RESEARCH REPORT

HEALTH EFFECTS INSTITUTE

Number 129
September 2005

Particle Size and Composition Related to Adverse Health Effects in Aged, Sensitive Rats

Fletcher F Hahn, Edward B Barr, Margaret G Ménache, and JeanClare Seagrave

Includes a Commentary by the Institute's Health Review Committee

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HEI STATEMENT
Synopsis of Research Report 129

Lung Inflammation and Exposure Spikes of Fine and Ultrafine Particles in Aged and Young Adult Rats

Epidemiologic studies have established an association between short-term increases in ambient levels of particulate matter and increases in morbidity and mortality, especially in older individuals with preexisting cardiovascular or respiratory disease. Despite the consistency of the epidemiologic findings, the biological mechanisms underlying these associations are not well understood. One set of research questions has focused on the role of particle attributes (such as size, surface properties, and composition) in causing adverse health effects.

In 1998, HEI issued Request for Applications (RFA) 98-1, "Characterization of Exposure to and Health Effects of Particulate Matter." In response, Dr Kristen Nikula and colleagues of the Lovelace Respiratory Research Institute proposed to systematically examine lung inflammation in young adult and old rats after inhalation of fine particles (< 2.5 μm in aerodynamic diameter) and ultrafine particles (< 0.1 μm in aerodynamic diameter) of different composition: relatively inert carbon and vanadium pentoxide (V_2O_5), which contains the transition metal vanadium, known to cause toxic effects upon inhalation in humans in occupational settings. In addition, they proposed to examine the effect of a short-term increase (spike) in particle exposure concentration on inflammatory response. The investigators hypothesized that a spike in particle concentration would cause a greater inflammatory response than exposure to a steady concentration at equal particle lung burden would. During the second year of the study, Dr Fletcher Hahn assumed primary responsibility for the research.

APPROACH

The investigators used a previously developed deposition model to estimate the relative deposition of fine and ultrafine particles. They had identified predetermined concentrations to which rats would be exposed and used the model to make adjustments according to relative deposition. The model was designed to address several issues: (1) differences in deposition rate between ultrafine and fine particles; (2) differences between carbon and vanadium particle characteristics that may affect particle deposition; (3) differences in lung deposition between young adult and old rats due to differences in lung morphology; and (4) the need to incorporate a spike in exposure concentration into the model. Revisions to the model were made during the study, to add a clearance component, for example.

Rats were exposed for 5 hours/day for 17 consecutive days via nose-only inhalation to ultrafine or fine V_2O_5 or ultrafine carbon particles. Control groups were exposed to filtered air. On day 15, all rats were intratracheally instilled with endotoxin to produce mild inflammation. On days 16 and 17, some rat groups were exposed to higher concentrations of particles to mimic a short-term increase in air pollution. The other groups remained exposed to the same concentration as on days 1 through 15. Ultrafine V_2O_5 (35 nm count median diameter) concentrations were 50, 100, 150, or 200 μg/m^3; fine V_2O_5 (0.7 μm mass median aerodynamic diameter) concentrations were 150, 200, 500, or 1000 μg/m^3; ultrafine carbon (35 nm) concentrations were 150 or 200 μg/m^3. All rats were killed 18 hours after the

Continued

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr Fletcher F Hahn at the Lovelace Respiratory Research Institute, Albuquerque NM. The following Research Report contains both the detailed Investigators' Report and a Commentary on the study prepared by the Institute's Health Review Committee.

end of exposure on day 17. They were examined for lung morphology, lung particle burden, and markers of inflammation and oxidative stress in lung tissue and bronchoalveolar lavage fluid.

RESULTS AND INTERPRETATION

In this complex study, the investigators found that exposure for 17 days to fine and ultrafine particles caused an inflammatory response in aged rats with mild inflammation induced by endotoxin treatment. A greater mass of ultrafine V_2O_5 particles than fine V_2O_5 particles was deposited in the lung at the same inhaled mass concentration. On the basis of equal predicted lung burden, more neutrophils were found in bronchoalveolar lavage fluid from rats exposed to ultrafine V_2O_5 than from rats exposed to fine V_2O_5, indicating that ultrafine particles were more toxic. In general, exposure to ultrafine V_2O_5 caused greater lung responses (eg, LDH levels, neutrophil numbers, and production of mucus in small airways) than exposure to ultrafine carbon did. No major differences were found between aged and young adult rats exposed to fine V_2O_5. The results of this study suggest that, under the experimental conditions used, both particle size and composition are important for particle toxicity. However, more research using different kinds of particles and animal models is needed before generalizing these results to the effects of fine and ultrafine particles in the ambient air pollution mixture.

Some uncertainty remains about Hahn and colleagues' comparison of the effects of fine and ultrafine vanadium because of uncertainties in the model. The model accounted well for several aspects of the experimental design, but it may not have accounted for some differences in particle characteristics between V_2O_5 and carbon, such as in solubility, porosity, and tendency to aggregate into larger groups or chains, which would be difficult to incorporate into any model. The investigators used an innovative approach of analyzing data by predicted particle lung burden rather than exposure concentration to evaluate the effects of fine versus ultrafine V_2O_5. Direct validation of the model remained incomplete, however, because lung burden was determined in only a subset of the rats exposed to V_2O_5 and because the method to measure carbon lung burden did not yield valid results (although the predicted lung burdens for carbon exposures were not used for data analysis).

An interesting and novel finding was that an increase in exposure concentration for 2 days after 15 days of exposure caused greater inflammatory effects than exposure at a constant level (either at the original lower or the final higher concentration). The increase in exposure concentration was modeled after epidemiologic observations that a short-term increase in ambient particulate matter concentration is associated with an increase in deaths and hospital admissions. There are some indications that such an association may exist regardless of the initial concentration, because similar short-term associations have been found in cities with different levels of pollution. In the current study, the increased effect of a spiked exposure concentration was found in rats exposed to fine or ultrafine particles of different composition, but not for all concentrations tested. More research is needed to further investigate and confirm the increased effect of a short-term spike in exposure concentration.

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Library of Congress Catalog Number for the HEI Report Series: WA 754 R432.
The paper in this publication meets the minimum standard requirements of the ANSI Standard Z39.48-1984 (Permanence of Paper) effective with Report 21 in December 1988; and effective with Report 92 in 1999 the paper is recycled from at least 30% postconsumer waste with Reports 25, 26, 32, 51, 65 Parts IV, VIII, and IX, 91, 105, 117, and 128 excepted. These excepted Reports are printed on acid-free coated paper.

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Research Report 129

HEALTH EFFECTS INSTITUTE

Particle Size and Composition Related to Adverse Health Effects in Aged, Sensitive Rats

Fletcher F Hahn, Edward B Barr, Margaret G Ménache, and JeanClare Seagrave

Lovelace Respiratory Research Institute and University of New Mexico Health Science Center, Albuquerque, New Mexico

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

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RELATED HEI PUBLICATIONS

Publishing history: This document was posted as a preprint on *www.healtheffects.org* and then finalized for print.

Citation for whole document:

Hahn FF, Barr EB, Menache MM, Seagrave JC. September 2005. Particle Size and Composition Related to Adverse Health Effects in Aged, Sensitive Rats. Research Report 129. Health Effects Institute, Boston MA.

When specifying a section of this report, cite it as a chapter of the whole document.

INVESTIGATORS' REPORT

Particle Size and Composition Related to Adverse Health Effects in Aged, Sensitive Rats

Fletcher F Hahn, Edward B Barr, Margaret G Ménache, and JeanClare Seagrave

ABSTRACT

Small increases in concentrations of ambient particulate matter (PM*) have been linked to adverse health effects, especially in older people and people with preexisting respiratory disease. Some epidemiologic studies have shown the association to be stronger with PM less than 2.5 μm in aerodynamic diameter ($PM_{2.5}$) than with PM less than 10 μm in aerodynamic diameter (PM_{10}). Some scientists and regulators suggest that 2.5 μm might be an arbitrary cutoff and that the effects might be more pronounced for PM less than 0.1 μm in aerodynamic diameter (ultrafine PM).

Our first aim was to determine the relation between size of respirable particles and particle toxicity, as well as the health effects of short-term increases (spikes) in particle concentration against backgrounds of relatively low or high baseline exposures.

Our second aim was to determine the effect of spikes in concentration of fine particles (0.7 μm in mass median aerodynamic diameter [MMAD]) and ultrafine particles (35 nm in count median diameter [CMD]) of disparate composition: vanadium pentoxide (V_2O_5) and carbon black. The relative toxicity of these particles was determined in aged rats with mild pulmonary inflammation induced by instilled endotoxin.

Our third aim was to determine the influence of age (aged vs young adult) on particle-induced toxicity in these rats.

We selected endpoints to explore plausible mechanisms for differences in toxicity related to particle size and composition, mild inflammation, or rat age.

Our results suggest four basic conclusions concerning particles. (1) A greater mass of ultrafine than fine V_2O_5 particles was retained in the lungs of rats exposed to aerosols at similar mass concentrations. (2) Inhaled ultrafine V_2O_5 particles caused significantly higher levels of neutrophils (indicative of inflammation) than inhaled fine V_2O_5 particles in bronchoalveolar lavage (BAL) fluid of aged, endotoxin-treated rats, when compared on the basis of equal predicted lung burden, and a few response variables changed differently depending on particle size. (3) Two-day spikes in particle exposure concentration after a constant 15-day exposure resulted in significantly greater responses in lungs of aged, endotoxin-treated rats than in lungs of aged, endotoxin-treated rats exposed to a constant carbon black or V_2O_5 level for 17 days. (4) Aged, endotoxin-treated rats did not differ from young adult rats in their responses to relatively high concentrations of V_2O_5.

These results add further evidence that ultrafine particles in the air may be more hazardous than fine or coarse particles under certain conditions. They also suggest, for the first time, that spikes in PM concentration may initiate adverse responses in the lung at relatively low exposure concentrations (150 $\mu g/m^3$ fine particles or even 50 $\mu g/m^3$ ultrafine particles).

INTRODUCTION

A recent US Environmental Protection Agency (EPA) air quality criteria document for PM (EPA 1996a) notes that "many epidemiological studies have shown statistically significant associations of ambient PM levels with a variety of human health endpoints, including mortality, hospital admissions, respiratory symptoms, and illness measured in community surveys, and changes in pulmonary mechanical function." For example, Schwartz and Dockery (1992) and Schwartz (1994) analyzed data for Philadelphia residents from 1973 to 1980 that focused on

* 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 129, 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 Fletcher F Hahn, Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr SE, Albuquerque NM 87108; *FHahn@lrri.org*.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Award R82811201 to the Health Effects Institute, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency, and no official endorsement by it should be inferred. The contents of this document also have not been reviewed by private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views or policies of these parties, and no endorsement by them should be inferred.

effects associated with total suspended particulate (TSP). They found a 6.5% increase in mortality for a short-term change in TSP from 50 to 150 μg/m^3 and an even greater effect for people older than 65 years and for deaths related to respiratory causes.

A more recent evaluation of mortality and air pollution in the 90 largest cities in the United States reaffirmed the association of increased PM_{10} concentration with increased mortality (Samet et al 2000b). The PM_{10} effect was slightly greater for cardiorespiratory mortality than for total mortality at 1 day after exposure. The association between PM_{10} and mortality was not influenced by inclusion of gaseous pollutants in the analysis. In general, studies that have examined associations between short-term changes in PM concentration and cause-specific mortality have found higher relative risks for respiratory and cardiovascular causes of death than for total mortality, as well as higher risks for mortality in people older than 65 years of age than for mortality in the general population (EPA 1996a).

Similarly, of 13 studies that examined associations between short-term concentrations of PM and hospital admissions, 12 reported positive associations for respiratory and cardiac disease admissions (EPA 1996b). An evaluation of morbidity and air pollution in 20 US cities found that PM_{10} concentration was positively associated overall with hospital admissions of older people for cardiovascular disease, chronic obstructive pulmonary disease (COPD), and pneumonia (Samet et al 2000a). Populations consistently identified as being at greater risk for adverse health effects of PM exposures are children, individuals with respiratory disease, individuals with cardiovascular disease, and older individuals (EPA 1996a). Some respiratory diseases particularly associated with an increased risk are pneumonia, chronic bronchitis, asthma, and COPD.

A number of studies have consistently reported similar associations between increases in particle concentrations and disease. A variety of adverse health effects are associated with any range of particle concentrations studied. For example, similar mortality effects for each 10 μg/m^3 increase in PM_{10} are observed across settings where particle concentrations differ by an order of magnitude (Vedal 1997). This finding has led to the suggestions that short-term increases in particle concentration reflect temporal variability in some factors and that the observed associations between particle concentration and adverse health effects may be spurious (Vedal 1997).

The source of fine particles ($PM_{2.5}$) in the atmosphere also has been shown to affect daily mortality. Laden and colleagues (2000) found that the source of fine particles was indicated in sampled air by a silicon factor for crustal sources, a lead factor for mobile sources, and a selenium factor for coal combustion sources. Airborne particle concentrations were compared with daily mortality statistics in six eastern US cities. Fine particles from mobile or coal combustion sources, but not fine crustal particles, were associated with increased mortality.

Currently, few data can causally link exposure to low PM concentrations or small brief changes in PM exposure concentrations to premature mortality or to indicators of respiratory and cardiovascular disease. Proposed causal mechanisms include pulmonary inflammation with associated histologic alterations, impaired clearance and hypersecretion of mucus, aggravation of preexisting airway obstruction, effects on endothelial cells and altered cardiovascular function, and alterations in host defense mechanisms (EPA 1996a,b).

