BALF Caused by Endotoxin Alone in F344 Rats Principal pulmonary lesions induced by the endotoxin pretreatment were mild to moderate subacute bronchiolitis and alveolitis. Both large (proximal axial airways) and small bronchioles (distal axial and other small-diameter preterminal and terminal airways) and centriacinar regions of the alveolar parenchyma (alveolar ducts and adjacent alveoli) were affected by the instillation of endotoxin. Increased numbers of alveolar macrophages along with monocytes, lymphocytes, and neutrophils were present in the affected alveolar parenchyma. This was associated with a mild to moderate type II cell hyperplasia in the affected alveolar septa. A mixed inflammatory cell influx of neutrophils, eosinophils, and monocytes were also present in slightly edematous interstitial tissue surrounding the bronchioles

and blood vessels (pulmonary arteries and small veins). Larger aggregates of alveolar macrophages and epithelioid cells were occasionally present in proximal alveolar ducts along with thickened interalveolar septa due to epithelial cell proliferation and mild interstitial fibrosis.

Mild to moderate hypertrophy/hyperplasia of bronchiolar epithelium with increased numbers of AB/PAS-positive mucous goblet cells (mucous cell metaplasia/hyperplasia; predominantly large-diameter bronchioles) was also present in the endotoxin-pretreated pulmonary airways with a mixed inflammatory cell influx (bronchiolitis).

In the analyses of BALF, all endotoxin-pretreated rats had significant increases in the amounts of Muc5ac protein and elastase compared to saline-pretreated rats (Table 14). They also had increased numbers of total leukocytes, lymphocytes, and neutrophils compared to controls (Table 13).

Compared to the nose and lungs of endotoxin-pretreated rats examined after a 1-day exposure to filtered air or CAPs, rats exposed for 5 days had minimal endotoxin-related airway alterations; this suggests that endotoxin-induced airway inflammation and airway epithelial alterations had resolved by the end of 5 days. Endotoxin-pretreated rats exposed for 5 days (to either CAPs or air) had minimal peribronchiolar and perivascular edema and minimal accumulation of inflammatory cells, which consisted mainly of mononuclear cells (lymphocytes, monocytes, and plasma cells). Noticeably absent were accumulations of neutrophils and eosinophils in the interstitial regions around bronchioles and small blood vessels; such accumulations had been conspicuous in the lungs of endotoxin-pretreated rats exposed for only 1 day to filtered air or CAPs. In addition, mucous cell metaplasia and hyperplasia and thickening of the bronchiolar epithelium was slight or not evident. Endotoxin-induced centriacinar alveolitis and type II cell hyperplasia, which had been evident in the rats exposed for 1 day, were also minimal or absent in these rats; again, this suggests that this induced lung lesion had resolved by the end of 5 days of exposure.

Like the F344 rats pretreated with endotoxin in July, we noted marked hypertrophy of the respiratory epithelium that lines the middle septum due to a significant increase in IM compared to saline-pretreated rats.

F344 Rats, 1-Day Exposure (July 17) There were significant increases in the amounts of AB/PAS-stained IM in the proximal axial airways of these rats (Table 11). Similar increases in IM were evident in the middle septum of the proximal nasal airways of endotoxin-pretreated/CAPs-exposed rats. Endotoxin-pretreated/CAPs-exposed rats had 2.85 times more IM in the middle nasal septum compared to saline-pretreated/CAPs-exposed rats in this exposure protocol (Table 11).

Interestingly, endotoxin-pretreated rats had the same level of pulmonary histopathology and IM (caused by the endotoxin-pretreatment) regardless of whether they were exposed to CAPs or to filtered air. In addition, no microscopically detectable nasal or pulmonary injury was present in saline-pretreated/CAPs-exposed rats.

Exposure to CAPs did not enhance or attenuate the endotoxin-induced biochemical or cellular changes in BALF. Endotoxin-pretreated rats exposed to CAPs did have significantly greater numbers of alveolar macrophages compared to saline-pretreated/CAPs-exposed rats (Table 13). Measured parameters in BALF from saline-pretreated/CAPs-exposed rats were not significantly different from saline-pretreated/air-exposed rats, which indicates that a 1-day exposure to CAPs in rats without airway inflammation did not cause pulmonary injury detectable by lavage.

F344 Rats, 5-Day Exposure (July 18–22) We found no significant increases in the amount of nasal or pulmonary IM, and no CAPs- or endotoxin-related alterations in mucin-specific gene expression or in the DNA synthesis of bronchiolar epithelium (Tables 11 and 12). In the analyses of BALF, no significant cellular or biochemical changes related to endotoxin instillation or CAPs exposure were detected except for a mild endotoxin-induced increase in total leukocytes and macrophages in the F344 rats exposed to CAPs (Tables 13 and 14). In addition, there was a modest, but significant, CAPs-induced increase in the percentage of BrdU-labeled cells in the pulmonary axial airway epithelium in the rats pretreated with endotoxin (Table 12).

F344 Rats, 1-Day Exposure (September 18) Some of the endotoxin-pretreated rats also had an associated minimal to mild rhinitis characterized by small aggregates of mixed inflammatory cells (neutrophils and mononuclear cells) in the lamina propria of the septal mucosa.

The 1-day exposure to CAPs did not cause any significant alterations to the nasal epithelium except for a mild, but significant, loss of IM in the respiratory epithelium that lines the proximal nasal septum in saline-pretreated rats. This was not associated with any inflammatory response or any histologic evidence of epithelial degeneration. In addition, the 1-day exposure to CAPs did not change the endotoxin-induced increase of IM in the respiratory epithelium that lines the proximal septum.

Surprisingly, endotoxin-pretreated rats did not have any histologic alterations to the pulmonary conducting airways or alveolar parenchyma after a 1-day exposure to

CAPs or air. Furthermore, compared to control rats, neither endotoxin pretreatment nor CAPs exposure caused changes in the BrdU-labeling index (Table 12) of the surface epithelium in the pulmonary axial airways or overexpression of the *Muc5ac* gene (Table 11). Interestingly, endotoxin pretreatment caused a curious and unexplainable decrease in the expression of this gene in these rats.

In addition, compared to saline-pretreated rats, we noted no changes in the BALF of endotoxin-pretreated rats (Tables 13 and 14). CAPs-exposure also did not cause any histologic or biochemical changes compared to rats exposed only to filtered air.

F344 Rats, 4-Day Exposure (September 19–22) As with the F344 rats exposed for 1 day, no endotoxin-related lesions were evident in the lungs of rats exposed to either filtered air or CAPs.

F344 rats exposed to CAPs for 4 days in September had no CAPs-related alterations to the conducting airways or alveolar parenchyma. Exposure to CAPs for 4 days also did not cause any lesions in the nasal mucosa or changes in the IM in the respiratory epithelium that lines the nasal or pulmonary airways (Table 11).

Compared to control rats, neither endotoxin instillation nor CAPs-exposure caused changes in the gene expression of *Muc5ac* or in the BrdU-labeling index of the surface epithelium in the pulmonary axial airways (Tables 11 and 12).

In addition, no differences in the cellular or biochemical content of the BALF were evident between rats exposed to CAPs and those exposed to air (Tables 13 and 14).

$PM_{2.5}$ INTRATRACHEAL INSTILLATION COMPONENT

Characterization of Isolated $PM_{2.5}$ Used in Instillation Studies

To assess the efficiency of particle recovery from the high-volume $PM_{2.5}$ filters, extracted particles were analyzed for metal composition by ICPMS and the results compared with metal analysis from the ambient MOI samples. As previously described (see Methods / Trace Elements), extraction of particles from MOI sample filters involved successive acid washes over a 2-week period. Although this method yields more particles, the acidified sample is less suitable for intratracheal instillation than samples isolated in deionized water. As shown in Figure 22, ambient air concentrations estimated from analysis of MOI samples were quantitatively greater than those determined from samples collected from high-volume filters; however, the relative composition of metals was qualitatively similar. We therefore concluded that the $PM_{2.5}$ isolated from the high-volume filters by ultrasonication, which we intratracheally instilled into rats, accurately represented the composition of $PM_{2.5}$ in ambient air on the days during which samples had been collected.

Because of mechanical failures, our ability to collect ambient $PM_{2.5}$ samples was limited to the first 2 days of the 5-day CAPs inhalation exposure with BN rats in September. Thus, the particles instilled into animals were not derived from the same population of $PM_{2.5}$ as the animals breathing CAPs for 5 days (Table 15).

The chamber concentration of $PM_{2.5}$ averaged 291 μg/m^3 for BN rats, September 26–30 (Table 8). We assumed minute ventilation of approximately 0.2 L and CAPs deposition of

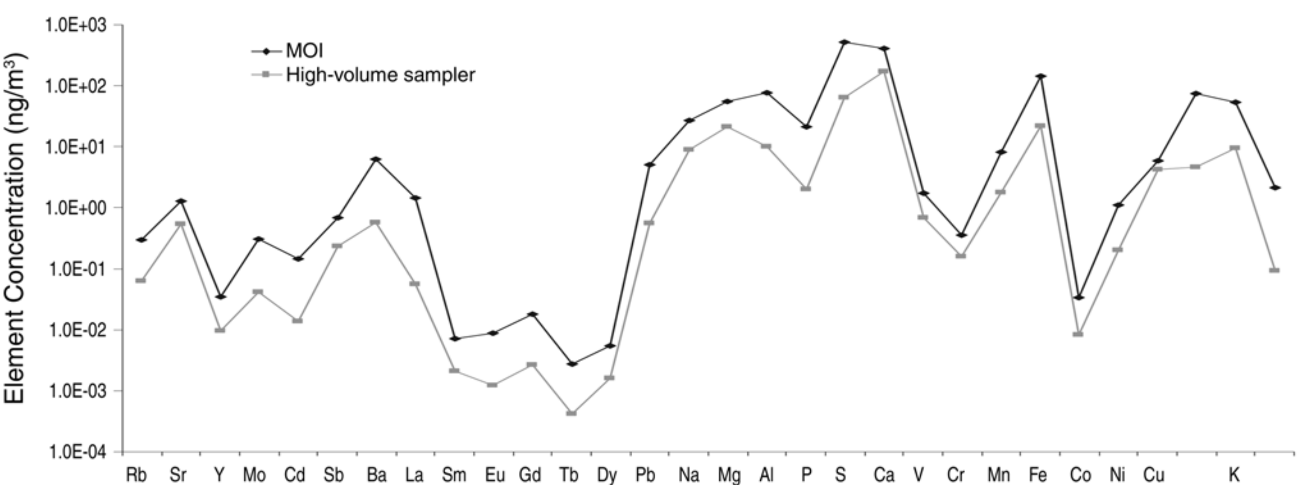


Figure 22. Comparison of the elemental composition of $PM_{2.5}$ collected on filters from the ambient MOI and from the high-volume sampler used to collect particles for the $PM_{2.5}$ instillation component. Samples from the MOI were isolated by acid extraction and those from the high-volume sampler by ultrasonication; elemental analysis was performed by ICPMS.

