HEALTH EFFECTS INSTITUTE

STATEMENT

Synopsis of Research Report 135

Mechanisms of Particulate Matter Toxicity in Neonatal and Young Adult Rat Lungs

BACKGROUND

Ambient particulate matter (PM) is a complex mixture of solid and liquid particles suspended in air. Depending on the source of pollution, PM varies in size, chemical composition, and other physical and biologic properties. On the basis of epidemiologic findings and supporting results of toxicologic studies, many governmental agencies have set regulatory standards or guidelines for concentrations of ambient PM. Particles ≤ 10 µm in aerodynamic diameter (PM_{10}) are of most concern because these particles are considered to be respirable by humans. To protect the general population and groups considered most vulnerable to adverse effects from PM in the United States, the U.S. Environmental Protection Agency monitors PM_{10} levels and has promulgated National Ambient Air Quality Standards for particles ≤ 2.5 µm in aerodynamic diameter ($PM_{2.5}$ or fine particles). Some scientists believe that ultrafine particles (≤ 100 nm in diameter) may be particularly toxic.

Much work has focused on understanding the effects of particles derived from combustion sources: these particles are in the fine and ultrafine range and generally are composed of an elemental carbon core that binds metals (such as iron, vanadium, nickel, copper, and platinum), organic carbon compounds, and sulfates. Critical questions in PM research involve understanding the mechanisms by which particles may cause effects and the key characteristics of particles, both physical and chemical, that are associated with toxicity. To address these questions, HEI issued RFA 96-1, *Mechanisms of Particle Toxicity: Fate and Bioreactivity of Particle-Associated Compounds,* in 1996.

APPROACH

In response, Dr. Kent Pinkerton and colleagues from the University of California–Davis, conducted a study to evaluate the effects of short-term exposure on the airways of rats using laboratory-generated ultrafine metal particles, either alone or in combination with soot. The rationale for the study was that combinations of metal and soot should provide an understanding of the interaction between components of combustion source-derived particles found in ambient air.

The major objective of this study was to determine if the biologic response to inhaled ultrafine particles depended on particle composition. The investigators focused on oxidative stress and inflammatory responses in rat lungs and airways after exposure to three different particle compositions: iron, soot, or a combination of iron and soot.

To accomplish these objectives, the investigators constructed a flame combustion system connected to an animal exposure inhalation unit and generated particles of iron, soot, and combinations of iron and soot. The investigators characterized the particles physically and chemically. Young adult male rats were exposed for six hours per day on three consecutive days to: iron particles alone (57 or 90 $\mu g/m^3$/day); soot particles alone (250 $\mu g/m^3$/day); a combination of iron and soot particles (250 $\mu g/m^3$/day total, with an iron concentration of 45 $\mu g/m^3$); or a filtered air control. Male and female neonatal rats were exposed for six hours per day for three consecutive days at age 10–12 days and again at age 23–25 days, but only to the combination of iron and soot particles. In one experiment the concentration of iron was 30 $\mu g/m^3$ and in the other it was 100 $\mu g/m^3$; the total concentration of the combination of iron and soot particles was 250 $\mu g/m^3$/day.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Kent Pinkerton at the Center for Health and the Environment, University of California–Davis. The complete report, *Mechanisms of Particulate Matter Toxicity in Neonatal and Young Adult Rat Lungs*, can be obtained from HEI or our Web site (see reverse side).

Dr. Pinkerton and coworkers obtained bronchoalveolar lavage fluid 2 hours and lung tissue 24 hours after the end of the third day's exposure, respectively. They measured several oxidative stress, inflammatory response, and lung injury endpoints.

RESULTS

The iron particles generated were predominantly ultrafine ferric oxide. Ultrafine soot particles were found (20 nm), but formed chains or clusters larger than 100 nm (fine particles). Most combinations of iron and soot particles had a mean diameter of 70–80 nm (ultrafine particles), but some were in the fine particle range. In the exposures to particle combinations of iron and soot, most of the iron particles appeared separate from soot particles but in close proximity. On average, combinations of iron and soot particles contained 60% elemental carbon and 40% organic carbon, but the organic carbon species were not characterized further.

In adult rats exposure to the higher concentration of iron particles had effects that were not observed after exposure to the lower concentration of iron (changes in some measures of oxidative stress and increases in levels of ferritin and the proinflammatory cytokine IL-1β). In neonatal animals, exposure to either combination of iron and soot particles affected some markers of oxidative stress; only the combination containing the higher concentration of iron was also associated with increased ferritin and IL-1β levels, increased lactate dehydrogenase activity (a marker of cellular injury), and decreased cell viability. Exposure of neonatal rats to combinations of iron and soot particles also impaired cell proliferation in the alveolar region of the lung. Overt evidence of inflammation was not found, however, in either young adult or neonatal rats.

The investigators also found that a low concentration of iron particles (45 $\mu g/m^3$) in the presence of soot, which by itself induced no changes compared with controls, had more biologic effects than a similar concentration of iron particles alone (57 $\mu g/m^3$). The pattern of endpoints changed by exposure to the combination of iron and soot was similar to the pattern of endpoints affected by the higher concentration of iron particles alone (90 $\mu g/m^3$).

SUMMARY AND CONCLUSIONS

Using a flame generation system Pinkerton and colleagues successfully generated particles of iron, soot, and combinations of iron and soot and characterized the particles. Iron particles and soot particles were predominantly in the ultrafine range, but soot particles formed chains or clusters that were in the fine particle range. The combinations of iron and soot particles generated contained a high percentage of commingled particles, with a bimodal distribution similar to that of soot particles alone. Combinations of iron and soot particles contained elemental and organic carbon.

In both young adult and neonatal rats the biologic response in the airways and lung tissue to inhalation of ultrafine particles depended on particle composition, specifically on the iron content of the particles. In adult rats changes in some markers of oxidative stress, an increased release of the proinflammatory cytokine IL-1β, and increased levels of ferritin were detected at a higher but not at a lower iron concentration. In neonatal rats more effects were detected after exposure to particles containing a higher proportion of iron than to particles containing a lower proportion of iron (increased levels of ferritin and IL-1β, decreased cell viability, and increased cell injury). The finding that cell proliferation in the alveolar region of the lung was impaired after neonatal rats were exposed to combinations of iron and soot particles suggests that exposure to air pollutants during a period of rapid lung development may affect lung growth. Overt evidence of inflammation was not found, however, in either young adult or neonatal rats. Particle effects were measured in the airways and lung tissue at only one time point after exposure, so it is possible that different results might have been obtained at different time points.

The investigators also found that the presence of soot particles enhanced the effects seen with iron particles alone, suggesting that the effects of soot and iron were synergistic. If reproduced in other studies, this would suggest that the interaction between different particles may affect their toxicities.

The investigators suggest that their combinations of iron and soot particles bore a generic similarity to diesel exhaust particles; both contain carbon and iron and have a similar size distribution. However, the investigators found that the particles differed in several other key physical and compositional characteristics; for example, the percentage of iron in diesel particles is much smaller than in the iron and soot mixtures evaluated in this study. Thus, although both iron and carbon particles are abundant in the atmosphere, the relevance of the particles generated in this study to the emissions of a particular source such as diesel is uncertain. Extrapolating the study findings to humans is difficult because of likely differences in oxidative stress responses between rats and humans and because the levels of particles were high compared with human ambient air exposures.

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