Most epidemiologic data are based on measurements of TSP or PM_{10} and do not identify the size fraction of respirable PM or the particle composition associated with adverse health effects. Thus, the default assumption for regulation of PM_{10} or $PM_{2.5}$ is that all particles within each size fraction have equal toxicity per microgram of mass regardless of their size and chemistry. Some epidemiologic data suggest that $PM_{2.5}$ derived from combustion processes is more clearly associated with adverse health effects than are either PM_{10} or TSP (Pope 1995). More recent epidemiologic data from studies in Europe suggest that increased ultrafine and fine particle concentrations in the atmosphere cause similar increases in mortality but that fine particles show a more immediate effect and ultrafine particles a more delayed effect (Ibald-Mulli et al 2002). These and other limited epidemiologic data suggest that the health effects of fine and ultrafine particles may be independent.

Studies in animals show that the inflammatory response to ultrafine particles deposited in the lungs is more severe than the response to fine particles of the same mass (Donaldson et al 2001; Oberdörster 2001). This point has been demonstrated in rats instilled with carbon black (Oberdörster et al 1998) or titanium dioxide (TiO_2; Li et al 1999). More importantly, findings concerning rat inhalation of fine and ultrafine TiO_2 aerosols are similar (Ferin et al 1992). However, effects on rats of inhaled cadmium chloride aerosols 30 to 1500 nm in size depended only on particle mass, not size (Cassee et al 2002). The paucity of information about effects of ultrafine particles on animals and humans and about relations between exposure pattern and response compounds the difficulty of setting National Ambient Air Quality Standards on the basis of size fractions and concentrations.

PM exposure regulations that aim to protect humans from PM-associated morbidity and mortality would be

more effective if the particle sizes and exposure patterns most likely to cause adverse health effects were known. In addition, evidence of toxicity that mechanistically explained the associations between PM exposure and morbidity and mortality would increase confidence that the associations are causal and are not merely markers of another exposure or factor.

SPECIFIC AIMS

In this study, we used young adult and aged rats, all with a mild respiratory inflammation induced by instilled endotoxin. We had three basic aims:

1. Determine the relation between size of respirable particles (ultrafine vs fine) and particle-induced toxicity as well as the effect of a short-term increase in particle concentration (spike).

   Two hypotheses underlie this aim:

   - Exposure of animals with induced mild pulmonary inflammation to aerosols of ultrafine particles causes greater cytotoxicity, inflammation, mucous cell metaplasia, and alveolar macrophage hyperplasia than aerosols of fine particles do. The rationale for this hypothesis is that each unit of particle mass deposited in the lung is composed of a greater number of ultrafine particles than fine particles and therefore provides more surface area for reaction and allows more dispersion within the lung.
   - An equivalent increase in PM concentration for a brief time (a 2-day spike) causes similar cytotoxicity, inflammation, mucous cell metaplasia, and alveolar macrophage hyperplasia in rats exposed to relatively low or high baseline particle concentrations before the spike. The rationale for this hypothesis is epidemiologic data that suggest that relatively small, short-term increases in particle concentrations are associated with similar increases in morbidity and mortality in urban populations, even though mean ambient concentrations vary among cities.

2. Determine the effect of a spike in concentration of particles of disparate composition: a transition metal (vanadium, in the form of V_2O_5) and carbon black.

   The underlying hypothesis is that ultrafine particles of a transition metal are more toxic than ultrafine carbon black particles at each exposure concentration. The rationale for this hypothesis is that a transition metal compound causes greater oxidative stress than carbon black, thus increasing pulmonary inflammation.

3. Determine the influence of age on particle-induced toxicity.

   The underlying hypothesis is that aged rats with mild pulmonary inflammation that inhale particles exhibit greater cytotoxicity, inflammation, mucous cell metaplasia, and alveolar macrophage hyperplasia than young adult rats do under the same conditions. The rationale for this hypothesis is that aged rats with pulmonary inflammation have a pulmonary environment that favors the generation of reactive oxygen species and further inflammation when exposed to toxic particles. Epidemiologic studies have shown adverse health effects in both young and older people when exposed to increased ambient particles, but increased mortality is observed primarily in older people. Therefore, adverse health effects are expected in both groups, but they should be more severe or pronounced in older rats.

STUDY DESIGN

ANIMAL MODEL: AGED F344 RATS WITH ENDOTOXIN-INDUCED PULMONARY INFLAMMATION

We induced low-grade pulmonary inflammation in aged rats by intratracheally instilling endotoxin to represent pathologic conditions in an aged, sensitive human population. We studied aged, ~100 week old, male F344 rats from the colonies maintained for the National Institute of Aging (Harlan Sprague Dawley, Indianapolis IN). Male rats in this colony have a median life span of approximately 105 weeks (Sprott 1997).

This animal model of inflammation was chosen for three reasons. First, epidemiologic studies have shown that older people and people with respiratory disease are at greater risk than others for adverse health effects due to PM exposure (EPA 1996a). In many cases, these populations may be one and the same (eg, individuals with respiratory diseases such as pneumonia, chronic bronchitis, and COPD may also be elderly). Second, the small increases in relative risk discovered in epidemiologic studies suggest that large numbers of animals would be needed to detect PM effects at low concentrations unless a sensitive animal population is used along with endpoints that are more sensitive than mortality. Third, toxicology studies using a variety of particle types have shown that healthy, young adult animals generally are not sensitive to low, environmental levels of PM.

For example, subchronic inhalation (6 hours/day, 5 days/week for 13 weeks) by rats of 1.1 mg/m^3 carbon black did not elicit any detectable adverse effect in the lung (Driscoll et al 1996a). In addition, rats exposed to diesel exhaust at 0.35 mg particles/m^3 for 24 months did not show a decrease in survival or alterations in pulmonary function, and they had little pulmonary inflammation at terminal killing (Mauderly et al 1988). In contrast, aged rats may be at greater risk for particle-induced effects because they have less ascorbic acid (Rikans and Moore 1988) and superoxide dismutase (Pérez et al 1991) in the lung, less inducibility of antioxidants (Gomi et al 1995), and less mucociliary clearance (Wolff et al 1987) than younger rats.

We chose endotoxin to induce inflammation in the airway and in pulmonary parenchyma. Endotoxins are lipopolysaccharide molecules released from the walls of gram-negative bacteria. They are believed to be the principal etiologic agents of acute inflammatory responses in gram-negative, bacteria-induced pneumonia and in sepsis. Many ambient aerosols are associated with surface-adhered endotoxin, which has been detected in biologically active concentrations in air humidification systems (Flaherty et al 1984; Rylander and Haglind 1984) and in commercial house dust extracts (Peterson et al 1964). Inhalation exposure to endotoxin may also be involved in several occupational diseases and syndromes, including byssinosis (Rylander and Nordstrand 1974), bagassosis (Salvaggio et al 1966), mill fever (Pernis et al 1961), and sick building syndrome (Burge et al 1987; Harrison et al 1992).

Intratracheal instillation into rats of high doses of endotoxin (5–16 mg/kg body weight) has been used as a model of adult respiratory distress syndrome (Domenici-Lombardo 1995; van Helden et al 1997). Intratracheal instillation of lower doses (0.025–2.5 mg/kg) has been used to study mechanisms of less severe pulmonary inflammation and mucous cell metaplasia (Xing et al 1994; Sugahara et al 1996; Tesfaigzi et al 1998). Endotoxin-induced mucous cell metaplasia of rat bronchial epithelium, which occurs rapidly after endotoxin instillation, has been compared to morphologic findings in airways of humans with chronic bronchitis, bronchopneumonia, and asthma (Harkema and Hotchkiss 1992, 1993).

Activation of alveolar macrophages by endotoxin in people with pneumonia could greatly increase their response to subsequent particle exposure. Driscoll (1990) showed that a low-toxicity dust (TiO_2) and a high-toxicity dust (quartz) can elicit similar levels of tumor necrosis factor secretion if macrophages are sufficiently activated. Endotoxin is a potent activator of macrophages. Killingsworth and colleagues (1997) suggest the importance of preexisting pulmonary inflammation to severity of response: They exposed rats by inhalation to fuel oil fly ash and found that death occurred after exposure only in rats with monocrotaline-induced preexisting pulmonary inflammation.

More recent studies have shown that endotoxin priming affects the pulmonary response to inhaled ultrafine particles in both young and old rats (Elder et al 2000). Rats were exposed to ultrafine carbon black particles and to ozone alone or in combination for 6 hours. Inhalation of a low dose of endotoxin was used to induce respiratory tract infection. The endotoxin increased the effects of the inhaled particles and ozone in both age groups.

MODEL PARTICLES

Two types of PM were chosen for this research: the relatively toxic compound V_2O_5 and the relatively innocuous compound carbon black. This range of toxicity was intended to elicit a range of responses.

V_2O_5 is a toxic constituent of occupational and community air pollution and is representative of transition metal air pollutants. V_2O_5 is the most ubiquitous and commercially important compound of vanadium (reviewed in Knecht et al 1985). Occupational exposure to V_2O_5 occurs during cleaning of oil-fired boilers and furnaces, during handling of catalysts in chemical plants, and during refining, processing, and burning of vanadium-rich fossil fuels (Zenz 1994). The time-weighted mean threshold limit value for V_2O_5 is 0.05 mg/m^3 (American Conference of Governmental Industrial Hygienists 2001). Inhaled vanadium irritates the upper and lower respiratory tracts and causes cough, wheezing, mucus production, chest tightness, runny nose, and sore throat (Kiviluoto et al 1979; Woodin et al 2000). Boilermaker's bronchitis results from exposure to high concentrations of vanadium compounds (> 1 mg/m^3; Levy et al 1984).

In the United States, estimates of rural airborne concentrations of vanadium range from 0.4 to 500 ng/m^3, with the highest concentrations in relatively industrialized areas (Mamane and Pirrone 1998). The burning of fuel oils that contain high concentrations of vanadium produces considerable amounts of V_2O_5 and fly ash (containing 100 to 1000 µg vanadium/g). Ambient airborne PM_{10} from Mexico City contains vanadium, which may contribute to its toxic effect on lung macrophages and myofibroblasts in vitro (Bonner et al 1998). Pritchard and coworkers (1996) showed that the degree of inflammation after instillation of particulate pollution from nine different sources in vivo correlates with the transition metal content as well as the generation of free radicals by the particulate in vitro.

A number of transition metals are community air pollutants. About 1.6 million pounds of vanadium and vanadium compounds were released into the air via various industrial processes in 2001 (EPA 2003). About the same

quantity of manganese, copper, and nickel compounds were released in the same year, as well as about one-third that amount of zinc compounds (EPA 2003). These transition metals differ in their abilities to induce pulmonary inflammation experimentally. When instilled into rat lungs in equimolar concentrations, copper was the most inflammatory metal, followed by manganese and nickel, whereas vanadium, iron(II), and zinc induced similarly lower levels of inflammation, as determined by BAL (Rice et al 2001). Other studies, which used morphologic techniques in addition to BAL, have shown that zinc and copper are equally toxic when instilled into mouse lungs: both are more toxic than vanadium, iron, nickel, and lead (Prieditis and Adamson 2002).

Vanadium is an important transition metal in determining the health effects of air pollutants, as shown with residual oil fly ash particles (Kodavanti et al 1998). Fly ash collected from different sites in an oil-burning power plant contained different amounts of water-leachable metals. When the fly ash was instilled into rat lungs, inflammatory response correlated positively with the amount of water-leachable vanadium but not the amounts of other leachable metals. Other studies have shown that vanadium is the metal primarily responsible for mucin production and secretion after exposure to residual oil fly ash particles (Jiang et al 2000; Longphre et al 2000).

Carbon black has distinctly different physical and chemical properties than V_2O_5: It is relatively nontoxic and causes no inflammatory response in the lung when ultrafine particles are inhaled at concentrations of 110 to 250 $\mu g/m^3$ for 6 hours for 1 or 3 days, respectively (Oberdörster et al 2000; Zhou et al 2003). Investigators have used carbon black as a surrogate for diesel soot (Nikula et al 1995) because it is similar to the carbon core of soot produced by combustion (such as black smoke, a common constituent of $PM_{2.5}$; Sunyer et al 1996; Gielden et al 1997; Knox et al 1997; van Vliet et al 1997). Ambient airborne carbon particles may contain transition metals, such as vanadium, which may amplify toxic effects in the lung (Dye et al 1997).

EXPOSURE REGIMEN

The purpose of our study was to simulate the exposure of the general population to air pollution incidents. The 2-day concentration spike used in our exposures was consistent with the lag time of interest in epidemiologic studies. Results from more than 60 time-series mortality studies suggest that increased mortality occurs within 1 to 5 days after an increase in PM_{10} concentration (Pope 2000). The lag for hospital admissions varies somewhat according to the disease. For example, the effect of PM_{10} decreases continuously over days 0 to 2 for pneumonia admissions, increases over days 0 to 1 for COPD admissions, and decreases to a modest effect by days 1 and 2 for cardiovascular admissions (Zanobetti et al 2000a).

With this in mind, the regimen we chose was to expose rats daily for 5 hours to relatively low concentrations of particles for 15 straight days. On days 16 and 17, the particle concentrations were increased.

To simulate community air pollution, the lowest exposure concentrations were set near the ambient concentrations in the exposure animal housing facility. The higher concentrations were multiples of these lowest concentrations.