Table 15. Trace Element Concentrations in Ambient $PM_{2.5}$ During the September 5-Day Exposure Protocol Compared with the 2-Day Collection of $PM_{2.5}$ for the Instillation Component[a]

Element	First 2 Days[b] (Sept 26–27)	All 5 Days[c] (Sept 26–30)	Ratio of 5 Days:2 Days (Sept 26–30: Sept 26–27)
Rb	0.294	0.238	0.808
Sr	1.366	1.827	1.337
Y	0.036	0.041	1.133
Ag	0.041	0.106	2.582
Cd	0.147	0.756	5.146
Sb	0.682	0.666	0.976
Cs	0.035	0.029	0.817
Ba	6.581	7.069	1.074
La	1.340	0.335	0.250
Ce	0.393	0.170	0.432
Pr	0.029	0.018	0.623
Nd	0.052	0.051	0.975
Sm	0.008	0.009	1.177
Eu	0.009	0.010	1.067
Gd	0.018	0.014	0.775
Tb	0.003	0.003	0.993
Dy	0.006	0.006	1.063
Er	0.004	0.005	1.172
Yb	0.003	0.003	1.023
Tl	0.022	0.041	1.854
Pb	5.265	6.547	1.244
Na	24.255	34.135	1.407
Mg	57.378	61.545	1.073
Al	72.913	54.140	0.743
P	20.125	27.099	1.347
S	487.200	793.391	1.628
Ca	417.226	365.748	0.877
Ti	2.547	1.989	0.781
V	1.558	1.786	1.146
Mn	8.556	8.014	0.937
Fe	151.839	167.191	1.101
Co	0.035	0.066	1.882
Ni	0.976	1.086	1.113
Cu	6.073	4.216	0.694
Zn	71.985	43.397	0.603
K	50.418	52.930	1.050
As	1.924	2.773	1.442

[a] Values are given as ng/m^3.

[b] Particles were collected with the Anderson high-volume sampler onto Teflon-coated glass fiber filters.

[c] Particles collected with the MOI.

10%. Based on these assumptions we estimated that approximately 3.3 µg CAPs were deposited in the lungs during a single 10-hour exposure (0.2 L/min × 600 min/day of exposure × 277 µg/m^3 × m^3/1000 L × 0.1 pulmonary deposition = 3.3 µg CAPs per day of exposure). Therefore, the 100-µg dose of $PM_{2.5}$ that we instilled intratracheally was equivalent to 6 times the amount of CAPs estimated to be deposited in the lungs of rats exposed by inhalation for 5 days.

Analyses of BALF

Numbers and Types of Cells Significant particle-induced changes in BALF cellularity in saline-instilled rats were limited to increased neutrophils after instillation of total $PM_{2.5}$ (5-fold increase) or insoluble $PM_{2.5}$ (11-fold increase) (Figure 23). By comparison, numbers of neutrophils from OVA-challenged rats were unchanged after particle instillation compared to OVA-challenged/saline-instilled controls.

OVA-challenged animals had increased numbers of eosinophils (2.5-fold increase) and lymphocytes (3-fold) compared to saline-challenged/saline-instilled rats (Figure 23). Instillation of total, soluble, or insoluble $PM_{2.5}$ fractions significantly suppressed eosinophilic responses, whereas only the total and soluble fractions blocked lymphocyte accumulation. Accumulation of macrophages in OVA-challenged/saline-instilled rats were the same as in saline-challenged/saline-instilled animals (Figure 23). However, instillation of soluble or insoluble $PM_{2.5}$ fractions caused significant increases in macrophages in OVA-challenged rats compared with OVA-challenged/saline-instilled rats.

Muc5ac Protein Mucus hypersecretion was determined by measuring the amount of Muc5ac protein. Mucus secretion in saline-challenged animals was not altered by instillation of any fractions of $PM_{2.5}$ (Figure 24). OVA-challenged rats demonstrated mucus hypersecretion with approximately 2-fold increases in Muc5ac protein compared to their respective saline-challenged counterparts. Instillation with particles neither increased nor decreased OVA-associated mucus secretion.

Total Protein Instillation of insoluble $PM_{2.5}$, but not the total or soluble fractions, caused increased protein in saline-challenged rats compared with saline-challenged/saline-instilled rats (Figure 25). OVA-challenged/saline-instilled rats had similar protein concentrations as saline-challenged/saline-instilled rats. In OVA-challenged rats, instillation of total or soluble $PM_{2.5}$, but not the insoluble fraction, caused increased protein compared with their saline-challenged counterparts.

Elastase and β-Glucuronidase The amount of elastase and the concentration of β-glucuronidase was the same in

saline-challenged and OVA-challenged rats and was unaffected by $PM_{2.5}$ instillation (Figure 26).

Pulmonary and Nasal Histopathology and IM

IM Instillation of particles in saline-challenged rats caused no changes in the amount of stored IM in the proximal or distal pulmonary airways (Figure 27). Airways of OVA-challenged rats had significantly more IM in the distal (but not in the proximal) airways than their saline-challenged counterparts. Particle instillation in OVA-challenged airways neither increased nor decreased the amount of IM.

BrdU-Labeling of Epithelial Cells Incorporation of BrdU in airway epithelial cells undergoing DNA synthesis was similar in saline-challenged and OVA-challenged rats (Figure 28). Intratracheal instillation of $PM_{2.5}$ fractions had no effect on BrdU uptake in either saline- or OVA-challenged rats.

Pulmonary Mucin Gene Expression The expression of rat *Muc5ac* mRNA in pulmonary axial airways was assessed only in rats instilled with total $PM_{2.5}$. Saline-challenged/saline-instilled rats had basal mucin gene expression. Increases in rat *Muc5ac* mRNA in saline-challenged $PM_{2.5}$-instilled rats were not significant (Figure 29). In OVA-challenged rats, levels of mucin gene expression were similar to those in saline-challenged rats and were unaffected by $PM_{2.5}$ instillation.

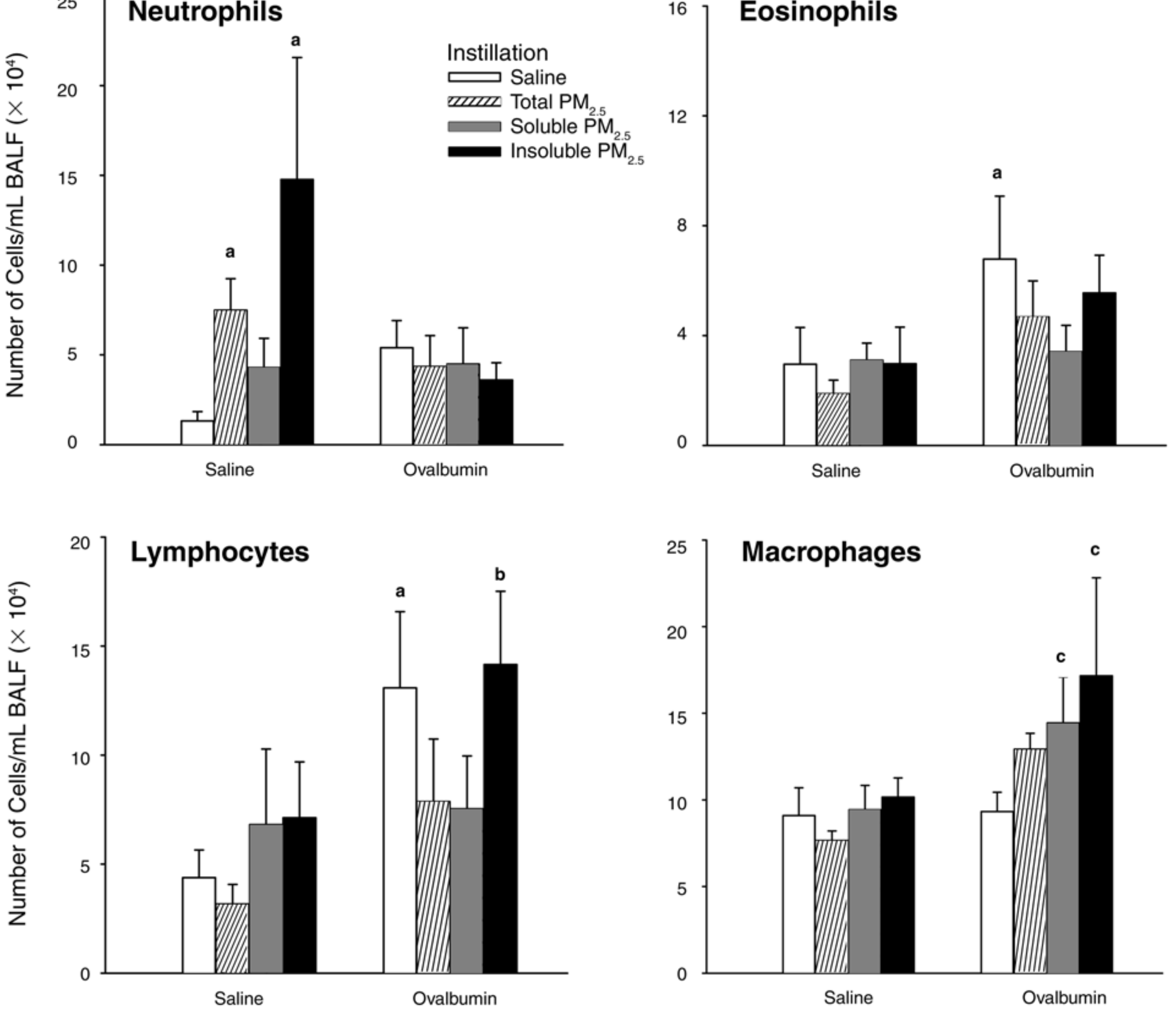


Figure 23. Effect of $PM_{2.5}$ instillation on the number and type of cells in the BALF from normal and OVA-challenged airways. BAL was performed 24 hours after particle instillation and cells were enumerated as described in the text. a = significantly different from saline-challenged/saline-instilled rats; b = significantly different from saline-challenged/insoluble $PM_{2.5}$–instilled rats; c = significantly different from OVA-challenged/saline-instilled rats; $P \leq 0.05$.

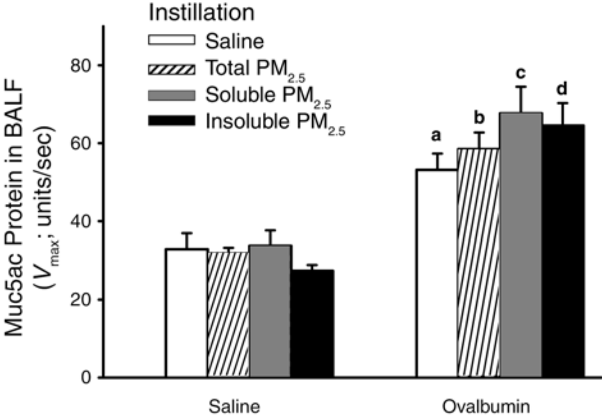


Figure 24. Effect of $PM_{2.5}$ instillation on mucus secretion in BALF from normal and OVA-challenged airways. BAL was performed 24 hours after particle instillation and mucin glycoprotein Muc5ac was determined by ELISA as described in the text. Letters denoting significant relations all compare the OVA-challenged rats with the equivalent instillation group among the saline-challenged rats; $P \leq 0.05$.

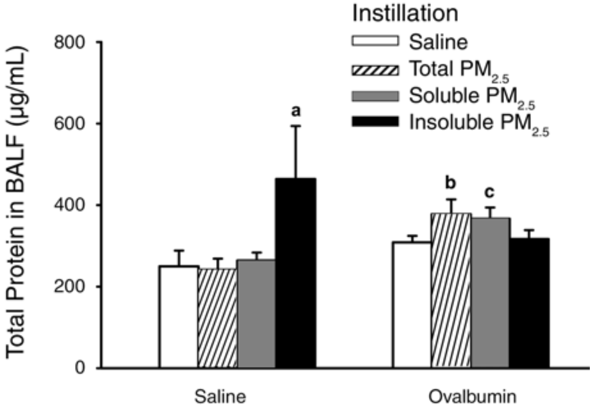


Figure 25. Effect of $PM_{2.5}$ instillation on total protein in BALF from normal and OVA-challenged airways. BAL was performed 24 hours after particle instillation and protein in lavage fluid was determined as described in the text. a = significantly different from saline-challenged/saline-instilled rats. Letters b and c denoting significant relations compare the OVA-challenged rats with the equivalent instillation group among the saline-challenged rats; $P \leq 0.05$.

