



STATEMENT

Synopsis of Research Report 106

HEALTH
EFFECTS
INSTITUTE

Effects on Mice of Exposure to Ozone and Ambient Particle Pollution

INTRODUCTION

Epidemiologic studies have indicated that small short-term increases in the concentration of particulate matter (PM), the complex and variable mixture of particles in the atmosphere, are associated with short-term increases in human morbidity and mortality. Particularly at risk are elderly people and individuals with compromised cardiac or airway function, such as those with asthma.

Plausible biologic mechanisms that would link low-level PM exposure with pathophysiologic effects or would explain why people with asthma may be more sensitive than healthy individuals to PM exposure have not yet been established. Because PM is present with other airborne pollutants, it is also important to evaluate the health effects of simultaneous exposure to multiple pollutants.

APPROACH

Dr Lester Kobzik and colleagues at Harvard School of Public Health used a mouse model of asthma to evaluate how inhaling pollutants affects the airways. The mice were sensitized to the allergen ovalbumin and later challenged with the same aerosol to induce a lung condition in the mice similar to that found in people with asthma. This mouse model of asthma has some appropriate characteristics of human asthma (most notably, allergen-induced airway inflammation), but lacks others (eg, hypersecretion of mucus).

The investigators studied concentrated ambient particles (CAPs) of respirable size and ozone, a gaseous pollutant known to cause airway inflammation and to compromise respiratory function. Kobzik hypothesized that exposure to CAPs plus ozone would cause a synergistic (or greater-than-additive) response. To maximize possible CAPs effects, he used a new version of the Harvard Ambient Particle Concentrator to deliver particles at concentrations that were many times higher than those in ambient Boston air (varying from 12-fold to 67-fold on different days of the study). He exposed mice to ozone at 0.3 ppm, a concentration similar to levels found in urban ambient air. Kobzik also measured daily levels of elements in the PM and CAPs so that, if he found a change in airway function, he could associate it with the elemental composition of the particles.

Kobzik and colleagues evaluated two endpoints associated with the asthmatic response: airway responsiveness and airway inflammation. They evaluated airway responsiveness by challenging the mice with various aerosol concentrations of methacholine, a bronchoconstrictor or agent that narrows the small airways. Mice were challenged with the methacholine aerosol immediately after a 5-hour exposure to one or both pollutants (CAPs and ozone). In some experiments, airway responsiveness was also measured beginning 24 hours after the exposure.

One valuable aspect of the investigators' approach was that they used whole-body plethysmography to measure airway responsiveness. This technique is noninvasive and allows a large number of animals to be tested repeatedly. They placed a mouse in an exposure chamber that was connected to a reference chamber; as the mouse breathed the methacholine entering the exposure chamber, the difference in pressure between the two chambers was measured. From these pressure readings, they derived a recently defined parameter known as enhanced (enh) pause (P), or Penh.

They also evaluated the effects of the pollutants on airway inflammation 24 or 48 hours after the exposure by assessing cells in bronchoalveolar lavage fluid to determine whether (1) the total number of cells increased, and (2) the numbers of cells associated with an inflammatory response, such as eosinophils, increased.

In addition, Kobzik and colleagues evaluated *in vitro* how concentrated particles might induce the synthesis of cellular mediators associated with inflammation. They did this by resuspending particles collected on different days of the *in vivo* exposure study, adding them to lung cells (derived from rats), and measuring 24 hours later the levels of tumor necrosis factor α (TNF- α) and macrophage inflammatory protein-2 (MIP-2) produced by the cells.

RESULTS AND INTERPRETATION

Kobzik and colleagues used four statistical approaches to evaluate the effects of pollutants on Penh. One approach indicated that CAPs (without ozone) slightly increased Penh immediately after exposure, but values

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returned to baseline by 24 hours later. This suggests that the particles' effect on Penh is small and transient, which is consistent with the effects of various air pollutant components on airway function that have been described in other studies. Ozone alone did not increase Penh after exposure. Only one of the four statistical approaches suggested a synergistic effect of CAPs plus ozone, and the investigators were appropriately cautious in interpreting this finding.

Another approach—factor analysis—suggested that different elemental components of the CAPs were associated with different effects on Penh; in particular, that Penh increased on days with high aluminum and silicate levels in the particles. Some particle components also appeared to be associated with decreased Penh. These findings support the idea that the magnitude of a specific health outcome on a particular day is a function of the aggregate elemental composition and concentration of pollutants in ambient air on that day.

In vivo, CAPs had little or no effect on the numbers of cells in bronchoalveolar lavage fluid 24 or 48 hours after

exposure; ozone slightly increased cell numbers in a pattern consistent with inflammation, which was to be expected. In vitro, resuspended CAPs induced the synthesis of high levels (frequently more than 1000-fold increases above background levels) of TNF- α and MIP-2 from rat lung cells. These results confirm previous findings from the investigators that suggest that components present in resuspended particles can induce the release of mediators associated with lung inflammation.

Overall, the results indicate that respirable PM, even when concentrated to levels higher than normally found in ambient air, had little effect on the airway mechanical and inflammatory parameters measured in this small-animal model of asthma. In addition, the effects of exposure to CAPs plus ozone did not achieve synergy that could be convincingly demonstrated. Because this mouse model mimics only some characteristics of human asthma, which is a complex illness, we cannot be certain to what extent these results may or may not predict the effects of ambient PM and ozone exposure on people with asthma.

Effects of Combined Ozone and Air Pollution Particle Exposure in Mice

INVESTIGATORS' REPORT

Materials and Methods

- In Vivo Animal Studies
- In Vitro Analysis of Particle Effects
- Statistical Analyses

Results

- Airway Responsiveness: Overview of Data Analysis
- Airway Responsiveness: Starting 24 Hours After OVA or PBS Challenge
- Assessment of Cells in Bronchoalveolar Lavage Fluid
- In Vitro Cytokine (TNF- α and MIP-2) Production Caused by Particle Samples

Discussion

HEALTH REVIEW COMMITTEE'S CRITIQUE

Scientific Background

Technical Evaluation of Methods

- Sensitization and Aerosol Challenge of Mice
- Exposure to CAPs and O₃

- Measurement of Airway Responsiveness

- Bronchoalveolar Lavage

- Physical and Chemical Characterization of PM Components

- In Vitro Effects of Resuspended Concentrated Particles on Rat Cells in Bronchoalveolar Lavage Fluid

- Statistical Analysis

Results

- Exposure to CAPs, With or Without O₃, Induced a Small Increase in Penh

- Factor Analysis Identified Associations Between Components of CAPs and Changes in Penh

- CAPs Plus O₃ Worked Synergistically to Enhance Penh in an Analysis That Included Broncho-constrictor Concentration

- Exposure to CAPs With or Without O₃ Had Little or No Effect on Numbers of Cells in Lavage Fluid from OVA-Challenged Mice 24 or 48 Hours After the Last Challenge

- Resuspended CAPs Samples Stimulated Mediator Production in Lavaged Rat Cells in Vitro

Discussion