HEALTH EFFECT INDICATORS

The indicators of particle-induced toxicity used in this research emphasize oxidative stress, key cytokines and chemokines, and measures of inflammation. Mechanisms of lung injury after exposure to particulate air pollution have been postulated to involve oxidant generation catalyzed by metals associated with air pollution particles (Kadiiska et al 1997). Emission-source particles and ambient air pollution particles have been shown to generate oxygen-derived free radicals in vitro (Ghio et al 1996; Pritchard et al 1996). Emission-source particles that contain transition metals (including vanadium) have also been shown to generate oxygen-derived free radicals in vivo (Kadiiska et al 1997).

Metals that can exist in more than one stable valence state can participate in electron transfer reactions and can generate oxidants. Among those metals that can assume two stable valence states, first-row transition metals titanium, vanadium, chromium, manganese, iron, cobalt, nickel, and copper are found in greatest concentrations in the earth's crust and the atmosphere (Kadiiska et al 1997). These metals are also found in substantial concentrations in particulate air pollution (Lantzy and MacKenzie 1979). Exposure to these metals is associated with oxidative stress (reviewed by Kadiiska et al 1997).

The oxidant-sensitive activation of nuclear factor κB (NFκB) initiates the inflammatory cascade, including increased transcription and release of tumor necrosis factor α (TNF-α) and interleukin 1β (Blackwell and Christman 1997). Macrophage inflammatory protein-2 (MIP-2) expression can increase through TNF-α (Driscoll 1995; Driscoll et al 1997) or through direct action of particles on epithelial cells (Driscoll et al 1996b, 1997). These mediators function as chemotactic agents for inflammatory cells. Thus, after lungs are exposed to particulate air pollution, injury may result from catalyzed free radicals reacting directly with critical molecules in lung cells or

from proteases and endogenous oxidants generated by the recruited inflammatory cells.

PILOT EXPERIMENTS

Endotoxin Doses

The first pilot experiment was conducted to establish the dose of endotoxin instilled into aged rats that would induce moderate bronchopneumonia and bronchitis 1 day after instillation and that would subsequently resolve such that inflammatory and metaplastic changes were minimal to mild 3 days after instillation.

Male, 24-month-old, F344 rats (3 rats/group, 15 total) were intratracheally instilled with sterile, endotoxin-free saline or with one of two doses of endotoxin in saline (0.5 or 0.3 mg/kg body weight) and were killed 1 or 3 days after instillation. The endotoxin was lipopolysaccharide from *Escherichia coli* 0111:B4 (Sigma Chemical Co, St Louis MO). The volume of saline or endotoxin in saline was 0.5 mL. At the time of killing, the right lung lobes were lavaged with sterile, endotoxin-free saline and the left lungs were fixed in formalin. Total cell numbers and differential cell counts were determined in the BAL fluid. Sections of fixed lung were stained with hematoxylin and eosin and Alcian blue and examined by light microscopy.

In general, data for BAL fluid cells (cells per milliliter, differential cell counts, and total cell numbers) showed that both doses of endotoxin induced similar, substantial inflammation 1 day after instillation (Table A.1). Three days after instillation, the amount of inflammation was less than that 1 day after instillation in both endotoxin groups. The rats instilled with 0.5 mg endotoxin/kg still showed evidence of a marked inflammatory response 3 days after instillation, and the response in the rats treated with 0.3 mg endotoxin/kg was between that of the controls and the higher-dosed rats. Neutrophil data from rats treated with 0.3 mg endotoxin/kg tended to be more like the data for control rats than the data for the higher-dosed rats.

Microscopic examination of the left lung sections showed that rats instilled with 0.5 or 0.3 mg endotoxin/kg had moderate to marked bronchopneumonia and moderate bronchitis and bronchiolitis 1 day after instillation. Three days after instillation, bronchopneumonia was mild to marked in the rats instilled with 0.5 mg endotoxin/kg and mild to moderate in the rats instilled with 0.3 mg endotoxin/kg. Bronchitis and bronchiolitis were minimal to mild in the rats instilled with 0.5 mg endotoxin/kg and minimal in the rats instilled with 0.3 mg endotoxin/kg. Mucous cell metaplasia in bronchi and large bronchioles was mild to moderate in the rats instilled with 0.5 mg endotoxin/kg and mild in the rats instilled with 0.3 mg endotoxin/kg. Overall, 3 days after instillation, the histopathologic responses were slightly less in the rats instilled with 0.3 mg endotoxin/kg than in the rats instilled with 0.5 mg endotoxin/kg.

These data show that the dose of 0.3 mg endotoxin/kg induced responses in male 24-month-old F344 rats that were close to those desired for this study. Moderate to marked bronchopneumonia and moderate bronchitis and bronchiolitis occurred 1 day after instillation. Three days after instillation, the bronchopneumonia was mild to moderate and the bronchitis and bronchiolitis were minimal. The mucous cell metaplasia in bronchi and large bronchioles was mild.

We also desired, however, to induce an inflammatory response that was more resolved by 3 days after instillation. Therefore, we used a lower dose—0.2 mg endotoxin/kg—in the second pilot experiment.

Safety of Ultrafine V_2O_5 Exposure and Endotoxin Instillation

We conducted a second pilot experiment to ensure that the combination of ultrafine V_2O_5 nose-only exposure and endotoxin instillation was not too toxic, nor the procedure too stressful, for the aged rats. The experiment also served to confirm the endotoxin dose. Finally, it tested the vanadium exposure system with rats in place and acquired more data on the prevalence of mononuclear cell leukemia in aged rats from the supplier.

Male, 24-month-old, F344 rats were received from the colony maintained for the National Institutes of Aging. These rats were quarantined, conditioned to nose-only exposure tubes over two weeks, and exposed to a target concentration of 100 µg V_2O_5/m^3 or to filtered air 5 hours/day for 7 consecutive days. The target CMD for the aerosol was 30 to 40 nm. Rats were instilled with sterile, endotoxin-free saline or endotoxin in saline on exposure day 5 and killed 1 or 3 days later. At the time of killing, the right lung lobes were lavaged and the left lung lobes were fixed in formalin for histopathology.

As anticipated, the aerosol concentration and CMD had a large standard deviation during the first few days of the exposure, while the aerosol generation and exposure equipment was tested. On days 5 and 6, a stable ultrafine V_2O_5 aerosol was generated (32 nm mean CMD ± 1.7 SD; 1.46 mean geometric standard deviation [σ_g] ± 0.02 SD).

Endotoxin doses of 0.1, 0.2, or 0.5 mg/kg body weight were instilled into the lungs of the rats. The results indicate that the 0.1 mg/kg dose induced the desired amount of pulmonary inflammation: moderate inflammation 1 day after instillation that rapidly diminishes to minimal to mild inflammation 3 days after (Table A.2).

Lungs from 32 rats from the shipment used in this pilot study were examined for mononuclear cell leukemia. The presence of leukemic cells in the lung could presage increased deaths during the stressful events of shipment, conditioning, or exposures. In addition, cellular infiltrates could alter BAL fluid and histologic parameters important for this study. However, only 4 of the 32 rats had histologically evident mononuclear cell leukemia in capillaries and larger blood vessels of the lung, which had not yet invaded the pulmonary interstitial tissues. The low prevalence and relative mildness of the lesions (which were vascular but not extravascular or invasive) suggested that mononuclear cell leukemia would not be a major confounder and that large numbers of extra rats would not be needed to complete the study.

During the 2-week conditioning period, the 24-month-old rats lost weight, and the rate of weight loss accelerated during the exposure period. Some weight loss was expected on the basis of normal weight curves for these rats. In addition, the rats had no access to food during the exposure period.

Overall, the results of this pilot experiment indicate that the doses of endotoxin and V_2O_5 were appropriate and that the handling procedures would not compromise the experiments.

Ambient Aerosol Concentrations

Representative air samples were obtained from the Lovelace Respiratory Research Institute animal housing facilities to determine whether the rats would be inhaling considerable amounts of aerosols from the ambient air relative to the test aerosols. The first series of samples was collected in the room in which the rats were housed. A second series of samples was taken from inside one of the shoebox-style cages in which the rats were housed.

Ambient air samples from the animal room were collected on 47-mm diameter, open-face filters used to determine the mass concentration of particles. The filter samples were taken on 4 different days at flow rates of approximately 20 L/min for a minimum of 6 hours per sample. A scanning mobility particle sizer (SMPS; model 3934; TSI, St Paul MN) was used to determine particle size (CMD) and number concentration. SMPS samples were collected over separate 4-day periods. The room sampling results are summarized in Table 1.

Shoebox cages were located in the animal room. Four rats were housed in a cage on a bed of woodchips and were fed water and food. A mock sample probe was placed into the cage 1 week before the first sample collection day to acclimate the rats to it. The probe had a shroud around the inlet so that the rats would not be able to occlude the opening. The probe collected aerosol samples at a volumetric flow rate of approximately 2 L/min.

Filter samples were collected from the shoebox cage daily on 5 days on 25-mm Zefluor filters for a minimum of 5 hours per sample. SMPS samples were collected daily (6/day) for 3 days. The cage sampling results are summarized in Table 2.

Samples from the animal room and the shoebox cage showed that the rats were exposed to low particle concentrations in the Lovelace Respiratory Research Institute facility. However, the mean mass concentration of 39 $\mu g/m^3$ in the shoebox cage was close to the lowest planned aerosol concentration, 50 $\mu g/m^3$, indicating that lower exposure concentrations were not feasible or warranted in this environment.

Time Course of Endotoxin-Induced Pulmonary Inflammation

The time course of pulmonary inflammation after intratracheal instillation of endotoxin was determined for the dose (0.1 mg endotoxin/kg body weight) planned for

Table 1. Ambient Air Particle Characterization in Animal Room

	Mass Concentration ($\mu g/m^3$)[a]	CMD (nm)[b]	Particle Number Concentration (particles/cm^3)[b]
Mean	12	53	110
SD	4	22	59
N	4	60	60

[a] Collected daily on 4 days on 47-mm diameter, open-face filters at 20 L/min for a minimum of 6 hours per sample.

[b] Collected by SMPS over separate 4-day periods.

Table 2. Ambient Air Particle Characterization in Shoebox Cage

	Mass Concentration ($\mu g/m^3$)[a]	CMD (nm)[b]	Particle Number Concentration (particles/cm^3)[b]
Mean	39	67	80
SD	24	8	6
N	5	18	18

[a] Collected daily on 5 days on 25-mm diameter Zefluor filters for a minimum of 5 hours per sample. The cage contained bedding, food, water, and four rats.

[b] Collected by SMPS daily for 3 days.

the main experiments. The endotoxin was lipopolysaccharide from *E coli* 0111:B4 (Sigma Chemical Co).

Male, 21-month-old, F344 rats (3 groups, 24 rats/group, for a total of 72 rats) were used. One group was intratracheally instilled with 0.5 mL sterile, endotoxin-free saline and one group with 0.5 mL of endotoxin in saline resulting in a dose of 0.1 mg endotoxin/kg body weight. The second control group was sham instilled (anesthetized and intubated intratracheally but nothing instilled). Rats were killed at 5 hours, 1 day, and 3 days after instillation. At the time of killing, BAL analysis and histologic and morphologic analyses were performed as described in Table 3. The right lung lobes were removed and lavaged with sterile, endotoxin-free saline, and the left lungs were fixed in formalin. The findings are described in Results.

EXPERIMENTAL APPROACH

Three experiments were conducted, each focused on a specific aim. Each experiment was based on a similar exposure model. Because three exposure atmospheres and several exposure concentrations were used, a series of animal exposures was required. Aged rats (24 months old) were used in all three experiments. In addition, young adult rats (about 3 months old) were used in experiment 3 to examine the influence of age on particle-induced toxicity. Endotoxin in saline was instilled intratracheally into all rats to induce pulmonary inflammation before exposure to PM.

To evaluate the biological responses to the exposures, various groups of rats were examined by using BAL, histology, and quantitative morphology techniques (Table 3). BAL fluid was analyzed for lactate dehydrogenase (LDH) activity to assess cytotoxicity; for total and differential cell counts, protein levels, and TNF-α levels to assess inflammation; and for alveolar macrophage numbers, MIP-2 levels, and β-glucuronidase activity to assess alveolar macrophage activation. Histologic sections of the lungs were evaluated for airway mucus production and inflammatory changes. In addition, changes in lung and body weights were evaluated as general indicators of toxicity. All of these endpoints are associated with various mechanisms of action that have been hypothesized to explain morbidity and mortality observed in humans exposed to PM. Lung burdens of V_2O_5 and carbon black were also measured in some rats at the time of killing.

Experiment 1: Response of Aged Rats to Inhaled Fine or Ultrafine V_2O_5 and Effect of Short-Term Spikes in Particle Concentration

This experiment aimed to determine (a) the relation between size (fine or ultrafine) of inhaled V_2O_5 particles and particle-induced responses and (b) the response to a short-term increase (spike) in V_2O_5 concentration after low or relatively high baseline exposure concentrations. The experimental design for exposure of aged rats to ultrafine or fine V_2O_5 particles is illustrated in Figures 1 and 2, respectively. The first exposures were conducted with the low concentration of ultrafine V_2O_5 particles. Based on the results, different sets of concentrations were targeted for subsequent ultrafine and fine exposures.

