STATEMENT

Synopsis of Research Report 145

HEALTH EFFECTS INSTITUTE

Effects of Concentrated Ambient Particles and Diesel Emissions on Rat Airways

BACKGROUND

In the 1990s, results from several epidemiologic and controlled-exposure studies suggested an association between exposure to air pollution from traffic-derived particulate matter (PM) and increases in symptoms of airway diseases, including exacerbation of asthma. Some results also suggested that exhaust from diesel engines contributed to these effects. To address some of the questions raised by these findings, HEI issued Request for Applications 00-1, "Effects of Diesel Exhaust and Other Particles on the Exacerbation of Asthma and Other Allergic Diseases." In response, Dr. Jack R. Harkema, Michigan State University (MSU), and colleagues proposed a study to investigate how the inhalation of $PM_{2.5}$ (PM with an aerodynamic diameter $\leq$ 2.5 µm) concentrated from ambient air (concentrated ambient particles, CAPs) or diesel engine exhaust (DEE) would affect the airway inflammatory and allergic responses to the airborne allergen ovalbumin (OVA) in a rat model of asthma. Harkema and his colleagues proposed to expose rats to the pollutants at critical points in the induction of the allergic response, namely, the initial or sensitization phase and the second or challenge phase. The investigators hypothesized that inhalation of CAPs or DEE during sensitization or challenge would enhance inflammatory responses in the airways. The HEI Health Research Committee recommended the proposal for funding.

APPROACH

The study was conducted in two different locations: the CAPs study in Michigan (exposures in Detroit and analysis at MSU in East Lansing) and the DEE study at the Lovelace Respiratory Research Institute (LRRI) in Albuquerque, New Mexico. The same OVA sensitization and challenge regimen was used in both the CAPs and DEE studies: Brown Norway rats were *sensitized* by intranasal instillation of a 0.5% solution of OVA (or saline, as a control) on days 1 through 3; they rested for days 4 through 14 and were *challenged* intranasally with a 1.0% solution of OVA (or saline) on days 15 through 17.

In Detroit, Harkema and colleagues used a mobile research facility that they had used in a previously funded HEI study to collect CAPs from an area of the city with a higher-than-average prevalence of asthma. On the same 3 days that groups of rats were challenged with OVA or saline (days 15–17), the investigators exposed the rats to CAPs for 8 hours per day in the mobile facility. The two 3-day exposures, conducted in two different weeks, are referred to as CAPs Experiments 1 and 2 in this document and the Critique prepared by the HEI Health Review Committee in its independent review of the study. The investigators made measurements of PM mass; elemental carbon and organic carbon (EC and OC); sulfate, nitrate, and ammonium ions; pollutant gases (ozone, carbon monoxide [CO], sulfur dioxide [SO_2], and total nitrogen oxides [NO_x]); several organic species including polycyclic aromatic hydrocarbons (PAHs); and several trace elements in both ambient air and CAPs. The mobile laboratory also collected meteorological measurements.

At the LRRI, a team led by Dr. Joe Mauderly produced DEE from a single-cylinder 5500-watt diesel engine generator using number 2 nationally certified diesel fuel. The PM and gaseous components of DEE were characterized physically and chemically as in the CAPs substudy. Whole-body

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Jack R. Harkema, Department of Pathobiology and Diagnostic Investigation, Room 218, National Food Safety and Toxicology Building, East Lansing, MI 48864, and colleagues. The complete report, *Effects of Concentrated Ambient Particles and Diesel Engine Exhaust on Allergic Airway Disease in Brown Norway Rats*, can be obtained from HEI or our Web site (see last page). **HARKEMA 145**

exposures to DEE at either 30 or 300 μg/m^3 PM (or to filtered air as a control) were conducted for 8 hours per day for 3 days on the same days as either sensitization (days 1–3) or challenge (days 15–17) with OVA or saline.