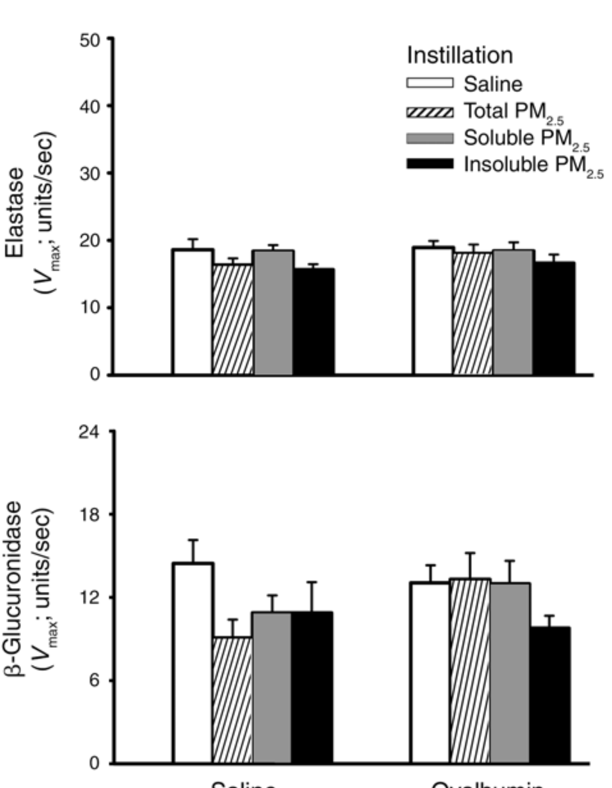


Figure 26. Effect of $PM_{2.5}$ instillation on elastase and β-glucuronidase in BALF from normal and OVA-challenged airways. BAL was performed 24 hours after particle instillation and elastase and β-glucuronidase in lavage fluid were determined as described in the text.

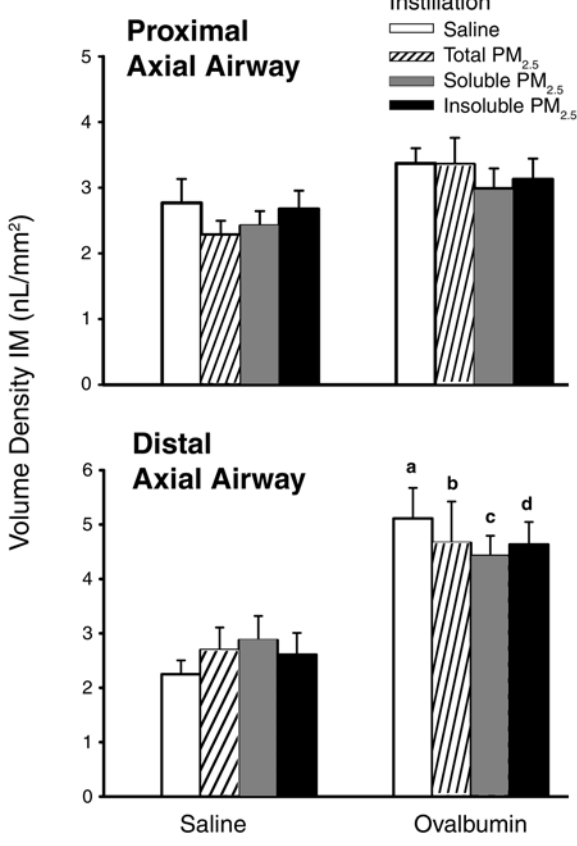


Figure 27. Effect of $PM_{2.5}$ instillation on IM in axial normal and OVA-challenged airways. Lung sections were removed 24 hours after $PM_{2.5}$ instillation and stained with AB/PAS and morphometry was performed as described in the text. Letters denoting significant relations all compare the OVA-challenged rats with the equivalent instillation group among the saline-challenged rats; $P \leq 0.05$.

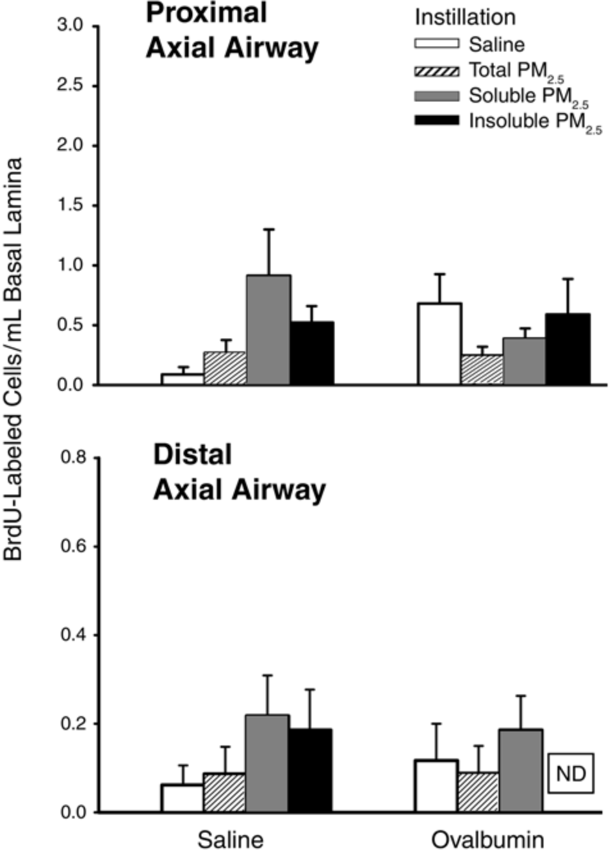


Figure 28. Effect of $PM_{2.5}$ instillation on cell proliferation in normal and OVA-challenged airways. Lungs were removed 24 hours after $PM_{2.5}$ instillation and sections were immunohistochemically stained for BrdU and positively stained cells were enumerated as described in the text. ND = no BrdU cells were detected.

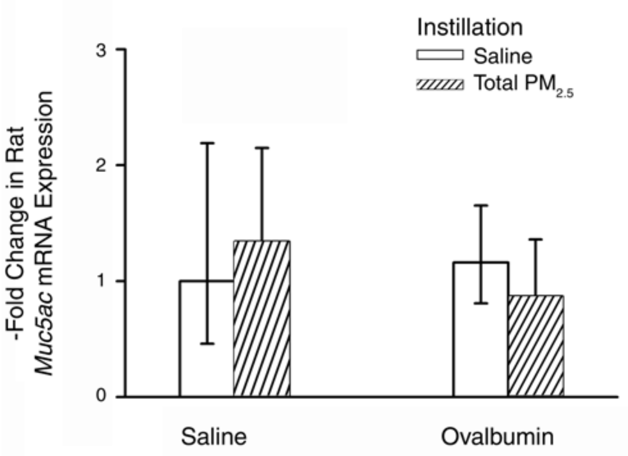


Figure 29. Effect of total $PM_{2.5}$ instillation on *Muc5ac* gene expression in normal and OVA-challenged airways. The saline-challenged/saline-instilled rats served as controls for the other three groups. Lungs were removed 24 hours after $PM_{2.5}$ instillation; RNA was isolated from microdissected axial airways and analyzed for *Muc5ac* by real-time PCR methods as described in the text.

DISCUSSION AND CONCLUSIONS

As part of this investigation, we conducted the most extensive physicochemical characterization of urban fine particulate air pollution in southwest Detroit communities to date. A primary focus of this work was to determine whether CAPs generated by the Harvard ambient fine particle concentrator reflected ambient particulate air pollution in a metropolitan Detroit community that has a high incidence of childhood asthma. Our characterization of the generated CAPs demonstrated that the Harvard ambient fine particle concentrator clearly concentrated the accumulation mode aerosols most effectively and, in general, the chemical characteristics of ambient particles were conserved through the concentrator and into the exposure chamber. Ultrafine aerosols (< 0.1 µm) were not significantly concentrated as they were at levels close to ambient in the exposure chambers.

This was the first project to our knowledge to use a mobile air research laboratory in the United States to conduct inhalation toxicology studies of laboratory rodents exposed to CAPs from an urban community. We have demonstrated that by using a well-designed mobile laboratory (such as AirCARE 1, equipped with the proper state-of-the-art exposure and monitoring equipment), a team of environmental scientists can successfully conduct animal inhalation toxicology “field studies” with naturally generated, “real world” air pollutants from urban communities where there are important public health concerns about daily exposures of children and adults to locally generated and transported airborne particulate matter from mobile vehicle and industrial sources.

The principal effects of CAPs exposure on the respiratory tracts of saline-challenged or OVA-challenged BN rats and saline-pretreated or endotoxin-pretreated F344 rats in the inhalation component of our investigation are summarized in Table 16. One of the most consistent results from all of the inhalation exposure protocols was the lack of CAPs-induced inflammation or alterations in the airway epithelium of the nose or lungs from saline-challenged BN or the saline-pretreated F344 control rats that had no histologic or biochemical evidence of preexisting nasal or pulmonary airway disease. Although these control rats with normal airways were exposed to the same chamber concentrations of CAPs as the endotoxin-pretreated or OVA-challenged rats, they had no detectable nasal or pulmonary injury resulting in airway inflammation or increases in airway epithelial mucosubstances that were measurably different from air-exposed rats. We conclude that these short-term exposures of CAPs from the outdoor urban environment did not cause adverse effects to the upper or lower

respiratory airways of normal BN or F344 rats. Similarly, there were no consistent findings in the histologic analyses, the morphometric measurements, or the analyses of the cellular or acellular components of BALF to suggest that CAPs caused an enhancement (or attenuation) of airway inflammation or airway epithelial alterations in the endotoxin-pretreated F344 rats.

The lack of CAPs-induced pulmonary alterations in normal laboratory rats is consistent with the findings of others (Gordon et al 2000; Kodavanti et al 2000). Gordon and coworkers (2000) found no indication of pulmonary inflammation or cytotoxicity in the lungs of male F344 rats exposed to a single (3-hour) or repeated (6 hours/day for 3 days) exposure to CAPs (132–919 μg/m^3) from New York City; nor did they find changes in pulmonary function. Similarly Kodavanti and colleagues in Research Triangle Park NC found no significant changes in the BALF of CAPs-exposed (650 μg/m^3, 6 hours/day for 2–3 days) male, 90- to 100-day old, Sprague Dawley rats compared to similarly aged rats exposed only to filtered air.