Groups of 12 to 14 aged rats were exposed nose-only, 5 hours/day, 7 days/week to ultrafine or fine V_2O_5 parti-

Table 3. Analysis of Biological Responses to Particle-Induced Injury

Procedure	Mechanism Analyzed	Variable		
		Primary	Key	Supporting
BAL	Cytotoxicity	LDH activity (IU/L)	None	None
	Inflammation	Neutrophils (number, number/mL, or %)	Cells (number or number/mL)	Protein (mg/dL), TNF-α (pg/mL), lymphocytes (number/mL or %)
	Alveolar macrophage activation	Alveolar macrophages (number, number/mL, or %)	β-glucuronidase activity (IU/L)	MIP-2 (pg/mL)
Histology	Inflammation	Total inflammation grade[a]	Alveolar, interstitial, and macrophage hyperplasia inflammation grades[a]	None
Morphology	Mucous cell metaplasia	Mucosubstances in airway generation 12 (nL/mm^2)	Mucosubstances in airway generation 4 (nL/mm^2)	None
Weighing	General toxicity	Lung weight (g)	Body weight (g)	None

[a] See Table 5 for grades.

cles. Control rats were exposed to filtered air by the same protocol. After 2 weeks of daily exposure (eg, on Monday after 2 weeks of Monday through Sunday exposures), the rats, including control rats, were intratracheally instilled with endotoxin in the morning about 2 to 3 hours before the exposures for that day. The next day (eg, Tuesday), the exposure concentration was increased (spiked) for two of the groups exposed to ultrafine or fine V_2O_5 (Figures 1 and 2). The exposure concentration was spiked for 2 days (two 5-hour exposure periods; eg, Tuesday and Wednesday). All rats were killed after 17 days of exposure (3 days after endotoxin treatment and 1 day after the last aerosol exposure).

Experiment 2: Response of Aged Rats to Inhaled Ultrafine Carbon Black and Comparison with Ultrafine V_2O_5

This experiment aimed to determine (a) the response to a spike in ultrafine carbon black particle concentration against a low or high baseline exposure concentration and (b) the relative toxicities of ultrafine V_2O_5 and ultrafine carbon black particles. We tested concentrations of ultrafine carbon black that matched a subset of the ultrafine V_2O_5 concentrations used in experiment 1. Figure 3 illustrates the experimental design for exposing rats to ultrafine carbon black particles.

Groups of 12 to 14 rats were exposed nose-only, 5 hours/day, 7 days/week to ultrafine carbon black particles. Control rats were exposed to filtered air by the same protocol. After 2 weeks of daily exposure, the rats, including control rats, were intratracheally instilled with endotoxin. The next day, the exposure concentration was spiked for 2 days (two 5-hour exposure periods) for one group exposed to ultrafine carbon black (Figure 3). All rats were killed after 17 days of exposure, 3 days after endotoxin treatment.

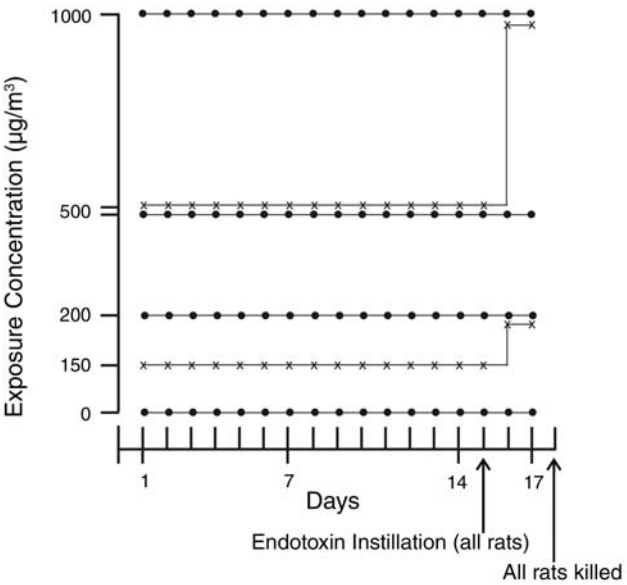

Figure 2. Design for experiment 1: exposure to fine V_2O_5 particles. Six aged rat exposure groups are represented: control rats exposed to 0 µg/m^3 (filtered air) for 17 days; rats exposed to 150 µg/m^3 for 15 days followed by 150+50 (200) µg/m^3 for 2 days; 200 µg/m^3 for 17 days; 500 µg/m^3 for 17 days; 500 µg/m^3 for 15 days followed by 500+500 (1000) µg/m^3 for 2 days; and 1000 µg/m^3 for 17 days. The endotoxin dose was 0.1 mg/kg body weight, administered 2 hours before the day 15 exposure. The 17-day 500 µg/m^3 exposure was conducted in experiment 3.

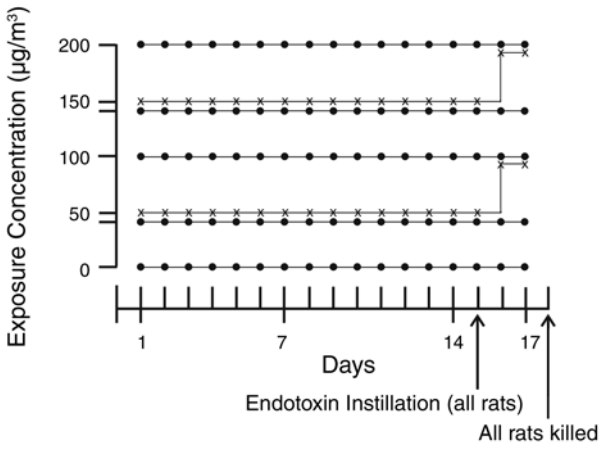

Figure 1. Design for experiment 1: exposure to ultrafine V_2O_5 particles. The seven aged rat exposure groups are represented: control rats exposed to 0 µg/m^3 (filtered air) for 17 days; rats exposed to 50 µg/m^3 for 17 days; 50 µg/m^3 for 15 days followed by 50+50 (100) µg/m^3 for 2 days; 100 µg/m^3 for 17 days; 150 µg/m^3 for 17 days; 150 µg/m^3 for 15 days followed by 150+50 (200) µg/m^3 for 2 days; and 200 µg/m^3 for 17 days. The endotoxin dose was 0.1 mg/kg body weight, administered 2 hours before the day 15 exposure.

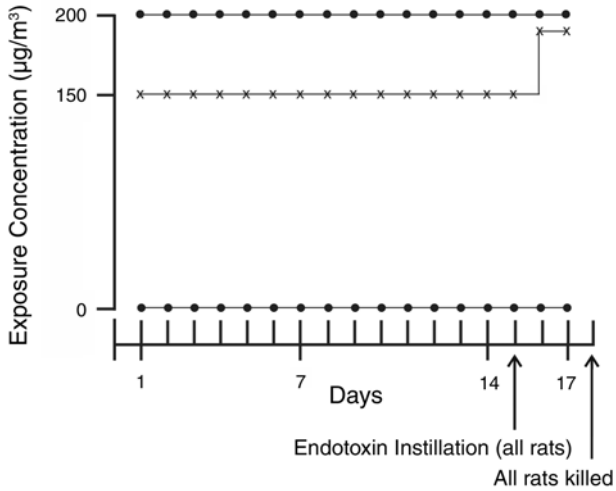

Figure 3. Design for experiment 2: exposure to ultrafine carbon black particles. The three aged rat exposure groups are represented: control rats exposed to 0 µg/m^3 (filtered air) for 17 days; rats exposed to 150 µg/m^3 for 15 days followed by 150+50 (200) µg/m^3 for 2 days; and rats exposed to 200 µg/m^3 for 17 days. The endotoxin dose was 0.1 mg/kg body weight, administered 2 hours before the day 15 exposure.

Data from these rats were compared with data for aged rats exposed to the same concentrations of ultrafine V_2O_5 in experiment 1.

Experiment 3: Responses of Aged and Young Adult Rats to Inhaled Fine V_2O_5

This experiment aimed to determine whether preexisting inflammation influences responses of aged rats and young adult rats to inhaled V_2O_5 fine particles and whether aged rats are more sensitive than young adult rats to the resulting adverse health effects. On the basis of experiments 1 and 2, fine V_2O_5 particles (500 µg/m^3) were chosen for the comparison of young adult and aged rats. This concentration was the lowest that gave a consistent toxicologic response.

Ten rats were placed in each age and exposure group. Rats were exposed nose-only, 5 hours/day, 7 days/week to 500 µg/m^3 fine V_2O_5. Control rats were exposed to filtered air by the same protocol. On day 15, all rats, including control rats, were instilled with endotoxin. All rats were killed after 17 days of exposure, 3 days after endotoxin instillation.

MATERIALS AND METHODS

AEROSOL GENERATION

V_2O_5

Ultrafine V_2O_5 particles (24 to 40 nm CMD) were generated by direct vaporization of V_2O_5 (Tu and Kanapilly 1979; illustrated in Figure 4). V_2O_5 powder was directly vaporized by loading it into a quartz boat and heating it to a temperature above its melting point of 690°C. Vapor concentration and final particle size were controlled by the high temperature furnace. V_2O_5 vapor was purged from the quartz boat by N_2 carrier gas at a flow rate of ~5 L/min. V_2O_5 ultrafine particles were formed in the condensation chamber. The chamber was designed around a 1-inch-diameter quartz tube perforated with small holes. The holes allowed cool dilution air to be injected into vapor-laden N_2 carrier gas and to condense particles with minimal losses due to impaction in the aerosol generation system. The resultant diluted, ultrafine particles were then delivered to the rat exposure chamber.

Fine V_2O_5 particles (0.7 µm in MMAD) were generated by nebulizing a V_2O_5 solution (Figure 5). V_2O_5 solution (0.1 g/100 mL distilled water) was nebulized using a compressed-air RETEC nebulizer. The resultant droplets were dried to form V_2O_5 particles by passing output through a sintered metal tube surrounded by dessicant. The tube allowed diffusion of water vapor to the dessicant while it separated the particles from the dessicant. The particles were further dried by removing a portion of the aerosol (via the aerosol dump line) and adding additional dry air (via the aerosol dilution line). The diluted aerosol was then delivered to the exposure chamber. Aerosol concentration was controlled by adjusting the aerosol dump and dilution air flows.

Carbon Black

An electric spark discharge generator supplied with graphite electrodes (model GFG-1000; Palas Co, Karlsruhe, Germany) was used to generate ultrafine carbon black aerosols (30 to 40 nm CMD). The Palas generator uses high voltage to generate sparks between two graphite electrodes. The graphite condenses to ultrafine particles of carbon black that coagulate while being purged from the electrodes with a flow of argon gas.

Recent studies (McDonald et al 2001) have demonstrated that the aerosols generated by this method are not pure carbon. Filter samples were collected and analyzed for carbon. We verified that approximately 70% of the mass was organic carbon and approximately 30% was elemental carbon. The composition of the small amount of remaining material was not determined, but it is most likely a composite of organic materials that are used as

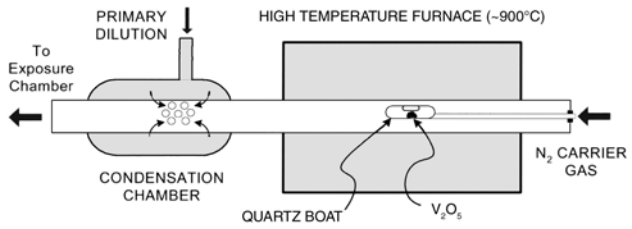

Figure 4. Ultrafine V_2O_5 aerosol generation system. Particles 24 to 40 nm in CMD were generated by direct vaporization of V_2O_5.

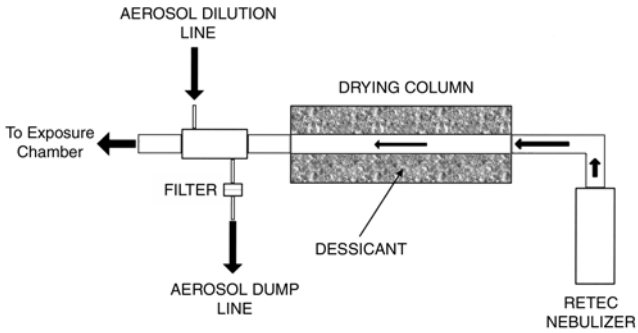

Figure 5. Fine V_2O_5 aerosol generation system. Particles 0.7 µm in MMAD were generated by nebulizing a V_2O_5 solution.

binders in the graphite electrodes (McDonald et al 2001). Total mass output and size distribution of the carbon black aerosols were controlled by spark frequency, argon gas flow, and air dilution flow.

NOSE-ONLY EXPOSURE SYSTEMS

The exposure systems for the three different aerosols (ultrafine V_2O_5, fine V_2O_5, and ultrafine carbon black) utilized a 96-port nose-only exposure chamber (In-Tox Products, Albuquerque NM) operated at a flow rate of ~30 L/min. The system design was basically the same for each aerosol (Figure 6). The test aerosol concentration was controlled by dumping a portion of the aerosol (via the chamber aerosol dump) and then diluting the remaining aerosol fraction with clean air (via the main chamber dilution). The addition of clean air was adjusted to maintain the chamber at a slightly negative pressure (0.5 inch water column) with exhaust flow of ~30 L/min (via the main exhaust).

The length of the aerosol delivery system was minimized as much as possible to reduce residence time in the delivery system and to minimize losses due to impaction in the system. The actual system losses were not measured, but experience with similar systems suggested losses of about 50%. The residence times in the exposure chamber were not measured either but were estimated to be about 36 seconds on the basis of a 40 L/min flow rate through the 24-L exposure chamber. Residence time within the generation line (the tube through which test aerosol is supplied to the chamber) should be considerably less (2–4 seconds).