In both the CAPs and DEE studies, rats were killed on day 18 and airway tissues from the nose and lung were harvested and bronchoalveolar lavage fluid (BALF) was collected. Total and differential cell counts and levels of secreted mucin glycoprotein (Muc5AC), total protein, elastase, β-glucuronidase, multiple cytokines, and other soluble mediators including OVA-specific immunoglobulin E (IgE) were evaluated in the BALF. Fixed nasal, and proximal and distal axial pulmonary airways were examined morphometrically to assess characteristics of inflammation in the airways, which included mucus cell metaplasia and an increase in stored intraepithelial mucosubstances (IM) in airway epithelial cells. The investigators also used bromodeoxyuridine (BrdU) labeling to assess the number of surface epithelial cells synthesizing DNA in the axial airways. Levels of RNA specific for mucin and multiple cytokines were also assessed in lung tissue.

RESULTS

The concentrator in the mobile laboratory in Detroit preferentially concentrated particles around 0.6 μm and smaller in diameter from ambient air, and by 20- to 31-fold based on $PM_{2.5}$ mass. Some PM components in the CAPs (e.g., some trace metals) were concentrated in proportion to their mass in ambient air, but others, including EC and OC, showed some variation. In both CAPs Experiments 1 and 2 the proportions of the major identifiable components of CAPs — OC, sulfate, nitrate, and ammonium ions — were similar, but levels of several trace metals — including Rb, Ni, Fe, and Al — were higher during CAPs Experiment 1 than Experiment 2. Ambient concentrations of several components of the PM mix that were not concentrated — ultrafine particles (diameter $\leq$ 0.1 μm), PAHs, and the pollutant gases SO_2 and NO_x, were also higher during CAPs Experiment 1 than Experiment 2.

At the LRRI most of the particles derived from the diesel generator were in the ultrafine range, and the major chemical component was EC, followed by OC (6%–14%), with small amounts of inorganic ions and trace metals. Levels of NO_x and CO in the high-diesel-exposure atmosphere were approximately 4 ppm.

At both study sites OVA sensitization and challenge of rats resulted in several features of an inflammatory response in the nasal and pulmonary areas. In the absence of sensitization and challenge with OVA, exposure to either CAPs alone or DEE alone had few biologic effects.

Exposure to CAPs during OVA challenge in CAPs Experiment 1 increased some features of the OVA-induced inflammatory response in the lungs — the severity and extent of distribution of detected pathology in the bronchi and alveoli; total number of cells (particularly eosinophils and lymphocytes); levels of total protein, Muc5AC, and tumor necrosis factor α in BALF; and levels of IM in proximal and distal pulmonary airways — but decreased levels of several cytokine-specific RNAs in lung tissue. Levels of nearly all measured cytokines and OVA-specific IgE (a characteristic of an allergic immune response) in BALF were not affected. Few, if any, effects of CAPs during OVA challenge on inflammatory or immune endpoints were detected in CAPs Experiment 2. No effects of CAPs during OVA challenge were detected in the nose in either CAPs experiment.

The effects of DEE exposure on OVA sensitization and OVA challenge were mild, and the pattern of DEE-associated changes was quite complicated. First, in both sensitization and challenge substudies, greater effects were observed at the lower DEE exposure concentration, 30 μg/m^3 PM, than at the higher, 300 μg/m^3 PM; few effects were detected at the high-level DEE exposure. Second, exposure to the low-level DEE during OVA sensitization predominantly *enhanced* inflammatory endpoints induced by OVA alone, whereas exposure to the same level of DEE during OVA challenge *attenuated* inflammatory endpoints induced by OVA alone. No changes in levels of OVA-specific IgE were detected, and no effects of DEE exposure during either OVA sensitization or challenge were detected in the nose.

SUMMARY AND CONCLUSIONS

In its independent review of the study, HEI's Health Review Committee thought that Harkema and colleagues successfully designed and conducted a descriptive study to evaluate the effects of two pollutants — CAPs (concentrated $PM_{2.5}$) and DEE — in a rodent model of asthma. The model had some but not all characteristics of the human disease, so caution should be used in extrapolating data obtained in the model to humans.