On the other hand, others have reported that short-term exposures of normal, Sprague Dawley male rats to CAPs from Boston (515 μg/m^3 average; 5 hours/day for 3 days) had significant increases in protein and inflammatory cells (neutrophils and lymphocytes) in the BALF compared to air-exposed controls (Clark et al 1999). This same group of investigators (Saldiva et al 2002) have also reported that the Boston CAPs–induced increase in neutrophils was an

Table 16. Summary of Principal Pathological Changes Induced by CAPs Exposure[a]

	Airway Status and Exposure Atmosphere[b]	Average CAPs	IM: Nasal Septum	IM: Pulmonary Airway	Mucin-Specific (*Muc5ac*) Gene Expression	BALF: Mucin-Specific (Muc5ac) Protein	BALF: Elastase	BALF: Acid Phosphatase	BALF: Leukocytes	Epithelial Cell Density in Pulmonary Airway
BN Rats, July										
1 Day	Sal/CAPs	59.5	—	—	—	—	—	—	—	—
	OVA/CAPs	59.5	—	—	—	↑	↑	—	—	—
4 Days	Sal/CAPs	615.7	—	—	—	—	—	—	—	—
	OVA/CAPs	615.7	↑	—	—	—	—	—	—	—
BN Rats, September										
1 Day	Sal/CAPs	79.7	—	—	—	—	—	—	—	—
	OVA/CAPs	79.7	—	—	—	—	—	—	—	—
5 Days	Sal/CAPs	291.0	—	—	—	—	—	—	—	—
	OVA/CAPs	291.0	—	↑	↓	—	—	↑	—	↑
F344 Rats, July										
1 Day	S/CAPs	266.3	—	—	—	—	—	—	—	—
	E/CAPs	266.3	—	—	—	—	—	—	—	—
5 Days	S/CAPs	71.0	—	—	—	—	—	—	—	—
	E/CAPs	71.0	—	—	—	—	—	—	—	—
F344 Rats, September										
1 Day	S/CAPs	175.8	—	—	—	—	—	—	—	—
	E/CAPs	175.8	—	—	—	—	—	—	—	—
4 Days	S/CAPs	327.7	—	—	—	—	—	—	—	—
	E/CAPs	327.7	—	—	↓	—	—	—	—	—

[a] Changes noted are in commparison to rats with the same airway status but exposed to filtered air: ↑ = increase; ↓ = decrease; — = no change

[b] Sal = saline-challenged rats; OVA = OVA-challenged rats; S = saline-pretreated rats; E = endotoxin-pretreated rats.

influx predominantly in the centriacinar region, rather than the more peripheral alveolar parenchyma of the rat lungs. Furthermore, they determined that the Boston CAPs–induced pulmonary inflammatory reaction observed in several rat studies was primarily influenced by the particle composition and not the mass concentration of the CAPs.

The reasons for the variability observed in the pulmonary responses of rats exposed to CAPs from Detroit MI (present investigation), Boston MA, New York NY, and Research Triangle Park NC are unknown; but they may include differences in the physicochemical composition of the CAPs. It is interesting to note that all of these laboratories used a similarly constructed Harvard ambient fine particle concentrator and generally similar short-term exposure regimens (5–10 hours/day for 1 or 3–5 days). In addition, the daily variability of and the mean of CAPs mass concentrations were similar among the laboratories. Some differences in the responses of normal rats to CAPs, however, may also be due to differences in the age and strain or stock of the laboratory rats used by the various investigating laboratories. Only our investigation used BN rats.

In contrast to the lack of pulmonary responses to CAPs in saline-pretreated F344 rats, endotoxin-pretreated (compromised) F344 rats, and saline-challenged BN rats, the OVA-challenged BN rats exposed to CAPs for 5 days in September did have some biochemical and histopathologic evidence of exposure-related airway injury and inflammation. These OVA-challenged BN rats had increases in eosinophils, macrophages (monocytes), elastase, total protein, and acid phosphatase in BALF, as well as increases in IM stored in the airway epithelium that lines the pulmonary axial airway. Only these OVA-challenged/CAPs-exposed rats demonstrated significant increases in these inflammatory and epithelial parameters compared to control rats. OVA-challenged/air-exposed BN animals had markedly less severe allergic bronchiolitis, alveolitis, and IM than OVA-challenged/CAPs-exposed rats.

Although CAPs-induced increases of IM stored in the axial airways were evident in the OVA-challenged BN rats exposed for 5 days in September, the expression of mucin-specific *Muc5ac* mRNA was lower than in control animals. We speculate that this mucin gene may have been transiently over-expressed early in the 5-day exposure protocol. In previous studies in our laboratory (Cho et al 1999), we have seen early, but transient, increases in *Muc5ac* mRNA with rats repeatedly exposed to ozone; these increases were noted before the histologic evidence of marked mucous cell metaplasia with morphometric increases of copious amounts of stored IM in the nasal epithelium was seen. In those ozone-exposed rats, the mucous cell metaplasia and increased amounts of stored IM may have persisted beyond the exposure period without any noticeable elevations in *Muc5ac* mRNA. Although we were unable to verify it in this investigation, an early but temporal elevation in mucin-specific gene expression could explain the lack of detectable changes in this gene in the CAPs-exposed rats (they were killed more than a week after their last OVA challenge and at least 24 hours after their last CAPs exposure).

In addition, the CAPs-induced increase in stored IM and elevations in the estimators of pulmonary inflammation in the BALF were not accompanied by increases in epithelial cell density or BrdU-labeling index (both markers of cell proliferation). We found no evidence of epithelial cell injury with subsequent reparative cell proliferation in the axial pulmonary airways in these animals or in any of the other BN or F344 rats exposed to CAPs. This suggests that the increase in the stored IM in the OVA-challenged rats exposed to CAPs for 5 days in September was not due to a proliferation of new mucus-secreting cells but to an OVA- and CAPs-induced increase in the production and storage of IM in preexisting airway secretory cells.

All of the OVA-challenged rats from both September protocols had histologic evidence of mild allergic rhinitis, bronchiolitis, and alveolitis. OVA challenge caused significant increases in IM stored in the nasal epithelium of both CAPs- and air-exposed rats. However, the OVA-sensitization and challenge regimen did not cause a morphometrically detectable increase in IM in the pulmonary axial airways of air-exposed BN rats after either a 1-day or 5-day inhalation. These rats were killed either 3 or 7 days after their last OVA challenge. Therefore, it is possible that any early OVA-induced increase in the IM attenuated during the postchallenge period when these rats were exposed only to filtered air. This suggests that the CAPs exposure of the other OVA-challenged rats may have prevented some of the normal attenuation (recovery) of OVA-induced increases in airway IM that occurs with time after challenge.

Although the precise biological mechanisms responsible for this increase in airway IM in the OVA-challenged/CAPs-exposed rats are unknown, they may be related to the concurrent inflammatory responses induced by repeated CAPs exposures in the lungs of these animals. For example, we noted significant increases in the amount of elastase in the BALF of these rats compared to the controls; several other studies have demonstrated that elastase is a strong mucous secretogogue and has the ability to cause mucous cell metaplasia in rodent pulmonary airways (Breuer et al 1985; Christensen and Alonso 1996) and to induce the over-expression of mucin-specific genes in cultured airway epithelial cells (Voynow et al 1999; Fischer

and Voynow 2002). In addition, inflammatory cytokines, growth factors, or reactive oxygen species that we did not measure may have played a significant role in stimulating mucin production, which could have resulted in the increased storage of IM in these rats (Kohri et al 2002). Likely cellular sources for the increased amounts of elastase in the BALF were the macrophages or eosinophils or both that were concurrently increased in number. Neutrophils, though not significantly elevated, may also have contributed to elevated amounts of this protease in the BALF.

Interestingly, CAPs-induced pulmonary inflammation was evident in OVA-challenged BN rats exposed for 1 day in July and 5 days in September, but was not evident in OVA-challenged BN rats exposed for 4 days in July or for 1 day in September. The specific reasons for this difference in response among the OVA-challenged BN rats is not known, but may be due to differences in the amount of CAPs to which the animals were exposed (number of exposures and chamber concentrations), the physical characteristics of the particles, the chemical components of the CAPs, or any combination of these.

For BN rats in September, the daily and the average mass concentrations of CAPs for the 5-day exposure period were not significantly greater than those to which OVA-challenged BN rats were exposed in July. However, only the group of OVA-challenged BN rats exposed for 5 days to CAPs in September had significant pulmonary inflammation, increases in airway IM, and measurable elevations in the lung burdens of specific CAPs-associated metals (ie, La, Mn, and V) compared to controls. This suggests that the injurious response of this particular exposure may have been related to the unique chemical composition of the inhaled CAPs that was deposited in the lungs.

Although the inhalation component of our investigation was not designed to identify the mechanisms responsible for the CAPs-related pulmonary inflammation, others have suggested that specific metal components, such as V, may be the primary components of ambient particulate matter responsible for pulmonary injury and the inflammatory reaction (Kodavanti et al 1998; Saldiva et al 2002). Bonner and coworkers (2000) have reported that vanadium pentoxide intratracheally instilled in rats caused marked chronic pulmonary inflammation with mucous cell metaplasia in the conducting airways. ROFA particles containing V have also been shown to induce, in vitro, increases in the steady-state mRNA of *Muc5ac* in cultured airway epithelial cells (Longphre et al 2000). Jiang and colleagues (2000) reported that ROFA particles enhanced mucin secretion and generated toxicity to airway epithelium in vitro via mechanisms involving the generation of oxidant stress. They suggested that the V in the ROFA may have been important in generating these reactions.

Inhalation of CAPs for 5 days in September caused significant responses of epithelial and inflammatory cells in OVA-challenged BN rats. During the first 2 days of that exposure protocol, we collected $PM_{2.5}$ on filters to use in the intratracheal instillation component of the investigation. We found that the primary effects of total, soluble, or insoluble $PM_{2.5}$ instillation in saline- and OVA-challenged BN rats was the induction of a mild inflammatory response with no significant effect on mucus secretion or storage. Despite an estimated sixfold increase in pulmonary $PM_{2.5}$ concentration from instillation compared to the average CAPs concentration during the 5-day inhalation exposure in September, intratracheal $PM_{2.5}$ instillation did not reproduce the effect of inhaled CAPs that enhanced mucous cell metaplasia.

Fundamental differences between the protocols for intratracheal instillation and inhalation likely contribute to the disparate results we observed. The most critical difference was probably the duration of exposure to the particles. In the inhalation component, rats breathed CAPs for 50 hours over a 5-day period; in the instillation component, a single, bolus dose of $PM_{2.5}$ was instilled. The longer duration of breathing CAPs would allow more contact time between particles and epithelial cells, inflammatory cells, and cellular mediators. In response to the constant inflammatory stimulus of a 5-day particle exposure, the expression of mucin glycoproteins and their posttranslational modification may be prolonged; in contrast, a single particle stimulus may be readily cleared. Furthermore, pulmonary distribution of inhaled CAPs is probably more homogenous and reaches deeper into the alveolar regions than intratracheally instilled particles. It should be noted, however, that we assessed mucous cell metaplasia in the main axial conducting airways, a site easily penetrated by instilled $PM_{2.5}$. We are confident that the respiratory epithelium that lines the axial airway was exposed to $PM_{2.5}$ in the instillation component, although certainly for much less time than during CAPs inhalation. Daily instillation of $PM_{2.5}$ would have more closely approximated the 5-day CAPs inhalation exposure and may have produced similar results.

Our results suggest that the duration of particle exposure is more critical than particle concentration in inciting epithelial cell responses in allergic airways. Indeed, we estimate a sixfold difference in particle deposition between instillation and inhalation delivery protocols. Although this dose of instilled $PM_{2.5}$ exceeded the estimated inhaled CAPs exposure, it is less than particle concentrations used in instillation studies with ROFA particles (500–800 µg), carbon black (1000 µg), or diesel exhaust particles (250–1000 µg) that induced inflammation (Kodavanti et al 1999;

Yang et al 1999; Takano et al 2002). In preliminary studies using intranasal instillation of $PM_{2.5}$ in normal BN rats, we found that doses of 500 µg and 1000 µg induced a more robust neutrophilic and eosinophilic response in BALF than did a dose of 100 µg (unpublished data). Although the inflammation induced by intratracheal instillation with 100 µg $PM_{2.5}$ was greater than that produced by intranasal instillation with 100 µg $PM_{2.5}$, it was still less than that produced by intranasal instillation of 500 µg or 1000 µg $PM_{2.5}$. This suggests that intratracheal instillation with 100 µg $PM_{2.5}$ produced a submaximal inflammatory response, and that giving higher intratracheal doses of particles would have induced greater inflammation and might have exacerbated allergic responses similar to the effects noted in the inhalation component with OVA-challenged/CAPs-exposed BN rats in the September 5-day protocol.