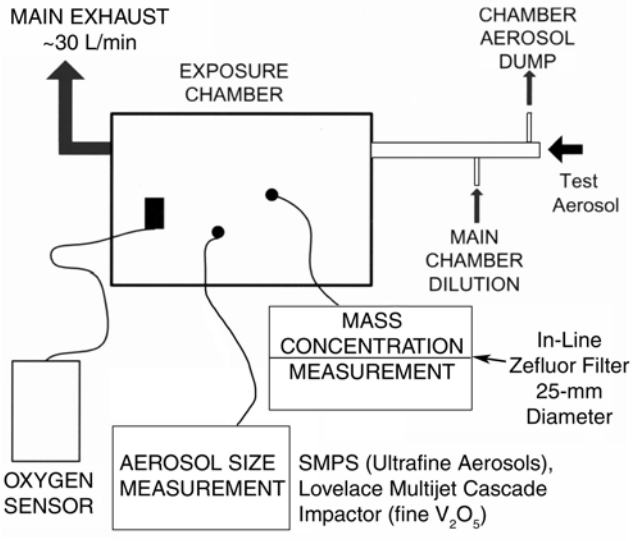

Figure 6. Nose-only exposure system for rats receiving filtered air or one of three aerosols (ultrafine V_2O_5, fine V_2O_5, or ultrafine carbon black). The 96-port nose-only chamber was operated at ~30 L/min.

CHARACTERIZATION OF TEST AEROSOLS

Mass concentrations in the chamber were determined from samples collected on Zefluor filters. To observe morphologies of fine and ultrafine particles, we collected samples from chambers by means of a point-to-plane electrostatic precipitator (In-Tox Products). The particles were collected on formvar-coated grids and examined by transmission electron microscopy. Size distributions of ultrafine and fine aerosols were measured by an SMPS (model 3934) and a Lovelace 7-stage multijet cascade impactor (In-Tox Products), respectively (Figure 6). Number concentration was measured for ultrafine aerosols only by using a condensation nucleus counter (model 302; TSI, St Paul MN).

Samples were extracted from the exposure chamber through a 1/4-inch-diameter conductive line to the SMPS or the cascade impactor. The sample line was as short as possible to minimize losses via diffusion. Samples were taken continuously during each exposure day, at the beginning, middle, and end of each exposure.

Spikes in mass concentration during exposures were achieved by using the condensation nucleus counter data to adjust the exposure system for ultrafine particles and a real-time monitor for fine particles (RAM-S; GCA Corp, Bedford MA).

Densities of the ultrafine V_2O_5 and carbon black aerosols could not be measured directly but were estimated by means of the particle number concentrations (Appendix B).

ANIMALS

All protocols were approved by the Lovelace Animal Care Committee. Animal care personnel followed all applicable standard operating procedures specified by the American Association for Accreditation of Laboratory Animal Care for its certified animal facilities. All rat husbandry was conducted under supervision of a laboratory animal veterinarian.

F344 male rats were obtained from the colony maintained for the National Institute of Aging (Harlan Sprague Dawley). A total of 225 aged and young adult rats were used in the three main experiments in this study. Aged rats were 23 months old upon receipt and 24 months old when the exposures ended. Young adult rats were 8 to 10 weeks old upon receipt and 12 to 14 weeks old when the exposures ended. Because the aged rats had reached their median life span at the time of receipt, deaths occurred from the time of receipt to the end of the conditioning and

exposure periods. On the basis of the survival curves for aged rats in this colony, 14 rats were ordered and placed on study for each of 14 exposure groups. The study groups were designed to have a minimum of 12 rats per group in addition to 3 control rats per exposure.

Rats were quarantined and acclimatized to housing conditions for 10 to 14 days before the study began. They were weighed and then randomized by weight for assignment to groups so that all groups had similar mean body weights and standard deviations. The mean weight was 421 ± 33.4 g for the aged rats and 233 ± 14.0 g for the young adult rats. Each rat was assigned a unique number and was identified by tail tattoo. One aged or two young adult rats were housed in each filter-topped polycarbonate shoebox-style cage on hardwood chip bedding (Laboratory Grade Sani-Chips; Harlan Teklad). The animal rooms were maintained at 22 ± 2°C with 40% to 60% relative humidity on a 12-hour light cycle (0600 to 1800). Food pellets (Certified Rodent Diet; Harlan Teklad) and water were available at all times except during exposures.

ANIMAL EXPOSURES

The exposure groups were (1) a spike group with a 15-day exposure to a baseline concentration followed by a 2-day exposure to the spike concentration; (2) a baseline group with a 17-day exposure to the baseline concentration; and (3) a spiked group with a 17-day exposure to the spike concentration. All exposures were conducted in 96-port rodent nose-only inhalation exposure chambers (Hoover and Mewhinney 1985). Before each 17-day exposure period, the rats were conditioned in the exposure tubes for 3 hours/day for 3 days. All rats were exposed to aerosolized particles (V_2O_5 or carbon black) or to filtered air for 5 hours/day for 17 consecutive days. Spikes in exposure concentration occurred over two 5-hour exposure periods on days 16 and 17. After each exposure period, the rats were returned to their cages.

ENDOTOXIN TREATMENT

Two hours before day 15 of aerosol exposures, all rats received 0.1 mg/kg body weight endotoxin (lipopolysaccharide from *E coli* 0111:B4, 1.5×10^6 endotoxin units/mg; Sigma Chemical Co) in 0.5 mL of saline via intratracheal instillation. All rats were lightly anesthetized by inhalation of 4% halothane in oxygen with nitrous oxide before instillation. Endotoxin dosages were calculated for each rat on the basis of preexposure body weights.

TERMINAL KILLS

All rats were killed with Euthasol (Delmarva Laboratories, Midlothian VA) 18 hours after the end of the exposure on day 17. The lung and distal two-thirds of the trachea were removed from each rat and weighed. In rats designated for BAL analysis, the left lung lobes were clamped with a vascular clip. The right lung lobes were then lavaged via the trachea by using two washes of sterile saline. In rats designated for lung burden measurement, the right lung lobes were tied off and removed, frozen in liquid nitrogen, and stored at −80°C for later analysis. The left lung lobes from all rats were fixed via the trachea under 20 cm of constant pressure for 1 hour with a 1% paraformaldehyde–0.1% glutaraldehyde solution. Each left lung was then immersed in a large volume of fixative until further processing, usually 1 to 2 days later. The spleen was also sampled from each rat and fixed for histologic indication of mononuclear cell leukemia.

LUNG BURDEN MEASUREMENT

Right lung tissues designated for vanadium content analysis were weighed and digested in an acid mixture (1 mL 14 M nitric acid, 0.5 mL 6 M hydrochloric acid, and 1 mL 30% hydrogen peroxide [H_2O_2]) per gram of tissue in 50-mL Teflon digestion tubes. The sample tubes were placed in a hot block set to 100°C for 4 hours. The vanadium contents of the digestate were measured by staff at Carlsbad Environmental Monitoring & Research Center (Carlsbad NM) using a low-resolution inductively coupled plasma mass spectrometer (ICP-MS; Elan 6000, PerkinElmer, Wellesley MA) according to EPA method 200.8 (EPA 1994a). The system was configured with a gem-tip cross-flow nebulizer and a Scott spray chamber. All samples were analyzed by using a nickel sampler and skimmer cone. Reported values are the mean of three replicate readings. The method detection limit for vanadium is 4 µg/L. Data are reported as micrograms of vanadium per gram of tissue.

Right lung tissues were analyzed for carbon black content by using a preparation technique and spectrophotometric method previously described (Driscoll et al 1996a). However, the procedure did not give valid results because of high background levels.

PRELIMINARY PARTICLE DEPOSITION CALCULATIONS

Because particles deposit differentially as a function of size, we performed preliminary deposition calculations to determine exposure concentrations that should result in similar thoracic deposition for the baseline exposures. The empirical model used for the deposition calculations is described in Appendix C and has been published elsewhere (EPA 1994b; Ménache et al 1996).

Table 4. Predicted Pulmonary Deposition of Fine and Ultrafine V_2O_5 Particles Inhaled by Rats

Particle Type / Exposure Level	Exposure Concentration (μg/m³)			Predicted Deposition (μg)		
	Baseline	Baseline +Spike	Spiked	Baseline	Baseline +Spike	Spiked
Ultrafine (0.03 μm CMD)						
Low	50	50+50	100	10	10+10	20
High	150	150+50	200	30	30+10	40
Fine (0.65 μm MMAD)						
Low	150	150+50	200	10.5	10.5+3.5	14
High	500	500+500	1000	35	35+35	70

The study design called for low concentrations of fine and ultrafine particles that were expected to result in similar lung burdens, as well as high concentrations of fine and ultrafine particles that would also result in similar lung burdens. Table 4 shows the predicted depositions calculated with assumptions about particle size (0.03 and 0.65 μm), particle density (3.36), and particle σ_g (2). The baseline exposures at the low concentration of 50 μg/m³ ultrafine V_2O_5 and of 150 μg/m³ fine V_2O_5 were predicted to result in a similar deposition of approximately 10 μg. Selection of the spiked exposures relied on the deposition calculations as well, but these exposures were designed to provide a wide range of burdens.

Deposition calculations were not made for carbon. At the conclusion of the experiments, lung burdens were predicted on the basis of dosimetric modeling that used actual values for the particle sizes and rat characteristics and incorporated the temporal effects of clearance. These results are shown in Table 7.

BAL FLUID ANALYSIS

The right lung lobes were lavaged through the trachea twice with sterile, pyrogen-free, physiologic saline without calcium or magnesium. The total amount of BAL fluid instilled for each right lung was 16 μL/g body weight (Henderson et al 1985).

An aliquot of cells from each BAL sample was stained with trypan blue and analyzed by using a bright line hemocytometer to determine cell viability and number. Cytocentrifuged cell preparations were treated with a Wright-Giemsa stain and analyzed at ×200 magnification on an Olympus BH2 microscope. At least 300 cells per sample were identified to determine percentages of the total cell count and absolute numbers of alveolar macrophages, neutrophils, and lymphocytes.

Cell-free preparations of the BAL fluid samples were analyzed for several indicators of damage. Total protein content, which indicates epithelial or endothelial cell permeability as part of inflammation, was determined by using a kit from Biotrol Diagnostics (Chennevieres-les-Louvres, France). LDH activity levels, which indicate cytotoxicity, were determined by using a kit from Instrumentation Labs (Lexington MA). Both LDH and total protein levels were determined on an automated chemical analyzer (Monarch Chemistry System 2000; Instrumentation Labs). β-Glucuronidase, an indicator of increased activity or increased lysis of alveolar macrophages, was analyzed by measuring the production of *p*-nitrophenol from its substrate 4-nitrophenylglucuronide by using a spectrophotometric method. (Reagents for this assay were purchased from Sigma Chemical Co.)

Aliquots of BAL supernatant were frozen at −80°C and analyzed later for the inflammation-associated cytokines, TNF-α and MIP-2, with enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Camarillo CA). ELISA plates were analyzed at 450 nm (7520 Microplate Reader; Cambridge Technology, Watertown MA).

Superoxide anion production by alveolar macrophages was determined from the slope of the cytochrome C reduction curve (Nasrallah et al 1983). BAL fluid was centrifuged to recover the cells. The cells were counted and resuspended (1×10^5/mL) in Earle buffered salt solution with 10 mM HEPES (pH 7.4). For each BAL sample, 90 μL of cell suspension was placed in each of four wells of a tissue-culture grade, 96-well plate and incubated at 37°C for 2 hours to allow the cells to adhere. The nonadherent cells were transferred to a fresh plate; the adherent cells were gently washed in the wells and recovered with the Earle–HEPES buffer. To each well, we added acetylated cytochrome C (25 μM final concentration) alone, with the inhibitor superoxide dismutase (3000 U/mL), with the stimulant phorbal myristate acetate (1 μM) or the stimulant zymosan (200 μg/mL, opsonized by preincubation with rat serum), or with both the inhibitor and a stimulant. Reduction of cytochrome C was monitored as the optical density at 550 nm reported from a kinetic 96-well plate reader (Versa Max; Molecular Devices, Sunnyvale CA; Pick and Mizel 1981). The rate of reduction of cytochrome C across the linear portion of the curve (usually about 1000 to 4600 seconds) was compared between samples from control and treated rats.

The luminol assay was used to measure H_2O_2 production by lavaged bronchoalveolar macrophages (Lincoln et al 1995). The assay is based on the horseradish peroxidase–catalyzed oxidation of luminol (Sigma Chemical Co) by H_2O_2, which results in the production of visible light. Cells are incubated with the reaction mixture in the presence or

absence of a stimulus (opsonized zymosan) and in the presence or absence of catalase (which converts H_2O_2 to H_2O and O_2). Briefly, 100 µL of lavaged cells (resuspended to 1×10^6 cells/mL in Roswell Park Memorial Institute medium with 1% glutamine and 10% fetal bovine serum) were added to each well of a 96-well clear-bottomed, white-walled tissue culture plate. After 1 hour of incubation at 37°C in 5% carbon dioxide, the cells were washed three times to remove nonadherent cells. Subsequently, 100 µL of 10 mM solution of luminol and 1250 U of horseradish peroxidase were added to each well. All samples were run in duplicate. To one pair of wells we added catalase (3200 U in 10 µL), to another pair opsonized zymosan (200 µg) and, to a third pair, opsonized zymosan and catalase. The control pair had no additions. Luminescence was monitored for 1 hour in an MLX Microplate Luminometer (Dynex Technology, Chantilly VA; Pick and Mizel 1981). Light intensity was integrated over the hour and was used as a measure of H_2O_2 production by the cells.