The most surprising findings were the relative lack of effect of high-level DEE (300 μg/m^3 PM) exposure in the model, which used rats sensitized and challenged with the airborne allergen OVA. In addition, low-level DEE exposure (30 μg/m^3 PM) during allergen sensitization mildly enhanced inflammatory responses, but the same exposure during allergen challenge attenuated several effects of exposure to OVA alone.

These findings differ from those of previous diesel-exposure studies, which have reported enhancement of inflammatory and allergic responses in humans when exposed and in animal models. Differences in findings could be the result of the lower levels of diesel emissions used in the current study compared with some previous studies. Another possible explanation is that in some prior diesel-exposure studies rodents and humans were administered diesel particles, rather than the whole emissions used in the current study. Thus, the presence of gases in the DEE used in the present study could be hypothesized to have inhibited responses to the diesel particulate fraction. On the other hand, some controlled human studies of exposure to DEE — which clearly contained both gaseous and particulate components — have shown limited *enhancement* of allergic and inflammatory responses, findings inconsistent with the notion that gases found in diesel emissions might have inhibited a diesel-particulate-mediated enhancement of allergic and inflammatory responses that would otherwise have occurred.

The Committee also thought that the investigators made good use of a mobile air research laboratory to expose rats to CAPs at a site where the prevalence of asthma in the population is higher than average. In CAPs Experiment 1, exposure to CAPs during the 3 days of OVA sensitization enhanced some allergic and inflammatory endpoints, a finding consistent with data from previous studies, but that was not observed in CAPs Experiment 2. Differences in the findings of the two CAPs experiments suggest that the observed differences in composition of CAPs, and hence sources of $PM_{2.5}$, in the different weeks that CAPs were collected may be factors in determining the pattern of response obtained. However, the investigators did not perform analyses to identify sources of pollutants. In addition, in contrast to the investigators, the Committee was not convinced that the multiple elements whose levels were elevated in CAPs in Experiment 1 were associated specifically with local stationary sources. Thus, the Committee did not believe that positive effects of CAPs in Experiment 1 could be attributed easily to any one set of PM components or type of source.

The Committee also cautioned that in this study, as in all others that use CAPs, the concentrated particles may not be representative of particles in ambient air. Concentrated and ambient particles may differ in either physical characteristics — selective concentration of a particular size of particle — or chemical composition — selective concentration of particular components. In a similar note of caution about the diesel-exposure results, the Committee noted that the diesel emissions emitted by the generator used in the current study differ from emissions derived from new diesel-powered vehicles, which are subject to recent regulations to reduce particulate emissions. Future studies to explore the effects of exposure to airborne pollutants will need to use more relevant exposure atmospheres and better models of human disease.

Research Report 145

Effects of Concentrated Ambient Particles and Diesel Engine Exhaust on Allergic Airway Disease in Brown Norway Rats

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INVESTIGATORS' REPORT *by Harkema et al.*
Specific Objectives
Study Designs, Methods, and Materials
Analytical Methods for Ambient Particles, CAPs, and DEPs
Urban Ambient Aerosol and Gaseous Pollutant Measurements
Statistical Analyses for Ambient Air, CAPs, and DEPs
Animal Necropsies and Tissue Selection for Analysis
Airway Tissue Selection and Processing for Airway Morphometry
Morphometric Analysis of IM in Airway Epithelium
Analyses of BALF
Gene Expression Analysis of Airway Mucin and Inflammatory Cytokines
Statistical Analyses of Biologic Endpoints
Results
Biologic Changes Related to DEE Exposures
Discussion
Conclusion

CRITIQUE *by the Health Review Committee*
Introduction
Background
Objectives and Specific Aims
Study Design
Results
HEI Evaluation
Summary and Conclusions

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