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Project Summary

Effect of Industrial Particulate Samples on Alveolar Macrophages

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Toxic ranking of the <3 μm size fraction of particulate samples in *in vitro* rabbit alveolar macrophage assay showed generally low to intermediate cytotoxicity for samples obtained from a steel foundry, an electric arc furnace steel plant and from four coal-fired power plants. Particulates obtained from an aluminum and from a copper smelter were highly toxic to alveolar macrophages as monitored by viability, total cellular protein and ATP levels. The two smelter samples also contained soluble components that significantly contributed to their cytotoxicity. None of the particulate samples tested were true emission or effluent samples, but were collected from in-plant control devices.

The copper smelter dust and the fluidized-bed coal fly ash chosen on the basis of their respective high and low *in vitro* cytotoxicity were used in aerosol exposures to examine their *in vivo* effects on the pulmonary free cells, bactericidal activity and resistance to respiratory infection in mice. The results of multiple daily 3-hour exposures to 2.0, 1.0 and 0.5 mg/m^3 of the pollutants closely correlated with the *in vitro* data. Inhalation of copper smelter particle aerosols produced significant changes in more of the parameters than inhalation of the coal fly ash. Thus the overall objective of these studies was realized by demonstrating the validity of prediction of inhalation hazard on the basis of the *in vitro* screening assay.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project which is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Alveolar macrophages protect the lungs principally by phagocytosis of inhaled particles and infectious agents. Adverse effects on the activity of the alveolar macrophages can lead to increased retention of non-biological particles, as well as to impairment in the defensive capacity of the lung and consequent increased susceptibility to respiratory disease. Since resistance to infection is known to be lowered by exposure to pollutants, changes in the functional characteristics of alveolar macrophages can be used to monitor effects of such exposure in the intact animal. However, since alveolar macrophages can be obtained easily by tracheobronchial lavage and maintained in culture, they are frequently used in *in vitro* toxicology to assess the potential inhalation hazard of various substances.

The advantages of *in vitro* screening assay in terms of cost and time efficiency are well known. The rabbit alveolar macrophage test, a rapid, *in vitro* assay has been used extensively at EPA and IITRI laboratories to evaluate the relative cytotoxicity of a variety of soluble compounds and particulates (1, 2, 3, 4, 5, 6,

7, 8). This bioassay system is capable of screening and toxicity ranking of a broad spectrum of materials and thereby identified not only the potentially hazardous, but also the inert compounds. Based on results of the *in vitro* assays the number of samples which must be studied further *in vivo* can be significantly reduced.

The objective of the studies was to determine if *in vitro* exposure of alveolar macrophages to a series of complex industrial particles resulted in the same relative toxicity ranking as *in vivo* aerosol inhalation exposures to these particles in intact animals. The studies were designed to examine the relative toxicity of a number of particulate samples. The *in vitro* studies provided initial information on the toxicity of the particles and these data formed the basis for selection of samples for the subsequent *in vivo* investigations. The test particles used in the studies were collected from various metal manufacturing plants and coal-fired power plants directly from in-plant control devices and were not post-control device emission samples. All samples were air classified; only particles in the 3μm size-fraction were used.

Conclusions

Particles provided by the EPA were collected as baghouse samples, or from electrostatic precipitators, or by cyclone sampling train from three conventional and one fluidized-bed coal fired power plants, and electric arc furnace steel plant, a steel foundry and an aluminum and copper smelter. None of the samples reported here were emission or effluent samples collected after pollution control devices and were not necessarily similar in composition or toxicity to emission samples. All samples were air classified and only particles in the <3 μm size-fraction were used.

The *in vitro* effects on rabbit alveolar macrophages were monitored in dose response experiments using cell viability, total protein and ATP levels as experimental parameters. Regression analysis applied to these data showed a significant negative linear dose response relationship for each parameter in all samples, thus enabling the evaluation of their relative cytotoxicity. The results indicated that particles from a steel foundry and an electric arc furnace steel plant, and coal fly ash samples from three conventional combusiton processes and one fluidized-bed system had a low to intermediate cytotoxic effect. Samples from a copper and an aluminum smelter ranked high in cytotoxicity relative to all others, with the copper smelter sample being the most toxic. In addition to the particles *per se* soluble components released from copper and aluminum smelter samples contributed to their cytotoxic effect on alveolar macrophages.