Protein carbonyls were measured in BAL fluid to assess oxidation of proteins (a measure of inflammation) in lung lining fluid. Protein carbonyls were measured by using the OxyBlot system (Oncor, Gaithersburg MD). BAL fluid was allowed to react with 2,4-dinitrophenylhydrazine to generate dinitrophenyl hydrazone groups at the sites of protein carbonyls. The proteins were then separated by polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The derivatized groups were detected with a monoclonal anti-dinitrophenyl antibody followed by horseradish peroxidase–conjugated secondary antibody and were made visible by using a chemiluminescent substrate (Amersham Biosciences, San Francisco CA). All BAL protein samples were handled at the same time: each was collected, frozen, and subjected to an OxyBlot test. The results were expressed as a percentage of a standard (oxidized, dinitrophenyl hydrazone-treated bovine serum albumin) run on the same gel.

MORPHOLOGIC AND HISTOLOGIC ANALYSES

Once their fixation was complete, the left lung lobes from rats designated for morphometric analysis of intraepithelial mucosubstances were microdissected (techniques of Plopper and colleagues 1983 as modified for rat airways by Harkema and Hotchkiss 1992). Dissections were performed under a high-resolution dissection microscope (Wild M-8 stereomicroscope; Wild Heerbrugg, Heerbrugg, Switzerland). Beginning at the lobar bronchus of the left lung lobe, the airway was split down the long axis of the main axial pathway. Each daughter branch off the main airway was numbered to ensure that only generations 4 and 12 were sampled. These cross-sections were excised and embedded in glycol methacrylate. Thin sections (2 µm) were cut and stained with Alcian blue (pH 2.5)–periodic acid Schiff sequence (AB/PAS) to identify intraepithelial mucosubstances.

The volume density of AB/PAS-stained mucosubstances in the chosen sites was determined by means of computerized image analysis and standard morphometric techniques. Morphometric analyses were performed with a semiautomatic, computerized image analysis system consisting of a BH2 light microscope (Olympus Corp, Lake Success NY) connected to a high-resolution camera (VE-1000 CCD System; Dage-MTI, Michigan City MI), an LG-3 digital image processing board (Scion Corp, Frederick MD), and a G4 Power Macintosh computer (Apple Computer, Cupertino CA) running the public-domain National Institutes of Health image analysis software program (NIH Image; available at *http://rsb.info.nih.gov/nih-image*). In a digitized image of the airways, the areas of AB/PAS-positive intraepithelial mucosubstances were calculated from the automatically circumscribed perimeter of the stained material. The underlying basal lamina was measured by manually tracing the contours. The method used to estimate the amount of stored intraepithelial mucosubstances per unit area of basal lamina is described in detail elsewhere (Harkema et al 1987). Data were expressed as the mean volume density (nanoliters per square millimeter of basal lamina) of AB/PAS-positive mucosubstances within the epithelium.

The left lung lobes from the remaining rats, designated for histologic analysis, were cut using a procedure adapted from Gundersen and associates (1988). The left lung lobe was cut perpendicular to the main axial airway every 3 to 4 millimeters, starting at a random point. Randomly selected sections were then chosen for standard histologic paraffin embedding and were cut into 5-µm sections for hematoxylin and eosin staining. Specimens were examined and graded in a blind fashion from 0 to 4 for degree of alveolar macrophage hyperplasia, alveolar inflammation, interstitial reaction, and perivascular edema by using the criteria shown in Table 5.

These individual lesion grades were summed for a total inflammation grade for each rat, which could range from 0 to 16. Rats were also evaluated for mononuclear cell leukemia involvement of the lung, a relatively common condition in aged F344 rats (Stromberg 1992). Rats with lung parenchyma invaded by leukemic cells were not used in analyses.

Table 5. Lung Lesion Severity

Grade	Diagnostic Criteria
Alveolar Macrophage Hyperplasia[a]	
0	Few scattered macrophages in alveoli, difficult to find
1	Minimal hyperplasia
2	Mild hyperplasia; macrophages easily found at high magnification (mean, 1 per alveolus)
3	Moderate hyperplasia; macrophages easily found at low magnification (mean, several per alveolus)
4	Marked hyperplasia; enlarged macrophages easily found at low magnification (mean, many per alveolus)
Alveolar Inflammation[b]	
0	No neutrophils or lymphocytes in alveoli
1	Few, scattered neutrophils or lymphocytes in alveoli
2	Low number of neutrophils or lymphocytes in alveoli or focal involvement of < 25% of section
3	Moderate increase of neutrophils or lymphocytes in alveoli or focal involvement of 25–50% of section
4	Marked increase of neutrophils or lymphocytes in alveoli or focal involvement of > 50% of section
Interstitial Reaction[c]	
0	None
1	Occasional foci: 5% of lung section
2	Low number of foci: 5–25% of lung section
3	Moderate number of foci: 25–50% of lung section
4	Marked number of foci: > 50% of lung section
Perivascular Edema[d]	
0	None
1	Occasional, scant edema
2	Mild edema: 5–25% of the structures involved
3	Moderate edema: 25–50% of the structures involved
4	Marked edema: > 50% of structures involved severely

[a] General increase in numbers of alveolar macrophages.

[b] General presence of neutrophils or lymphocytes in alveoli.

[c] Focal thickening of alveolar septa owing to accumulation of inflammatory cells or hyperplastic alveolar epithelial cells.

[d] Homogeneous proteinaceous material in the loose connective tissues around large veins and arteries in the lung.

STATISTICAL METHODS AND DATA ANALYSIS

Statistical analyses followed a consistent strategy for all experiments but differed in their exact implementation. In all cases, specific indicators of response were divided into groups associated with various general biological responses. Within each group, the indicators were identified as primary, key, and supporting (Table 3). The purpose of this stratification was to focus interpretation of the study results on the endpoints that were most reflective of biologically meaningful changes (primary and key variables) and to interpret results concerning the supporting endpoints as supportive (or not) of the primary and key indicators. A similar strategy has been employed elsewhere to evaluate the consequences of prolonged ozone inhalation in rats (Chang et al 1995).

Before analysis, the data were tested for homogeneity of variance and transformed as needed. For all statistical tests, a P value less than 0.05 was considered statistically significant.

The objective of experiment 1 was twofold: to determine whether comparable delivered doses of fine and ultrafine V_2O_5 result in similar responses and to evaluate whether a short-term increase (spike) in V_2O_5 results in responses that differ from those expected from a similar delivered dose without a spike. The predicted lung burdens were determined by using the empirical dosimetry model of Ménache and colleagues (1996) and empirical measurements of clearance reported by Raabe and Al-Bayati (1997) and by Yu (1996). Details of the predicted lung burden calculations are in Appendix C.

Predicted lung burden was the dose variable in this two-stage analysis. In the first stage, rats undergoing constant exposures over the 17 days were compared using an analysis of covariance (ANCOVA) in a linear model. A logistic model

was used for the inflammation responses. The covariate was predicted lung burden and the model allowed for different intercepts and slopes in the fine and ultrafine groups. In the second stage of the analysis, responses for the spike exposure groups were predicted using the fitted first-stage model, and these predictions (and 95% confidence intervals) were compared to means of the observed responses.

Experiments 2 and 3 were analyzed by two-way analysis of variance (ANOVA). The factors for experiment 2 were particle type (carbon black or V_2O_5) and particle concentration (0, 150+50, 200 μg/m^3 ultrafine). The factors for experiment 3 were age (young adult or aged) and particle concentration (0 or 500 μg/m^3 fine V_2O_5). The interaction term between the two main factors was included in both models.

LIMITATIONS OF STUDY

BAL Technique

The BAL technique removes cells and fluids from the lung. It is a well-established approach to accessing lung damage in animals (Henderson et al 1985) and humans (Linder and Rennard 1988). The presence of inflammatory cells and the ability to chemically analyze the fluids allow for a unique and valuable assessment of the health status of the deep lung.

Like all health evaluation techniques, however, the BAL procedure has limitations. BAL does not remove cells that are in the tissues (such as inflammatory cells in the bronchial epithelium, alveolar septa, or lung interstitium). The cells obtained are essentially all from the airway and alveolar spaces. The number of lavages performed does influence the number of cells obtained. Multiple lavages (up to 15) in normal rats do not remove all alveolar macrophages from the lung (Brain 1970). In addition, inflammatory reactions in the lungs may cause some adherence of cells in the deep lung, although this factor has not been studied in detail. Serial lavages reduce the concentration of protein components in BAL fluids. Two lavages remove about 70% of the protein that is removable by the technique (Henderson 1989). These limitations are the basis for the standardized number (two) and volume (16 μL/g body weight) of the lavages used in our study.

Endotoxin Treatment

Pneumonia, chronic bronchitis, and COPD are associated with the increased mortality ascribed to increased PM levels in community air. We chose to use endotoxin instillation because it causes both airway and parenchymal inflammation of the lung and can be administered with a fair degree of reproducibility. The extent of injury depends on the amount and type of endotoxin instilled (Foster et al 2003). Instillation of 1 mg of lipopolysaccharide from *E coli* 055:B5 per kilogram of body weight has been used to model acute lung injury in animals (O'Leary et al 1996). The resulting pathologic findings include a protein-rich, fibrinous alveolar exudate and a diffuse alveolitis. This model mimics the febrile, airway-obstructive, and pulmonary reactions of humans to organic dust inhalation. Endotoxin from *E coli* 0111:B4 has been used to induce airway changes with similarities to chronic bronchitis. Harkema and Hotchkiss (1991) administered 500 μg lipopolysaccharide (~2.5 mg/kg body weight) via intranasal instillation once a day for 3 days. They found neurophilic intraepithelial infiltrate, secretory hyperplasia, and a dramatic increase in intraepithelial mucosubstances, particularly in the epithelium lining the main axial airways. The amount of intraepithelial substances increased considerably 1 day after the last instillation. The rats also exhibited acute bronchopneumonia at that time.

In our pilot experiments, endotoxin doses of 0.1, 0.2, or 0.5 mg/kg body weight were instilled into the lungs. The results indicated that the 0.1 mg/kg dose induced moderate inflammation 1 day after instillation, which rapidly diminished to minimal to mild inflammation 3 days after instillation.

The primary limitation of the endotoxin model is the lack of persistence of the inflammation and the predominance of neutrophils in the inflammatory response, which mirrors the lack of persistence. Other possible limitations are variability in biological activity of the endotoxin, distribution of the instilled endotoxin, and biological responses, which are limitations for any administered material. However, these factors have not been quantified.

Measures of Oxidative Stress

The measurements of oxidative stress were not profound. Oxidative stress could not be measured at exposure concentrations lower than 1000 μg/m^3 of fine V_2O_5. A full presentation of the data for oxidative stress is in Appendix D.

RESULTS

PILOT EXPERIMENT: TIME COURSE OF ENDOTOXIN-INDUCED PULMONARY INFLAMMATION

Data from the BAL and lung morphology analyses for all exposure groups are given in Table E.1. Marked acute inflammation was noted 5 hours after instillation of endotoxin, as illustrated by the increase in TNF-α and protein levels and numbers of cells, primarily neutrophils, in the BAL fluid (Figure 7). Protein and TNF-α concentrations in

endotoxin-instilled rats decreased by 1 day after instillation and were near control (sham instillation) values at 3 days after. Neutrophil numbers remained elevated at 1 day and had markedly decreased by 3 days after but were still significantly higher than in controls. The alveolar macrophage numbers in BAL fluid did not increase at any time after endotoxin instillation (Figure 7). They did increase, however, at 5 hours after saline or sham instillation and returned to normal concentrations at 1 and 3 days after instillation.

The β-glucuronidase concentrations increased twofold at 5 hours, nearly sixfold by 1 day, and decreased again by 3 days after instillation (Table E.1, Figure 7). β-Glucuronidase is found in lysosomes, a cellular organelle, and is particularly abundant in alveolar macrophages and neutrophils. It is released into the alveolar space in response to inflammatory agents or phagocytosis. β-Glucuronidase levels in response to endotoxin instillation in this experiment, however, did not correlate with neutrophil percentages or neutrophil numbers or with TNF-α or protein concentrations, two indicators of acute inflammation. LDH concentrations followed a similar pattern: a modest increase at 5 hours, a nearly sixfold increase at 1 day, and a decrease to threefold levels at 3 days after instillation (Figure 7). The increase in LDH level is related to the release of the enzyme from damaged or necrotic cells, likely due to acute inflammatory response.