The different outcomes between the inhalation and instillation components of our investigation might be due to the different compositions of particles to which each group of animals was exposed. Instilled $PM_{2.5}$ consisted of particles from only the first 2 days of the 5-day CAPs exposure in September. Thus, the intratracheally instilled animals were not exposed to the same particles as the animals that inhaled CAPs. Metals with potential pulmonary toxicity were higher for the 5-day CAPs inhalation than for the 2 days during which particles were collected for the instillation component (Table 11). Notable among these were Cd, As, and S. Because only particles from the first 2 days were collected, intratracheally instilled animals were exposed to lesser relative concentrations of these metals. It should be noted, however, that most ambient concentrations of metals were slightly higher during the first 2 days when samples were being collected. Therefore, exposures to such metals as La, Cu, and Zn were relatively higher in intratracheally instilled animals than in animals exposed to CAPs by inhalation over 5 days. It is reasonable to suggest that differences in metal composition might contribute to the disparate results in inhalation versus instillation components. Because of the variability in metal concentrations that exist in each exposure regimen, it is difficult to interpret how this factor impacts the outcomes of these two components.

It has been suggested that preexisting pulmonary inflammation, particularly in susceptible individuals with chronic pulmonary and cardiovascular diseases, may be an important mechanism by which ambient airborne particles adversely affect human health. Though most toxicology studies to date have used healthy adult animals to explore the toxicity of inhaled particles, more recently some investigators have used compromised animal models with various types of preexisting pulmonary inflammation (Clarke et al 1999; Kodavanti et al 2000; Saldiva et al 2002); in their compromised rodent models, CAPs exposure enhanced the ongoing inflammatory reaction. Clarke and coworkers (1999) reported that rats with SO_2-induced bronchitis had greater pulmonary inflammatory responses to short-term CAPs exposure (5 hours/day for 3 days; 206, 733, 607 µg/m^3 for days 1, 2, and 3) than healthy rats exposed to CAPs and bronchitic rats exposed only to filtered air. Kodavanti and associates (2000) also observed increased protein and neutrophil influx in CAPs-exposed rats with SO_2-induced bronchitis compared to similarly compromised rats exposed only to filtered air. In both of these studies, the short-term CAPs exposures appeared to enhance the neutrophilic influx into the lungs of rats with this type of preexisting inflammation.

In contrast, we did not find that short-term exposures to CAPs from Detroit enhanced the neutrophilic inflammation in the lungs of F344 rats exposed to bacterial endotoxin, a potent inducer of neutrophilic inflammation when inhaled. The reasons for this difference in response is not known, but may be due in part to differences in the SO_2-induced and endotoxin-induced inflammatory responses in the two rodent models, differences in the physicochemical makeup of the CAPs from the various urban centers, differences in the exposure regimens (eg, daily concentrations, exposure durations), or a combination of these.

We did observe that the 5-day September CAPs exposure enhanced the eosinophilic inflammation and associated increase in airway mucosubstances in the lungs of BN rats with preexisting allergic airway disease caused by OVA sensitization and challenge. It is interesting that we found a CAPs-related increase in eosinophils, the leukocyte most commonly associated with allergic airway diseases (eg, asthma and allergic rhinitis), rather than an increase in neutrophils, the leukocyte more commonly associated with nonallergic acute and chronic active pulmonary airway diseases (eg, bacterially induced bronchopneumonias and chronic bronchitis). The CAPs from southwest Detroit in September enhanced (or maintained) the preexisting allergen-induced inflammation in our BN rats rather than inducing a neutrophilic inflammatory reaction that has been previously observed by others in other stains of rats (F344 or Sprague Dawley) with or without neutrophilic airway inflammation. Our inhalation component was not designed to investigate the mechanisms for the CAPs-induced enhancement of the OVA-induced mucous cell metaplasia and eosinophilic inflammation. T_H2-cytokines (eg, interleukins 4, 5, 9, and 13) that are most associated with the pathogenesis of allergen-induced airway inflammation and increased production of airway mucus may have been directly or indirectly affected by the CAPs exposure. Studies specifically designed to

investigate the cellular mechanisms involved in the particle-induced enhancement of allergic airway response need to be conducted in the future.

Few toxicology studies have used animal models of allergic airway disease (eg, asthma) to explore the effects of ambient $PM_{2.5}$ or other air pollutants on compromised hosts. The present investigation is the first to study the effects of CAPs exposure on BN rats with or without OVA-induced allergic airway disease. Others have shown that ROFA particles enhance the allergic sensitization of BN rats to house dust mite antigen (Lambert et al 1999, 2000). Goldsmith and colleagues (1999) tested the hypothesis that CAPs or the leachate of ROFA particles can cause an amplification of pulmonary inflammation and airway hyperresponsiveness induced by OVA in mice. They found that OVA-challenged mice, when further exposed to CAPs, did not have any additional effects on airway hyperresponsiveness or pulmonary inflammation. However, OVA-challenged mice, when subsequently exposed to the leachate of ROFA particles, had enhanced airway hyperresponsiveness compared to their respective controls. Goldsmith and associates (2002) investigated the effects of coexposure to ozone and CAPs on mice with and without OVA-induced allergic airway disease. They found no effects of these pollutants on airway inflammation, but they did observe an immediate short-lived increase in airway hyperresponsiveness in the mice exposed to both ozone and CAPs that was associated with the Al-Si particle fraction.

Our observation that Detroit CAPs enhanced mucous cell metaplasia and eosinophilic inflammation in OVA-sensitized and OVA-challenged BN rats during the 5-day September protocol suggests that fine ambient urban particles may aggravate the symptoms of asthma by increasing airway mucous production and pulmonary inflammation. It is interesting that $PM_{2.5}$ associated with refinery sources was identified during this 5-day exposure protocol but not during the other exposure protocols.

This is the first use of a mobile air research laboratory equipped with a Harvard ambient fine particle concentrator and inhalation exposure chambers to conduct animal inhalation toxicology studies of real-time ambient air particles in an urban community (Detroit MI). The Harvard ambient fine particle concentrator effectively concentrated the fine ambient particles without significantly changing the physicochemical features of the atmospheric particles. Single or repeated exposures of CAPs did not cause adverse effects in the nasal or pulmonary airways of normal F344 or BN rats. In addition, CAPs-related toxicity was not observed in endotoxin-pretreated F344 rats. Variable airway responses to CAPs exposure were observed in BN rats with preexisting allergic airway disease induced by OVA sensitization and challenge. Only OVA-treated rats exposed to CAPs for 5 days in September had significant increases in airway IM and airway eosinophilic inflammation (compared to saline-challenged/air-exposed control rats). OVA-treated BN rats exposed to CAPs for 4 days in July showed neither increased IM nor pulmonary inflammation. These results demonstrate that short-term exposures to CAPs from this southwestern Detroit community caused variable and mild adverse effects in only those rats previously sensitized and challenged with OVA. The CAPs-related pulmonary alterations in these OVA-treated rats appeared to be related to the chemical composition, rather than the mass concentration, of the particles to which the animals were exposed.

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ABBREVIATIONS AND OTHER TERMS

AB/PAS	Alcian Blue (pH 2.5)/Periodic Acid Schiff Sequence
ABS	automation buffer solution
AirCARE 1	a specially designed mobile air research laboratory
ANOVA	analysis of variance
APS	aerodynamic particle sizer
BALF	bronchoalveolar lavage fluid
BrdU	bromodeoxyuridine
CAPs	concentrated fine urban air particulates OR concentrated ambient particles
CEF	concentration efficiency factor
CO	carbon monoxide
C_T	in PCR, the cycle in which the dye output exceeds the threshold level
EC	elemental carbon
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency (US)
H^+	aerosol strong acidity
HEPA	high-efficiency particulate air [filter or workstation]
HNO_3	nitric acid

HONO nitrous acid
HYSPLIT HYbrid Single Particle Lagrangian Integrated Trajectories model
ICPMS inductively coupled plasma mass spectrometry
IM intraepithelial mucosubstances
LOD limits of detection
MOI microorifice impactor
MQ Milli-Q water
mRNA messenger RNA
NH_3 ammonia
NH_4^+ ammonium
NIST National Institute of Standards and Technology (US)
NO nitric oxide
NO_2 nitrogen dioxide
NO_3^- nitrate
NO_x nitrogen oxides
OC organic carbon
OVA ovalbumin
PM particulate matter
$PM_{2.5}$ PM ≤ 2.5 µm in aerodynamic diameter
PM_{10} PM ≤ 10 µm in aerodynamic diameter
ROFA residual oil fly ash
RT-PCR reverse transcriptase–polymerase chain reaction
SMPS scanning mobility particle sizer
SO_2 sulfur dioxide
SO_4^{2-} sulfate
TEOM tapered element oscillating microbalance
T_H2 helper T cell type 2
TWA time-weighted average
UMAQL University of Michigan Air Quality Laboratory
V_{max} maximal velocity

COMMENTARY
Health Review Committee

INTRODUCTION

Ambient particulate matter (PM*) is a complex mixture of particles suspended in the air. The size (ranging from approximately 0.005 to 100 µm in aerodynamic diameter), chemical composition, and other physical and biological properties of these particles vary spatially and temporally. The variability in PM characteristics derives from differences in the sources of pollution; these may be natural in origin—the result of geographical conditions, weather, or seasonal patterns—or generated by human activities such as driving vehicles and operating manufacturing or power plants.

Although the characteristics of PM vary in different places, epidemiologic studies in many diverse locations have reported that short-term increases in low levels of PM are associated with short-term increases in morbidity and mortality (Health Effects Institute 2001). The strongest of these associations have been found with individuals who have compromised cardiac or airway function (reviewed in US Environmental Protection Agency [EPA] 1996). On the basis of these findings, many governmental agencies have set regulatory standards or guidelines for levels of ambient PM. To protect the general population and groups considered to be most vulnerable to adverse effects from PM, the EPA promulgates National Ambient Air Quality Standards for PM 2.5 µm or smaller in aerodynamic diameter ($PM_{2.5}$; fine particles) and monitors levels of PM 10 µm or smaller.

Several critical concerns in PM research remain: (1) the size and chemical composition of particles that may cause harmful human health effects, (2) the potential biologic mechanisms that underlie the epidemiologic associations reported between exposure to PM and changes in morbidity and mortality, and (3) the groups of people who may be particularly sensitive to the effects of PM. For example, people with asthma, especially children with asthma, are considered to be a susceptible population (Health Effects Institute 2002).

In 1998, the Health Effects Institute issued Request for Applications 98-1, *Characterization of Exposure to and Health Effects of Particulate Matter*. A key component of RFA 98-1 was the need to evaluate the health effects of ambient particles in humans and in relevant animal models of human conditions. One area of interest was studies involving controlled exposures to concentrated ambient particles (CAPs): A technology had recently been developed to concentrate fine particles and expose humans and other species to the concentrated particles (Sioutas et al 1995, 1997). The Health Effects Institute Research Committee proposed to make this concentrator technology available to some funded investigators. Preliminary studies with animals exposed to CAPs had reported changes in the proportions of subsets of peripheral blood leukocytes in rats (Gordon et al 1998) and changes in electrocardiogram readings in dogs (Godleski et al 1997).