To confirm the relevance of the *in vitro* assay the inhalation hazard of these particulate samples was studied in intact animals. Mice were exposed 3 hr/day, 5 days/week for up to 4 weeks to aerosols of the copper smelter dust and of the fluidized-bed coal fly ash, i.e., particles that had shown high and low cytotoxicity *in vitro*. Aerosol mass concentrations used were 2040 and 1010 $\mu g/m^3$ for the coal fly ash and 2050, 1020 and 540 $\mu g/M^3$ for the more toxic copper smelter dust. The effects of inhalation on the pulmonary defense system were determined after 5, 10 and 20 exposures by examination of the pulmonary cellular lavage, bactericidal activity in the lungs and the resistance to experimentally induced respiratory bacterial infection. Results of these studies substantiated the *in vitro* observations by demonstrating that inhalation of copper smelter dust was significantly more deleterious (i.e., increased susceptibility to streptococcus infection and decreased pulmonary bactericidal activity) than of the fluidized-bed coal fly ash. The major objective of these studies was accomplished by demonstrating the feasibility of predicting the potential inhalation hazard of a particulate substance on the basis of the *in vitro* alveolar macrophage screening assay.

Recommendations

Copper smelter dust which contained 13% arsenic in addition to such other major trace metal constituents as lead, copper, iron, antimony and zinc showed high *in vitro* cytotoxicity in the rabbit alveolar macrophage assay. These *in vitro* studies also indicated that much of the arsenic can be solubilized from the particles and the leachate *per se* is toxic to alveolar macrophages. Inhalation of copper smelter dust (<3 μm aerodynamic diameter) significantly reduced the pulmonary bactericidal activity and the resistance to respiratory bacterial infection in mice. If the arsenic content is used as one basis of assessment of the health hazard of the copper smelter dust, the experimental results suggest that five daily 3-hr aerosol exposures to an equivalent of 266 $\mu g/m^3$ of arsenic resulted in significantly increased mortality rates from streptococcal pneumonia. Moreover, a significant depression of pulmonary bactericidal activity was seen after five daily exposures to 133 $\mu g/m^3$ of arsenic. These concentrations are considerably lower than the TLV of 500 $\mu g/m^3$ of arsenic.

Most the arsenic in the atmosphere is the consequence of emissions from copper, lead and zinc smelters. At smelting operating temperatures arsenic trioxide is formed that, upon cooling, condenses on the surface of small particulate effluents. Since soluble arsenic trioxide adsorbed on particulates of <3 μm may easily penetrate into the gas-exchange region of the lung, it can be released there. Larger aerosol particles containing arsenic in soluble and/or non-soluble form can partially be adsorbed in the upper respiratory tract and the conducting airways.

Thus, studies should be conducted to determine if particulate aerosols that contain components potentially soluble in the respiratory tract *a priori* studies are necessary to explain how the respiratory defense systems are affected by the form of arsenic or by the particle size of the inhaled aerosol. Since the copper smelter dusts also contain other metals such as lead, copper, zinc and antimony that can potentially contribute to their toxicity, studies should be undertaken to compare the effects of inhalation of smelter dusts with those of aerosol exposures to such trace metals in a soluble form and at chemically equivalent concentrations.

The *in vivo* studies indicated increased susceptibility to respiratory infection in mice exposed to copper smelter dust particles. Further studies are necessary to determine the effects of inhalation of such toxic particulate aerosols on the cellular and humoral immune systems and thereby elucidate the changes in the immune mechanisms due to particulate pollutant-induced immunotoxicity.

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The complete report, entitled "Effect of Industrial Particulate Samples on Alveolar Macrophages," (Order No. PB 81-150 963; Cost: $6.50, subject to change) will be available only from:

National Technical Information Service
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Telephone: 703-487-4650

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