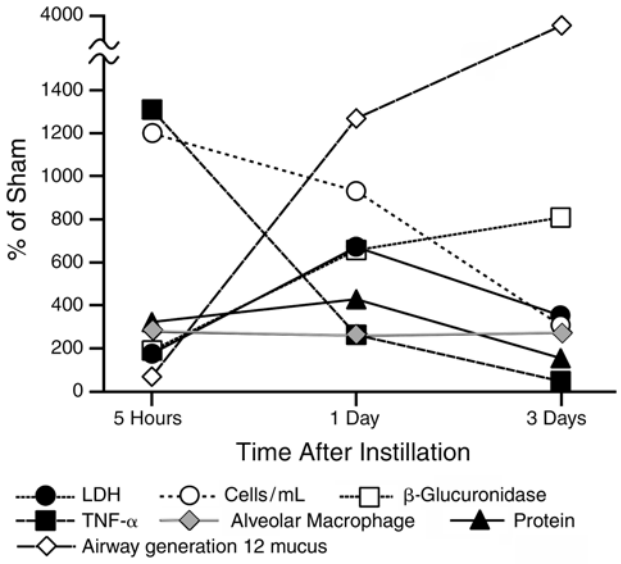

Figure 7. Pilot experiment: time course of changes in lung damage indicators in rats instilled with endotoxin. The endotoxin dose was 0.1 mg/kg body weight. Data are expressed as the mean percentage of the values from sham-instilled rats. Data are from analysis of BAL fluid except for mucus. See Table E.1 for summary data and units for each biological indicator.

Histologic changes did correlate with changes in the indicators of acute inflammation in BAL fluid but showed a more prolonged response. Edema and accumulation of neutrophils in the alveoli were prominent at 5 hours after instillation (Figure E.1), and accumulation of neutrophils in the interstitium and alveolar macrophage hyperplasia were increasingly prominent at 1 and 3 days after (Figure E.2). Mucous cell metaplasia in airway generation 12 significantly increased only 3 days after, reflecting the delayed nature of this response (Figure 7).

Results of this pilot experiment emphasize the dynamics of the pulmonary reaction to intratracheal instillation of endotoxin. On the basis of these results, we anticipated that protein, neutrophils, LDH, and possibly β-glucuronidase would increase in BAL fluid 3 days after endotoxin instillation in our main experiments. Histologic changes of inflammation and mucous cell metaplasia were also anticipated to increase at 3 days after endotoxin instillation.

EXPERIMENT 1: RESPONSE OF AGED RATS TO INHALED FINE OR ULTRAFINE V_2O_5 AND EFFECT OF SHORT-TERM SPIKES IN PARTICLE CONCENTRATION

The summary data for experiment 1 are given in Appendix F. The text tables and figures were developed from these data.

Achieved V_2O_5 Exposure Concentrations and Sizes

The aerosol concentrations and sizes measured during each exposure to V_2O_5 are shown in Table 6. Actual mean concentrations were reasonably close to target concentrations. All but one were within approximately 5% of the targeted mean: the fine V_2O_5 200 μg/m^3 spike exposure fell short of the targeted value by nearly 10%.

The sizes of the actual ultrafine particles were close to the target sizes. The fine particles were about one-third of the target size; however, they were still considered to be fine (not ultrafine) particles. Particle size is a function of the nebulizer solution concentration. Because of exposure system constraints and the relatively low exposure concentrations desired, solution concentration could not be increased sufficiently to increase the size of the fine V_2O_5 aerosol.

Lung Burden Measurements

The amount of vanadium in the lung at the end of the exposure period (after 17 days) for all groups is shown in Table 7. For example, exposure of aged rats to 50 μg/m^3 of ultrafine V_2O_5 resulted in elevated mean vanadium lung burdens compared with control concentrations. The spike in exposure concentration did not increase the lung burden of vanadium, regardless of the baseline exposure

Table 6. Experiments 1–3: Aerosol Exposure Concentrations and Particle Sizes

Exposure Days	Mass Concentration (μg/m^3) Target Mean	Actual Mean	Actual SD	Number Concentration[a] (mean number/m^3)	Particle Size[b] (μm) Target	Actual	Actual σ_g
Experiment 1—Aged Rats							
Ultrafine V_2O_5							
1–17	50	50	3	6.80×10^5	0.035	0.033	1.40
1–15	50	51	20	3.79×10^5	0.035	0.031	3.35
16–17	100 (spike)	104	11	6.31×10^5	0.035	0.038	2.47
1–17	100	102	16	2.59×10^5	0.035	0.024	0.62
1–17	150	157	26	2.70×10^6	0.035	0.032	1.97
1–15	150	151	27	2.66×10^5	0.035	0.032	1.83
16–17	200 (spike)	212	1	2.50×10^5	0.035	0.036	0.15
1–17	200	200	32	2.12×10^5	0.035	0.038	1.68
Fine V_2O_5							
1–15	150	153	6		2.0	0.7	2.12
16–17	200 (spike)	181	2		2.0	0.7	2.12
1–17	200	206	20		2.0	0.7	2.12
1–15	500	504	33		2.0	0.7	2.12
16–17	1000 (spike)	1010	75		2.0	0.7	2.12
1–17	1000	939	103		2.0	0.7	2.12
Experiment 2[c]—Aged Rats							
Ultrafine carbon black							
1–15	150	149	12	5.90×10^6	0.035	0.035	1.48
16–17	200 (spike)	213	10	7.04×10^6	0.035	0.035	1.47
1–17	200	196	17	6.31×10^6	0.035	0.035	1.48
Experiment 3—Aged and Young Adult Rats							
Fine V_2O_5							
1–17	500	504	33		2.0	0.7	2.12

[a] Measured for ultrafine particles only.

[b] Particle size is CMD for ultrafine V_2O_5 and carbon and MMAD for fine V_2O_5.

[c] Data from experiment 2 were compared with data from the aged rats exposed to the same concentrations of ultrafine V_2O_5 in experiment 1.

concentration. Figure 8 shows the relation between V_2O_5 exposure concentration and vanadium lung burden for fine and ultrafine particles. At similar exposure concentrations, the ultrafine V_2O_5 particles resulted in a twofold greater lung burden compared with fine particles. This difference is expected because of differences in particle size, and it demonstrates why lung burden is the preferred dose metric for analysis of the data from experiment 1.

Calculation of Predicted Lung Burdens

One objective of this research was to evaluate various biological responses as a function of actual lung burden rather than exposure concentration. Actual lung burdens would have been the ideal dose metric; however, we could not measure lung burden in all rats. As a result, the predicted lung burden used as the dose metric for the analysis of the data from experiment 1 was determined by dosimetry modeling.

Empirical equations for deposition and for clearance and retention were fit to the data to estimate lung burden for each rat. The development of each component of the lung burden model is described in detail in Appendix C. A sample lung burden calculation is also shown there.

For purposes of statistical analyses, the predicted lung burdens for all the rats were adjusted as follows: for fine particles, adjusted burden = 0.45 × predicted burden; for ultrafine particles, adjusted burden = 0.40 + 0.2 × predicted burden.

Table 7. Experiments 1–3: Measured and Predicted Lung Burden of Test Particles

Exposure Concentration (μg/m³)			Lung Burden						
			Measured[a] (μg/g lung)				Predicted[b] (μg/g lung)		
Baseline	Baseline + Spike	Spiked	Control[c]	Baseline	Baseline + Spike	Spiked	Baseline	Baseline + Spike	Spiked
Experiment 1—Aged Rats									
Ultrafine V_2O_5 (0.03 μm CMD[d])									
50	50+50	100	0.09 ± 0.02	0.72 ± 0.07	0.64 ± 0.04	0.97 ± 0.08	0.68 ± 0.01	0.79 ± 0.01	0.67 ± 0.01
150	150+50	200		1.19 ± 0.04	1.20 ± 0.14	1.36 ± 0.05	1.28 ± 0.02	1.24 ± 0.04	1.49 ± 0.03
Fine V_2O_5 (0.7 μm MMAD)									
ND	150+50	200	0.10 ± 0.005	ND	0.54 ± 0.04	0.64 ± 0.04	0	0.45 ± 0.01	0.60 ± 0.01
ND	500+500	1000		ND	1.50 ± 0.04	2.64 ± 0.14	ND	1.55 ± 0.03	2.48 ± 0.04
Experiment 2[e]—Aged Rats									
Ultrafine carbon black (0.035 μm CMD)									
ND	150+50	200	—	ND	—	—	0	33.5 ± 1.2	44.7 ± 1.5
Experiment 3—Aged and Young Adult Rats									
Fine V_2O_5 (0.7 μm MMAD)									
Aged Rats									
500	ND	ND	0.10 ± 0.008	2.43 ± 0.20	ND	ND	1.27 ± 0.04	ND	ND
Young Adult Rats									
500	ND	ND	0.04 ± 0.003	1.33 ± 0.11	ND	ND	1.77 ± 0.02	ND	ND

[a] Data are mean ± SEM and are based on analysis of lungs from 3–7 rats per group. ND = not done. — = Measurements were not valid.

[b] Data are mean ± SE and are based on model predictions (Appendix C). ND = not done.

[c] Control rats were exposed to 0 μg/m³ particles (filtered air).

[d] CMD averaged over all six exposure concentrations. Actual CMDs per exposure concentration were 0.024–0.038 (Table 6).

[e] Data from experiment 2 were compared with data from the aged rats exposed to the same concentrations of ultrafine V_2O_5 in experiment 1.

Mean adjusted predicted lung burdens for each exposure are shown in Table 7. The relation between the adjusted predicted burdens and the measured burdens are illustrated in Figure 9. There and hereafter, the predicted burdens given are the *adjusted* predicted burdens.

Biological Responses

Survival and Weights In experiment 1, all rats were between 22 and 24 months of age and all were instilled with endotoxin 3 days before the end of exposure. A total of 137 aged rats were used in the 10 V_2O_5 exposures in experiment 1. Ten exposed rats either died or were removed from the study before analyses were performed. These deaths and removals were related to diseases of aging or experimental manipulations, not to V_2O_5 toxicity. Rats exposed to 1000 μg/m³ of fine particles (predicted lung burden = 2.5 μg/g lung tissue) were generally lethargic at the end of the 5-hour exposure period. No other groups of exposed rats showed notable clinical signs.

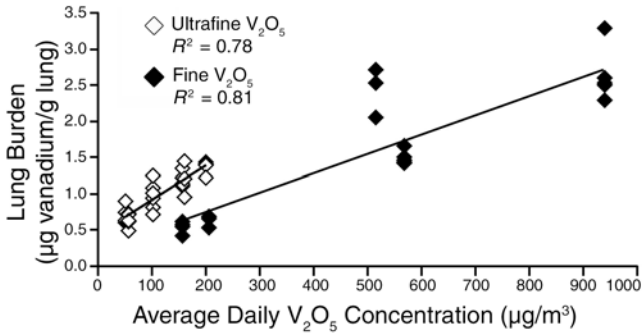

Figure 8. Experiment 1: lung burdens of aged rats and exposure concentrations of V_2O_5 particles. Data points represent the means over 17 days of exposure (with or without a spike in concentration during the last 2 days) for individual rats. R^2 is coefficient of determination for multivariate analysis.

Fifty control rats were instilled with endotoxin but were not exposed to V_2O_5. Eight of these rats either died or were removed before the end of the study for diseases related to aging, primarily leukemia or testicular tumors.

Body and lung weights are shown in Table F.1 and the *P* values from the ANCOVA of key weight parameters are shown in Table 8. The body weights of all rats decreased during the 17 days of the experiment (as well as in subsequent experiments). The stress of the nose-only exposure procedure most likely contributed to the weight loss. Although all groups underwent the same nose-only exposures, however, the degree of weight loss was significantly greater in those groups exposed to the ultrafine particles but was not associated with the predicted lung burden ($P = 0.40$). Lung weights increased with increasing concentration and increased more rapidly during exposure to ultrafine particles compared with fine particles. No interaction of particle size and predicted lung burden was found for any weight parameter.

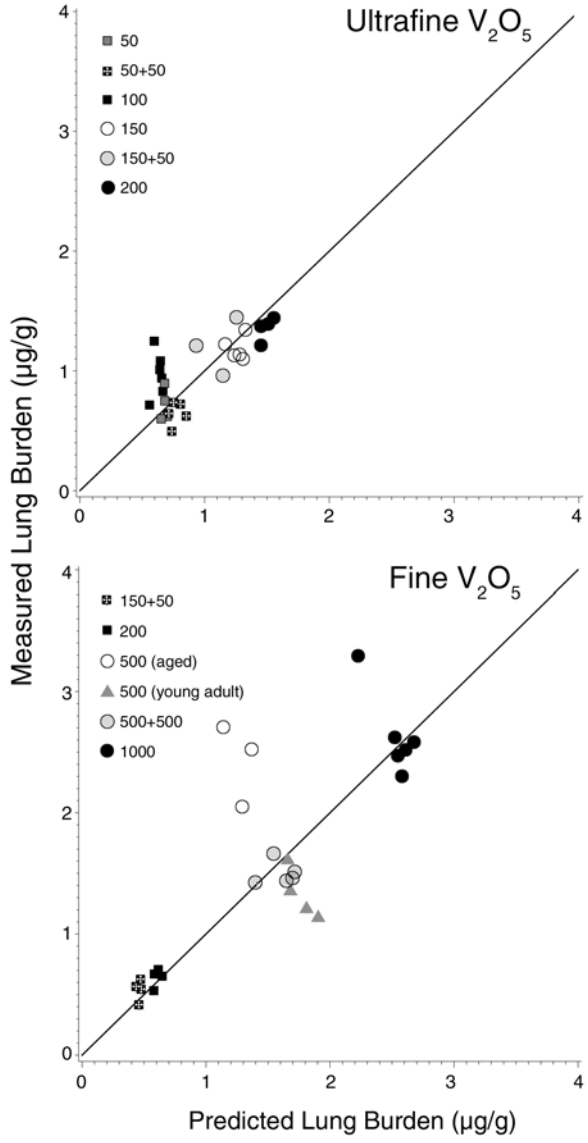

Figure 9. Experiment 1: predicted and measured lung burden. The lines indicate the slope at which measured lung burden would be equal to predicted lung burden. Data are for individual rats and are given for each particle exposure (µg/m^3 particles). The 500 µg/m^3 data sets are from experiment 3.