In response to the RFA, Dr Jack Harkema and colleagues proposed a 2-year investigation to evaluate the short-term effects of exposing rats to (1) inhaled CAPs derived from the air in an area of Detroit, Michigan, where the incidence of childhood asthma was high; or (2) intratracheal instillations of $PM_{2.5}$ collected on filters at the exposure site and resuspended. They proposed to examine CAPs effects on healthy rats and on two different groups of rats that had been induced to have airway mucus hypersecretion, which mimics airway conditions in people with asthma or chronic bronchitis; for the intratracheal instillations, only the rat model of asthma would be used. To conduct the exposure component of this investigation, the researchers also proposed to use non-HEI funds to build a mobile air research laboratory—a trailer that could be moved to the exposure site—that would contain equipment for monitoring ambient air, the particle concentrator and inhalation exposure chambers, and animal laboratory facilities.

The HEI Research Committee recommended funding Harkema's investigation on the basis that important data could be acquired from examining the effects of CAPs derived from an area with high asthma incidence in animal models of susceptible human populations.† The Committee also recommended providing the investigator with a particle concentrator. HEI commissioned Petros Koutrakis (Harvard School of Public Health, Boston MA) to construct the concentrator (identical to concentrators used at Harvard; Sioutas et al 1995, 1997; Godleski et al 1997). At the same time that the Committee recommended Harkema's investigation for funding, it also recommended funding

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

This document has not been reviewed by public or private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views of these parties, and no endorsements by them should be inferred.

† Dr Harkema's 2-year study, "Effects of Inhaled Urban Air Particulates on Normal and Hypersecretory Airways in Rats", began in August 1999. Total expenditures were $844,218. The draft Investigators' Report from Harkema and colleagues was received for review in August 2002. A revised report, received in August 2003, was accepted for publication in October 2003. 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.

two other sets of investigators to perform controlled exposure studies with healthy people and individuals with asthma: Henry Gong and colleagues (Los Amigos Research Facility, Los Angeles CA), who exposed people to CAPs derived from air in the Los Angeles area, and Mark Frampton and colleagues (University of Rochester School of Medicine and Dentistry, Rochester NY), who exposed people to ultrafine (≤ 0.1 µm diameter) carbon particles. The study by Gong and associates has been published (Gong et al 2003); the study by Frampton and colleagues has been completed and is scheduled for publication in 2004.

SCIENTIFIC BACKGROUND

Asthma is a chronic disease of the lower airways characterized by inflammation, mucus hypersecretion, reversible airway obstruction, and airway hyperresponsiveness (exaggerated responsiveness to a bronchoconstrictor, an agent that narrows the opening of a bronchus or bronchiole). Numerous epidemiologic studies have reported that short-term exposure to particulate pollution exacerbates the symptoms of asthma (eg, Schwartz et al 1993; Lipsett et al 1997) and decreases lung function in people with asthma (eg, Pope et al 1991, Peters et al 1997).

The mechanism or mechanisms by which particles may exacerbate asthma symptoms are unknown, however. In controlled exposure studies with animal models of human illness, different types of particles found in ambient air induced or exacerbated airway inflammation. In an early study (Gearhart and Schlesinger 1989), rabbits that underwent prolonged exposure to a high concentration of sulfuric acid aerosol (250 µg/m^3 for 1 hour/day, 5 days/week for up to 52 weeks) showed airway inflammation, including higher numbers of mucus secretory cells than were found in control animals. Mice sensitized to allergen showed increased inflammatory responses after exposure to diesel exhaust particles via inhalation (Miyabara et al 1998). In vitro studies of human cells also supported the idea that particle exposure affected inflammation: bronchial epithelial cells (from healthy and asthmatic people) exposed in vitro to diesel exhaust underwent physical and biochemical changes associated with cell activation and the production of mediators characteristic of the inflammatory response (Bayram et al 1998a,b). Furthermore, rats exposed by intratracheal instillation to high levels of residual oil fly ash—a highly toxic combustion-generated urban particulate rich in metals—developed airway hyperresponsiveness and potent inflammatory responses; these were characterized in part by epithelial cell hyperplasia and increases in mucus-producing cells (goblet cells) in the pulmonary airways (Dreher et al 1997; Gavett et al 1997). Soluble leachates from the residual oil fly ash—which contained a high proportion of transition metals such as iron, nickel, and vanadium—were as potent as the starting materials at inducing airway inflammation (Dreher et al 1997; Gavett et al 1997).

The development of an instrument that would concentrate ambient fine particles and allow animals (and subsequently humans) to be exposed to CAPs made it feasible to evaluate the effects of exposure to a representative and more complete spectrum of ambient particles (Sioutas et al 1995, 1997). At the time the current investigation was funded, however, few results had been obtained. In Gordon and colleagues' study (1998), a small number of healthy rats that inhaled CAPs—concentrated approximately sixfold from ambient New York City air using a different concentrator—showed little or no effects in the airways but modest changes in the distribution of peripheral blood leukocytes (Gordon et al 1998). That preliminary finding—an increase in the percentage of neutrophils and a decrease in the percentage of lymphocytes in the blood compared with control animals—suggested that CAPs exposure might induce inflammatory effects outside the airways and especially in the circulation. In fact, preliminary data from another study demonstrated that dogs exposed intratracheally to a CAPs aerosol derived from Boston air—concentrated up to 60-fold from ambient fine particles—underwent cardiac changes (Godleski et al 1997).

Rats primed with the allergen ovalbumin (OVA) and subsequently challenged in the airways with aerosolized OVA share some of the characteristics associated with human asthma (Haczku et al 1996; Schneider et al 1997), and thus have been used as an animal model of asthma. When Harkema proposed to work with rats as a model of human asthma and expose them to CAPs, no other studies had been conducted along this design, nor had anyone else used other rat models of airway conditions that might increase the sensitivity to particles. Thus, it was not known whether CAPs effects might be different or the same in conditioned animals and in healthy animals. The investigation by Harkema and colleagues was intended to address this issue with rats exposed to CAPs derived from an urban US location with high childhood asthma incidence. In addition, the investigation would provide information about the responses to CAPs exposures at a site where the constituents of particulate pollution were likely to differ from those used in prior CAPs studies, because of differences in sources, weather, and other factors. Since the Harkema investigation began, many CAPs studies have been undertaken in different locations.

SPECIFIC AIMS

Harkema and colleagues hypothesized that inhalation of CAPs would induce inflammatory responses and alterations to epithelial cells in the airways of rats, and that greater effects would be induced in rats with hypersecretory conditions than in healthy rats. The investigators also postulated that the magnitude of CAPs effects would depend on the level of soluble components in the particles, particularly metals.

The aims of this investigation were:

1. Using the outdoor air of a Detroit community with a high incidence of childhood asthma and elevated concentrations of outdoor pollutants during the summer months, examine the physical and chemical characteristics of $PM_{2.5}$, CAPs, and other airborne pollutants.
2. Using healthy rats and rats with preexisting hypersecretory airway conditions that model asthma and chronic bronchitis (which are both characterized by mucus hypersecretion), determine the effects of inhaled CAPs on the airway epithelium—particularly changes in mucus secretion and indicators of inflammation.
3. Using healthy rats and rats with a preexisting airway condition that models human asthma, intratracheally instill suspensions of $PM_{2.5}$ collected from ambient air onto filters, and identify the physical and chemical components of $PM_{2.5}$ responsible for airway inflammation and epithelial alterations.

INVESTIGATION DESIGN

LOCATION AND TIMING

The investigators built a Mobile Air Research Laboratory where the inhalation component of this investigation was carried out. The mobile laboratory was moved to an elementary school in a southwest Detroit neighborhood that Harkema and colleagues describe as having "moderately high to very high infant childhood asthma hospitalization rates and consistently high proportions of households living below the poverty level." The investigators also noted that the school is located within 2.5 km of a major border crossing for diesel trucks between the US and Canada, steel and chemical refineries, and the intersection of two large highways. The location and layout of the Mobile Air Research Laboratory are shown in Figures 1 through 3 of the Investigators' Report.

Dr Koutrakis and his colleague Mr Stevenson built and installed in the mobile laboratory a three-stage fine-particle concentrator that was similar in design to the three-stage concentrators used in controlled exposure studies at Harvard School of Public Health (Sioutas et al 1995, 1997; Godleski et al 2000). The three concentrator stages provided a theoretical 27-fold maximal enrichment of fine particles from ambient air, which was drawn in through a size-selective inlet mounted on top of the mobile laboratory. Because of the size of the inlet, particles greater than 2.5 µm in diameter theoretically do not pass through the concentrator.

Airflow from the concentrator was directed into one of two whole-body inhalation chambers, which could hold 16 rats. Airflow to the other chamber (also with 16 rats) was directed through a high-efficiency particulate air filter for control exposures. Animals were exposed in July and September 2000 because the investigators thought that PM mass concentrations would be high in summer and early fall.

The intratracheal instillation component of this investigation was conducted in the investigators' laboratory at Michigan State University. For that component, ambient particles were collected from the location in southwest Detroit during an exposure week in September. In their laboratory, the $PM_{2.5}$ was suspended and separated into soluble and insoluble fractions.

ANIMAL MODELS

To generate a model of asthma in which the infiltrating cells would be predominantly eosinophils, the investigators intranasally sensitized male BN rats with 0.5% OVA daily for 5 consecutive days and, starting 14 days later, challenged them intranasally on 3 successive days with 1% OVA. (They chose male rats to avoid estrous-cycle hormonal changes that modify mucus secretion in the airways of female rats [Hayashi et al 1979]). Control animals for this model were sensitized and challenged intranasally with saline. To generate a model intended to mimic human bronchitis in which the infiltrating cells would be predominantly neutrophils, the investigators pretreated male F344 rats by intratracheally instilling 20 µg endotoxin 48 hours before CAPs exposure. Control animals for this model were intratracheally instilled with saline. Animals were anesthetized for each administration of allergen, endotoxin, or saline.

For the inhalation component, both BN and F344 rats were exposed to CAPs; for the instillation component, only BN rats were administered resuspended particles. (Table 3 in the Investigators' Report provides details of the animal groups.)

CHARACTERIZATION OF AMBIENT AIR

During the exposure component of this investigation, Harkama and associates analyzed the size and mass of ambient particles (8 am–6 pm) using various instruments:

- a tapering element oscillating microbalance continuously measured $PM_{2.5}$ mass;
- cyclone samplers collected $PM_{2.5}$ and PM_1 on Teflon filters;
- a microorifice impactor and Teflon filters collected six size fractions of particles ranging from smaller than 0.18 µm to larger than 5 µm in aerodynamic diameter;
- an aerodynamic particle sizer measured the number of particles in the range of 0.5 µm to 20 µm; and
- a scanning mobility particle sizer measured the number of particles in the range of 20 nm to 800 nm.

The investigators also analyzed other characteristics of particles:

- a quartz filter gathered elemental and organic carbon;
- an annular denuder system collected the gaseous particle species nitrous and nitric acids, sulfur dioxide, and ammonia, and the inorganic fine particulate ions sulfate, nitrate, ammonium, and H^+;
- three instruments measured levels of the gaseous pollutants ozone, carbon monoxide, and nitrogen oxides; and
- a dichotomous sequential sampler used Teflon filters to gather particles, which were analyzed by inductively coupled plasma–mass spectrometry to measure multiple trace elements, including (1) several metals in nitric acid extracts of the particles, and (2) water-soluble trace elements in an aqueous fraction of collected resuspended particles.

CHARACTERIZATION OF CAPs

To analyze the CAPs during the 10-hour exposure periods, the investigators used many of the same kinds of instruments as those used to analyze ambient air: a microorifice impactor collected six size fractions of particles after the second stage of the concentrator; a tapering element oscillating microbalance continuously measured CAPs mass after the third stage of the concentrator and before the air stream entered the exposure chamber; the aerodynamic particle sizer and the scanning mobility particle sizer alternated sampling between ambient particles and chamber CAPs to compare particle characteristics as concurrently as possible. Except for the cyclone samplers that collected $PM_{2.5}$ and PM_1, all other measurements described above were also conducted on CAPs.

EXPOSURE TO CAPs VIA INHALATION OR $PM_{2.5}$ VIA INTRATRACHEAL INSTILLATION

For the inhalation component, Harkema and colleagues exposed four groups of 8 rats to CAPs or filtered air for 10 hours on 1 day or on 4 or 5 different consecutive days in July or September.

For the instillation component of the investigation, Harkema and colleagues collected ambient $PM_{2.5}$ on Teflon-coated glass fiber filters during the first 2 days of the September 5-day CAPs inhalation exposure. In their laboratory, they separated the total $PM_{2.5}$ into soluble and insoluble fractions by low-speed centrifugation: the soluble $PM_{2.5}$ fraction was the supernatant after centrifugation and the insoluble $PM_{2.5}$ was the pellet resuspended in saline.

The investigators intratracheally administered one 100-µg dose of total $PM_{2.5}$, soluble $PM_{2.5}$, or insoluble $PM_{2.5}$ (or the same volume of a saline control) to groups of 8 BN rats—four groups had been sensitized and challenged intranasally with OVA and four groups had been sensitized and challenged intranasally with saline (both OVA-challenged and control groups were instilled with saline, total $PM_{2.5}$, soluble $PM_{2.5}$, or insoluble $PM_{2.5}$; see Table 3).

Harkema and colleagues calculated that the 100-µg dose used for instillation was equivalent to sixfold more $PM_{2.5}$ mass than the rats had been exposed to during the 10-hour/day, 5-day September inhalation exposure during which the ambient particles had been collected for the instillation component.

EFFECTS RELATED TO CAPs EXPOSURE

Inflammatory and Mucus Secretion Endpoints

Animals were killed 24 hours after the final inhalation exposure or the intratracheal instillation. Nasal and lung airway tissues were dissected from each animal; samples were prepared and analyzed in multiple biochemical, morphometric, and molecular biological assays. The investigators lavaged the left lung lobes and the lavage fluids were analyzed for total and differential cell counts, levels of mucin protein Muc5ac, total protein, elastase, alkaline and acid phosphatases, and β-glucuronidase.

The investigators also made multiple morphometric measurements of airway and nasal epithelia:

- levels of intraepithelial mucosubstances contained in different regions of the respiratory epithelium that lines the airways;
- the density of epithelial cells;
- the number of epithelial cells that were proliferating in the axial airways (using bromodeoxyuridine labeling); and
- *Muc5ac* messenger RNA expression.

Inhaled Trace Elements

Using inductively coupled plasma–mass spectrometry, the investigators measured levels of several elements in the lungs of BN rats (OVA-sensitized and OVA-challenged rats as well as saline-treated controls) exposed to CAPs in July and September. The elements they measured included lanthanum, vanadium, phosphorus, sulfur, and manganese.

STATISTICAL ANALYSES

The investigators expressed all airway inflammatory data as the mean group value ± the SEM (n = 8/group). They performed two-way analysis of variance to test the airway status (ovalbumin, endotoxin, or saline) and the type of inhalation exposure (CAPs or filtered air). They conducted multiple comparisons using the Student-Newman-Keuls post hoc test. To make variances homogeneous, they used log or arcsin transformations as needed. Harkema and colleagues considered $P \leq 0.05$ the criterion for statistical significance.

The HEI Review Committee, however, thought that the true standard errors were likely larger than those reported by the investigators and concluded that the investigators should have used either of two statistical approaches that would have calculated standard errors more accurately: A hierarchical statistical model would calculate the appropriate standard errors for effect estimates and test statistics; such a model would take into account the number of statistical cells on which rat-specific estimates were computed coupled with the nested structure of the data (eg, that the measurements of different endpoints in lavage fluid from the same rat are correlated). Alternatively, the investigators should have computed robust standard errors using standard methods in SAS or other statistical packages. Furthermore, the Review Committee concluded that, to provide symmetry to the sampling distribution of estimates, all statistical analyses would have been more appropriate if conducted in the log scale.

RESULTS

CHARACTERIZATION OF PARTICLES

Ambient $PM_{2.5}$ and Gases

The daily average ambient $PM_{2.5}$ concentrations were similar in July and September, averaging 15.5 μg/m^3 (range 5 to 34 μg/m^3) in July and 13 μg/m^3 (range 4 to 27 μg/m^3) in September.

Of $PM_{2.5}$ *mass*, PM_1 was the major constituent (approximately 60%–90%) during both July and September exposures. approximately particles ($PM_{0.18}$), on the other hand, comprised only 6% to 35% of $PM_{2.5}$ mass, but made up the major fraction of PM_1 *number* (> 75% on all days except two in one of the multiple-day July exposure protocols). Spikes in $PM_{0.18}$ particle numbers were detected during morning and afternoon rush hours.

Mass concentrations of the main constituents of ambient $PM_{2.5}$ were found to be similar during July and September; namely, organic and elemental carbon comprised approximately 25% of the $PM_{2.5}$ mass; sulfate 22%; nitrate 10%; ammonium 8%; and the crustal elements iron, aluminum, silica, and calcium 8% (approximations based on data in Table 6). H^+ comprised approximately 5% of the mass in July and 2% in September. The concentrations of trace elements varied from day to day but generally within specific ranges. For example, calcium, iron, and sulfur levels were almost always higher than 100 ng/m^3; magnesium, sodium, potassium, and aluminum were generally detected at levels between 10 and 100 ng/m^3; vanadium, titanium, lead, and nickel were in the range 1 to 10 ng/m^3; many other elements were detected at levels lower than 1 ng/m^3.

Concentrator Performance

CAPs mass concentrations ranged from 20.5 to 926.2 μg/m^3 during the July exposures and from 75.9 to 754.7 μg/m^3 during the September exposures (Tables 8 and 9). The average mass enrichment factor for CAPs from ambient air was 18.6 over the course of the investigation. As described in other concentrator studies (eg, Godleski et al 2000; Gong et al 2003), however, the enrichment factor varied depending on meteorological conditions, concentrator slit alignment, and ambient particle levels. For example, the mass enrichment factor was 5 to 10 on July days with dry air and low ambient particle levels, but approximately 20 on humid July days with higher particle levels. Tables 8 and 9 indicate that the average CAPs mass for all July and all September exposure days was about the same (~283 μg/m^3 for July, ~262 μg/m^3 for September); however, for the multiple-day exposure protocols for BN rats (the asthma model), the CAPs mass was twice as high in July as in September (598 μg/m^3 vs 276 μg/m^3; time weighted averages in Table 8).

Several major PM constituents—including sulfate, nitrate, ammonium, and crustal elements—were found in the same proportions in CAPs as in total ambient $PM_{2.5}$ mass. The investigators indicate that the trace elements iron, vanadium, and antimony were all concentrated at the same relative levels in CAPs as were found in ambient air (Figure 13).

By contrast, organic carbon levels in CAPs were higher than expected from its levels in ambient air. The investigators

suggest that previously described artifacts in organic carbon sampling were likely responsible (Turpin et al 1994). (In support of this supposition, similar problems with organic carbon sampling were also noted in a recent CAPs exposure study with humans [Gong et al 2003].) In addition, elemental carbon levels in CAPs were not proportionate to its levels in ambient air; the investigators speculate that elemental carbon was associated with particles smaller than 0.2 µm, which were not concentrated.

Based on mass, the concentrator effectively concentrated fine particles in the range of 0.18 µm to 2.5 µm; within this range, particles 0.6 to 1.0 µm predominated. Consequently, on most exposure days, the proportion of total CAPs mass that was contributed by a particular size fraction (eg, 0.6 to 1.0 µm) differed from the proportion of total ambient particle mass that was contributed by the same size fraction (shown in Figure 12). Based on number, particles $\leq$ 0.18 µm were enriched only about twofold (Figure 11).

Chamber concentrations of ozone were on average only 36% of ambient concentrations, which parallels findings from previous studies in which the metal tubing of the concentrator removed most of the ozone (Godleski et al 2000; Gong et al 2003). Carbon monoxide concentrations in ambient air and chamber air did not differ.

CHARACTERIZATION OF THE ANIMAL MODELS

Pretreatment of animals induced hypersecretory conditions in the airways: In F344 rats, pretreatment with endotoxin induced a mild to moderate subacute bronchiolitis and alveolitis; these were characterized by inflammatory features including (1) an infiltration of monocytes, lymphocytes, and neutrophils into affected alveolar septa; (2) a mixed-cell influx of neutrophils, eosinophils, and monocytes into tissues surrounding bronchioles, pulmonary arteries, and small veins; and (3) epithelial cell hyperplasia and hypertrophy were also detected. These endotoxin-induced effects were evident in rats exposed for 1 day to air or CAPs in both July and September. In animals exposed for 4 or 5 days in each month, however, inflammatory and airway epithelial changes induced by endotoxin were minimal, which suggests that the inflammation had resolved during the multiple-day exposure protocols.

In BN rats, sensitization and intranasal challenge with OVA resulted in allergic alveolitis and bronchiolitis, characterized by airway inflammatory features such as increased intraepithelial mucosubstances in the axial airways (compared with saline-challenged animals) and the presence of an inflammatory infiltrate containing eosinophils.

CAPs-ASSOCIATED EFFECTS

Inhalation Component

In healthy and endotoxin-pretreated F344 rats, no differences were noted in inflammatory endpoints between CAPs-exposed and air-exposed animals.

In BN rats sensitized and challenged with OVA, exposure to CAPs for either 1 day or 4 or 5 days was associated with few effects on pulmonary and nasal inflammatory endpoints (Tables 11–14 and Figures 18–21). Comparing endpoints in CAPs-exposed animals to the same endpoints in air-exposed animals, the CAPs-associated effects were modest (summarized in Table 16):

- In animals exposed for 1 day in July: approximately 25% higher levels of elastase and Muc5ac protein in lavage fluids.
- In animals exposed to CAPs for 4 days in July: 40% more intraepithelial mucosubstances in the nasal septum.
- In animals exposed for 5 days in September: 30% to 50% higher levels of intraepithelial mucosubstances and epithelial cell density, and a 75% lower level of RNA expression of the mucin gene *Muc5ac* in the pulmonary airways; approximately 30% higher levels of acid phosphatase and of the numbers of eosinophils, macrophages, and total leukocytes in lavage fluids.

An additional effect was noted after the 5-day September exposure. The investigators detected higher levels of some elements in the lung tissues from CAPs-exposed animals than in those from air-exposed animals (Figure 14): fourfold higher levels of the trace metal lanthanum (approximately 5–7 pg/g dry lung tissue) in both OVA-challenged rats and healthy animals; vanadium levels were also higher in both sets of CAPs-exposed animals compared with air-exposed controls, but the percentage of change in vanadium was not as high as the percentage of change in lanthanum; and a weakly significant increase in sulfur levels. No CAPs-associated changes were detected in levels of the essential elements iron and phosphorus. The investigators reported that they did not find trace elements retained in the lungs of rats exposed in July.

Instillation Component

This component of the investigation also used BN rats that had been sensitized and challenged with either OVA or saline. Intratracheally instilling 100 µg total $PM_{2.5}$, water-soluble $PM_{2.5}$, or water-insoluble $PM_{2.5}$ fractions had little or no effect on many inflammatory endpoints, including elastase, β-glucuronidase, and Muc5ac protein in lavage fluids, intraepithelial mucosubstance levels, epithelial cell proliferation, and mucin gene expression (Figures 24 and 26–29).

The effects noted were inconsistent. Some endpoints were increased by only one type of particle tested but not the other two, and in only one group of animals (eg, see Figure 25; insoluble $PM_{2.5}$ increased the levels of total protein in lavage fluids from saline-challenged animals but not from OVA-challenged animals). Instillation of particles increased the numbers of some cells in the lavage fluids from one group but not the other (eg, see Figure 23; total $PM_{2.5}$ and insoluble $PM_{2.5}$ increased neutrophil numbers in saline-challenged but not in OVA-challenged animals).

DISCUSSION OF FINDINGS

Harkema and colleagues designed this investigation to ascertain the effects of CAPs derived from the air of a Detroit neighborhood with high childhood asthma incidence on the airways of healthy rats and rats with induced hypersecretory conditions that mimicked asthma and bronchitis. To accomplish their goals, the investigators successfully built and operated a mobile laboratory in which they collected and concentrated ambient fine particles and then exposed animals via inhalation to the concentrated particles. They performed a rigorously designed study that carefully characterized ambient and concentrated particles and made an extensive set of observations of airway morphometry in control and particle-exposed rats.

Their findings, that CAPs (concentrated up to 30-fold from ambient air) had no effect on the airways of healthy rats, are similar to the negative findings in lavage endpoints for healthy rats exposed to CAPs derived from the air of New York City (Gordon et al 2000) and Research Triangle Park NC (Kodavanti et al 2000). Healthy rats exposed to CAPs derived from Boston air, however, did show signs of airway inflammation, measured as an increased number of neutrophils in lavage fluids (Clarke et al 1999) and an increased density of neutrophils in the alveolar walls at the bronchoalveolar junction and in more peripheral alveoli (Saldiva et al 2002). As Harkema and colleagues describe, the reasons for the differences in findings among studies may involve differences in the duration and concentration of CAPs exposure, the strain of rats used, and the characteristics of the particle constituents at different locations. Short-term exposures of healthy rats to CAPs have also been associated with changes in endpoints that were not measured in the current investigation, namely, vasoconstriction of small pulmonary arteries (Batalha et al 2002; Boston), rapid production of reactive oxygen species (Gurgueira et al 2002; Boston), and small changes in peripheral blood leukocyte percentages (Gordon et al 1998; New York City). Healthy dogs exposed to Boston CAPs also showed small changes in the number of neutrophils in lavage fluids (Clarke et al 2000; Godleski et al 2000.)

The investigators' hypothesis that animals with induced hypersecretory conditions would show heightened responses to CAPs compared with healthy animals was not supported by the findings in endotoxin-pretreated rats, the groups intended to model humans with chronic bronchitis. Harkema and colleagues reported that the endotoxin-pretreated rats exposed for 1 day to CAPs developed features of mild bronchitis—a mild to moderate bronchiolitis and alveolitis. (Animals examined after the multiple-day exposures showed minimal characteristics of inflammation or hypersecretion, which indicates that the inflammatory response had resolved within 4 or 5 days.) Thus, even though the endotoxin-pretreated animals had developed a mild airway inflammatory hypersecretory condition, no CAPs-associated effects were detected.

These findings differ from those of other studies of rats with bronchitis exposed to CAPs in Boston and Research Triangle Park, where researchers found heightened responses compared with those in healthy animals (Clarke et al 1999; Kodavanti et al 2000; Saldiva et al 2002). In addition to the possible reasons described above for differences in results among studies with healthy animals, the models of bronchitis in these studies also differ: in the current investigation, the endotoxin-induced hypersecretory condition was transient. By contrast, rats in the Boston studies (Clarke et al 1999; Saldiva et al 2002) were pretreated with sulfur dioxide weeks before CAPs exposure to induce a chronic form of bronchitis.

The investigators' hypothesis about the heightened responsiveness of animals with hypersecretory conditions was somewhat supported by the results in the rat model of allergic (asthmatic) inflammation: in contrast to healthy rats (ie, rats sensitized and challenged with saline), OVA-challenged animals showed small CAPs-associated changes in a limited number of airway inflammatory endpoints. CAPs had no effect, however, on many of the endpoints measured. In a study measuring similar endpoints after CAPs exposure in mice, Kobzik and colleagues had found no effect of short-term exposure to Boston CAPs in the lavage fluids from healthy young mice or from mice sensitized and primed with OVA (Kobzik et al 2001).

The findings from the present investigation need to be considered in conjunction with results reported for healthy humans and those with asthma after short-duration controlled exposures to CAPs in different locations (Ghio et al 2000; Gong et al 2000, 2003; Petrovic et al 2000). Those

studies have shown small effects on a limited number of systemic and airway inflammatory responses and cardiac and vascular parameters. For example, in people exposed to CAPs derived from the Los Angeles area, Gong and colleagues found that effects on airway inflammatory and vascular parameters were similar for mildly asthmatic volunteers and for healthy study participants (Gong et al 2000, 2003). Thus, in that first study to evaluate CAPs exposure of patients with asthma, people with asthma did not respond differently than healthy volunteers.

Finally, the investigators measured multiple characteristics of pollutants in ambient air and in CAPs in one urban area described as having a high incidence of childhood asthma. It is not clear, however, how any of the measurements of airborne pollutants or particle characteristics made in this study shed light on how asthma may be induced, or asthma symptoms exacerbated, in this area.

INTERPRETATION OF CAPs-ASSOCIATED FINDINGS IN OVA-CHALLENGED ANIMALS

Harkema and colleagues interpreted their findings in OVA-challenged rats to indicate that airway inflammatory responses associated with CAPs exposure differed between July and September 2000, with a higher level of response in September. They formed this conclusion based on the findings that OVA-challenged rats exposed to CAPs for multiple consecutive days in September showed increases in levels of both airway mucosubstances and some other measures of airway inflammation compared with control animals; OVA-challenged rats similarly exposed in July did not show increases in both sets of parameters. CAPs mass during the multiple-day July exposure protocol was approximately twofold higher than the CAPs mass in the parallel September exposure protocol; if CAPs mass were the parameter responsible for inflammatory effects, more significant changes might have been expected in July rather than September. Because this was not the case, the investigators inferred that CAPs-associated airway inflammatory responses were dependent on the chemical composition rather than the mass of the CAPs to which the animals were exposed. Harkema and colleagues speculated that the differences in CAPs-associated effects in July and September might result from differences in the levels and proportions of ultrafine particles, sulfate, or metals in ambient air during the two exposure protocols.

Harkema and colleagues' interpretation of the data may be correct—namely, that characteristics other than mass may determine particle toxicity—but the HEI Review Committee did not agree that the distinction they made between CAPs-associated responses in July and September was clear-cut: Animals exposed to CAPs for 1 or 4 days in July showed a small number of changes in either inflammatory or mucosubstance endpoints that were distinct from, but of similar magnitude to, the changes detected in animals exposed for 5 days in September. The Review Committee also thought that the current study presented little or no evidence to support a role for ultrafine particles, metals, or changes in the proportion of sulfates in the induction of adverse effects. For example, levels of ultrafine particles were 1.5 times higher in ambient air in September than in July, but this does not seem to be a difference of enough magnitude to justify the investigators' conclusion. In addition, ultrafine particles were not concentrated in the CAPs, so it is difficult to ascribe effects to them in these experiments. Thus, from the data presented, the Review Committee thought it difficult to conclude that any constituent—or possible source of PM emissions—could be held responsible for effects associated with CAPs exposure.

Harkema and colleagues also hypothesized that soluble constituents of CAPs—metals in particular—might be associated with CAPs effects. This hypothesis was based in part on the results of studies with rodents exposed to the soluble components of metals-rich residual oil fly ash (Kodavanti et al 1998; Lambert et al 2000) and some human studies that have explored the role of metals in emissions from a Utah steel mill (Ghio and Devlin 2001; Molinelli et al 2002; Pagan et al 2003).

Two sets of findings in the current study are relevant to the question of what role metals might have in the toxicity of $PM_{2.5}$: (1) the effects of intratracheally instilling the metals-enriched soluble fraction of $PM_{2.5}$, and (2) the detection of metals in the lungs of animals that inhaled CAPs. Harkema and associates' findings on this subject are difficult to interpret. First, the effects of instilling the metals-enriched fraction of $PM_{2.5}$ on inflammatory endpoints were sparse and inconsistent, so it is difficult to draw conclusions from these experiments. Second, the investigators detected levels of the trace metals lanthanum and vanadium in the lungs of BN rats exposed to CAPs and filtered air in September but not in July. These results are not easy to explain: Levels of lanthanum and vanadium in ambient air did not differ greatly between July and September (Table 7). The enrichment of vanadium in CAPs from ambient air appeared to be similar for several days in September (as depicted in Figure 13 for September). One would infer that lanthanum was enriched to the same extent as vanadium, and thus that both trace metals were at similar levels in CAPs in September, and probably in July as well. Thus, based solely on the levels of trace metals in the CAPs during the two different exposure protocols, it is

difficult to understand why detectable levels of these metals would be retained in the lungs in September but not in July. Furthermore, it appears unlikely that retention of these metals in the lungs is associated with inflammatory effects in the airways: Harkema and colleagues detected lanthanum and vanadium in the lungs of both OVA-challenged and healthy rats exposed to CAPs. CAPs-associated inflammatory effects, however, were detected *only* in the OVA-challenged (asthma-model) animals, not in the healthy animals.

CONCLUSIONS

This investigation was the first to examine the effects of inhaled CAPs on the airways of rats conditioned to model human asthmatic airways (BN rats sensitized and challenged with OVA); it showed that few inflammatory endpoints were affected. Those changes that were detected were small. The results of the current investigation are inconclusive about the toxicity of metal constituents of $PM_{2.5}$.

The investigation found no inflammatory responses in healthy rats or rats conditioned to model transient bronchitis (F344 rats instilled with endotoxin). Some prior CAPs exposure studies had detected airway inflammatory effects in healthy rats and in rats with a different, longer-lasting model of bronchitis. Differences in exposure protocols, strains of rats, models of bronchitis, and characteristics of the concentrated particles at the various study sites make comparing the results of these different studies challenging.

Harkema and colleagues showed that they could concentrate and characterize fine particles under different meteorological conditions in a mobile laboratory, expose rats to the concentrated aerosol, and evaluate many of the CAPs-associated responses on site. Thus, the current investigation demonstrated the ability of a mobile laboratory specifically designed to conduct CAPs exposures to assess the effects of controlled exposures to concentrated, real-world particles at specific locations of possible clinical or pollutant importance.

The study also underscored some of the limitations of the CAPs approach: First, the characteristics of ambient particles and hence of CAPs vary from day to day and during the day, which makes identifying potentially toxic particle constituents difficult. Second, the concentrator preferentially concentrates fine particles, particularly those in the range of 0.6 to 1 µm; thus, the inhalation component of this investigation evaluated the effects of exposure to particles in this size range. This skew toward concentrating particles of one size range distorted both the mass and the number distributions of the collected particles compared with the distributions of those particles in ambient air. Although the investigators indicated that the CAPs concentrations of many constituents were proportional to the concentrations in ambient air, it is likely that some constituents of the mix—such as trace metals—were preferentially concentrated because they are specifically associated with this size range of particles. The preferential concentration of a particular size range of fine particles also limits the conclusions that can be drawn about the characteristics of particles responsible for toxicity. In addition, because of this preferential concentration of particles based on size, some elements or compounds may be preferentially concentrated and others not.

Finally, by definition, the fine-particle concentrator does not assess the effects of ultrafine and coarse particles, which are outside the limits of the concentrator's size range. Carefully designed epidemiologic studies and controlled exposures with consistent animal models and endpoints are needed in this and other locations to assess the health effects of particles outside the size range concentrated in this study.

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