Analysis of Response Data The response data for all rats exposed to V_2O_5 particles and instilled with endotoxin, and for controls exposed to filtered air and instilled with endotoxin, are shown in Table F.2. Statistical significance from analyses of the primary variables, calculated with a two-way ANCOVA, is shown in Table 9. Generally, particle size and predicted lung burden of V_2O_5 significantly affected cytotoxicity, inflammation, alveolar macrophage activation, and mucous cell metaplasia in the airways. Little evidence of interaction was noted between particle size and predicted lung burden. Results of analysis of the influence of short-term spikes in exposure concentration are shown in Table 10. Mean response values were compared to the 95% confidence interval for the regression curve derived from the ANCOVA analyses.

LDH concentrations in BAL fluid, which are primary indicators of cytotoxicity, correlated significantly with increases in predicted lung burden (Table 9, $P < 0.01$). LDH responses to the fine and ultrafine particles also differed significantly. Spikes of 150+50 µg/m^3 fine particles increased LDH concentrations more than predicted but spikes of 150+50 µg/m^3 ultrafine particles were associated with lower concentrations of LDH than would be predicted on the basis of lung burden mass (Table 10).

Table 8. Experiment 1: Statistical Analysis of Body and Lung Weights

	ANCOVA *P* Values		
Variable	Particle Size[a]	Predicted Lung Burden[b]	Particle Size × Lung Burden[c]
Change in body weight	<0.01	0.40	—
Lung weight	<0.01	<0.01	—
Lung–body weight ratio	<0.01	<0.01	—

[a] *P* values indicate the difference between the intercepts for the fine and ultrafine V_2O_5 exposure groups.

[b] *P* values reflect the significance of the overall weight trend as a function of predicted lung burden.

[c] *P* values indicate the difference in the trend or slope according to whether the exposure was to fine or ultrafine particles. — indicates that the interaction term was not statistically significant and was excluded from the final model fit.

Neutrophil concentrations in BAL fluid, which are primary indicators of inflammation, showed a significant interaction of predicted lung burden and particle size. Ultrafine particles resulted in significantly higher neutrophil concentrations than fine particles at similar predicted lung burdens. Spikes of 150+50 μg/m^3 fine particles and 150+50 μg/m^3 ultrafine particles, but not the baseline of 50 or 500 μg/m^3, increased the neutrophils more than predicted, although neutrophils were decreased relative to the predicted values for the other two spike exposures (Table 10). Similar changes in total numbers of all cells in BAL fluid from rats exposed to these same spikes is further evidence of the spike effect.

Table 9. Experiment 1: Statistical Analysis of Effect of V_2O_5 Size and Predicted Lung Burden

	P Value[a]		
Mechanism / Variable	Particle Size[b]	Predicted Lung Burden[c]	Particle Size × Predicted Lung Burden[d]
Cytotoxicity			
LDH (IU/L)	<0.01	<0.01	—
Inflammation			
Neutrophils/mL	<0.01	<0.01	<0.01
Cells/mL	<0.01	<0.01	—
Protein (mg/dL)	<0.01	0.28	—
TNF-α (pg/mL)	<0.01	0.99	—
Lymphocytes/mL	<0.01	<0.01	—
Macrophage Activation			
Macrophages/mL	<0.01	<0.01	—
β-glucuronidase (IU/L)	<0.01	<0.01	—
MIP-2 (pg/mL)	<0.01	<0.01	—
Mucous Cell Metaplasia			
Mucus in airway generation 12 (nL/mm^2)	<0.01	<0.01	—
Mucus in airway generation 4 (nL/mm^2)	<0.01	0.15	—
Histologically Evident Inflammation			
Total grade	0.07	<0.01	—
Alveolar inflammation	0.26	0.02	—
Interstitial inflammation	<0.01	0.60	—
Macrophage hyperplasia	0.97	<0.01	0.04

[a] *P* values are from two-way ANCOVA except for the Histologically Evident Inflammation variables, which were analyzed by logistic regression and the *P* values generated from Wald chi-square tests.

[b] *P* values indicate the difference between the intercepts for the fine and ultrafine V_2O_5 exposure groups.

[c] *P* values reflect the significance of the overall endpoint trend as a function of predicted lung burden.

[d] *P* values indicate the difference in the trend or slope according to whether the exposure was to fine or ultrafine particles. — indicates that the interaction term was not statistically significant and was excluded from the final model fit.

Table 10. Experiment 1: Effect of Short-Term Spikes in V_2O_5 Exposure Concentration

Mechanism / Variable	Ultrafine V_2O_5		Fine V_2O_5	
Exposure concentration (μg/m^3)	50+50	150+50	150+50	500+500
Mean predicted lung burden (μg/g lung)	0.79	1.24	0.45	1.55
Cytotoxicity				
LDH (IU/L)	—[a]	↓[b]	↑↑[c]	—
Inflammation				
Neutrophils/mL	↓↓	↑↑	↑↑	↓
Cells/mL	↓↓	↑↑	↑↑	↓↓
Protein (mg/dL)	—	↓	—	—
TNF-α (pg/mL)	↑↑	↓↓	—	—
Lymphocytes/mL	↑↑	↑↑	—	—
Macrophage Activation				
Macrophages/mL	↓↓	↑↑	↑	↓↓
β-Glucuronidase (IU/L)	↓	—	— ↑↑	—
MIP-2 (pg/mL)	↑	↓↓	↑↑	↓↓
Mucous Cell Metaplasia				
Mucus in airway generation 12 (nL/mm^2)	↓	↑↑	—	—
Mucus in airway generation 4 (nL/mm^2)	↓	↑↑	↓	—
Histologically Evident Inflammation				
Total grade	—	↓↓	↑	↓↓
Alveolar inflammation	↓↓	↓↓	—	↓↓
Interstitial inflammation	—	—	↑↑	↑
Macrophage hyperplasia	—	↓↓	—	↓↓

[a] Mean response value is within (—) the 95% confidence interval.

[b] Mean response value is above (↑) or below (↓) the 95% confidence interval of the regression curve derived from ANCOVA. This indicates statistical significance.

[c] Mean response value plus (↑↑) or minus (↓↓) 1 SE is outside the 95% confidence interval of the regression curve derived from ANCOVA. This indicates statistical significance.

Alveolar macrophage concentrations in the BAL fluid, which are primary indicators of alveolar macrophage activation, correlated significantly with increases in predicted lung burden and were consistently higher in rats exposed to fine V_2O_5 than in those exposed to ultrafine V_2O_5. Spiked exposures resulted in macrophage concentrations above or below the predicted response, although no consistent pattern was observed (Table 10). For rats exposed to 150+50 μg/m^3 fine V_2O_5, β-glucuronidase and MIP-2 concentrations significantly increased, further evidence of a significant spike effect.

Mucus in the epithelium of airway generation 12, which is a primary indicator of mucous cell metaplasia, increased significantly with increased lung burden and was greater in rats exposed to ultrafine V_2O_5. A spike of 150+50 μg/m^3 of ultrafine particles increased the volume density of mucus in airway generation 12 epithelium more than predicted (Table 10). Increased mucus in the epithelium of airway generation 4 was further evidence of the spike effect.

Total grades for histologic reactions in the lung, which are primary indicators of lung inflammation, increased significantly with predicted lung burden overall, as did grades for alveolar inflammation and macrophage hyperplasia (also interaction; Table 9). Interstitial inflammation was affected by particle size but not by predicted lung retention. The spike exposures tended to be associated with decreased grades, although this pattern was not consistent (Table 10).

Histologic reactions in the lung were characterized by alveolar macrophage hyperplasia and alveolar inflammation (Figure 10), interstitial reaction with inflammatory cells and hyperplastic alveolar epithelial cells, and airway mucous cell metaplasia (Figure 11). Control rats, which were not exposed to V_2O_5, were instilled with endotoxin and had inflammatory lesions as noted in the histologic scoring.

EXPERIMENT 2: RESPONSE OF AGED RATS TO INHALED ULTRAFINE CARBON BLACK AND COMPARISON WITH ULTRAFINE V_2O_5

The summary data for experiment 2 are given in Appendix G. The text tables and figures were developed from these data. Experiment 2 compared data from exposures of aged rats to 0 μg/m^3, 150+50 μg/m^3, and 200 μg/m^3 ultrafine carbon black particles with corresponding exposures to ultrafine V_2O_5 particles in experiment 1.

Achieved Carbon Black Exposure Concentrations and Sizes

The carbon black aerosol concentrations and sizes measured in each exposure are shown in Table 6. As with the V_2O_5 particles, the actual concentrations and sizes were reasonably close to their targets. Generation of high concentrations of ultrafine carbon black particles was difficult. Analyses of the response variables indicated lesser responses to carbon black than to V_2O_5 for a given exposure concentration. The predicted lung burden for the carbon black particles, however, was approximately double that of the V_2O_5 particles for a given exposure concentration. Thus, neither exposures to lower concentrations of ultrafine carbon black nor exposures to fine particles of carbon black were performed.

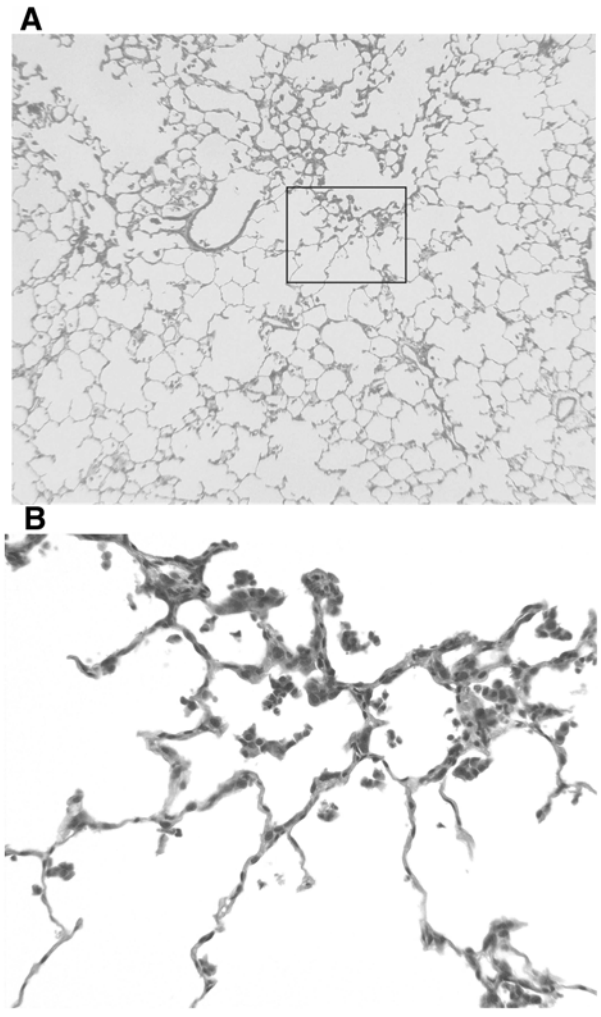

Figure 10. Experiment 1: alveolar inflammation and macrophage hyperplasia. Lung of an aged rat exposed to 500 μg/m^3 of fine V_2O_5 particles and instilled with endotoxin in experiment 3, used for comparison with experiment 1 exposures. ***(A)*** Alveolar inflammation (×4 magnification, 1 cm = 1 mm; H&E stain); and ***(B)*** alveolar macrophage hyperplasia and inflammatory cells in the alveoli (×20 magnification, 1 cm = 200 μm; H&E stain).

Biological Responses

Survival and Weights In experiment 2, all rats were between 22 and 24 months of age and all were instilled with endotoxin 3 days before the end of the exposure period. Forty rats (including 12 control rats) were used in the three carbon black exposures. Four exposed rats died or were removed from study before the exposure and analyses were completed. Losses of these rats were related to diseases of aging or to anesthesia administered before instillation. The other rats showed no marked clinical effects and none died of exposure to carbon black particles.

Body and lung weights are shown in Table G.1, and the statistical analyses of key weight parameters are given in Table 11. The body weights of all rats, regardless of exposure, decreased during the 17 days of the experiment. The degree of the weight loss was similar in all groups, giving no indication that the exposures caused a general toxicity. Additionally, lung weights increased (compared with control rats) in rats exposed to V_2O_5 but not in those exposed to carbon black.

Analysis of Response Data The response data for all rats exposed to carbon black particles and instilled with endotoxin are shown in Table G.2; comparative results from experiment 1 for rats exposed to ultrafine particles of V_2O_5 are shown in Table F.2. *P* values from a two-way ANOVA of the response variables are shown in Table 12. Although the predicted lung burdens from the carbon black exposures were approximately 30-fold greater than those from the V_2O_5 exposures, the V_2O_5 particles caused significantly greater responses in most variables than the carbon black particles did. For example, these endpoints were all significantly greater in V_2O_5-exposed rats: LDH in BAL fluid (the primary indicator of cytotoxicity; Figure 12A), neutrophils in BAL fluid (the primary indicator of inflammation; Figure 12B), and mucus increase in airway generation 12 (the primary indicator of mucous cell metaplasia; Figure 12C). Particle type and exposure concentration had a significant interactive effect on cytotoxicity, inflammation, and alveolar macrophage activation but not on airway mucous cell metaplasia or the total grade for histologically evident inflammation.

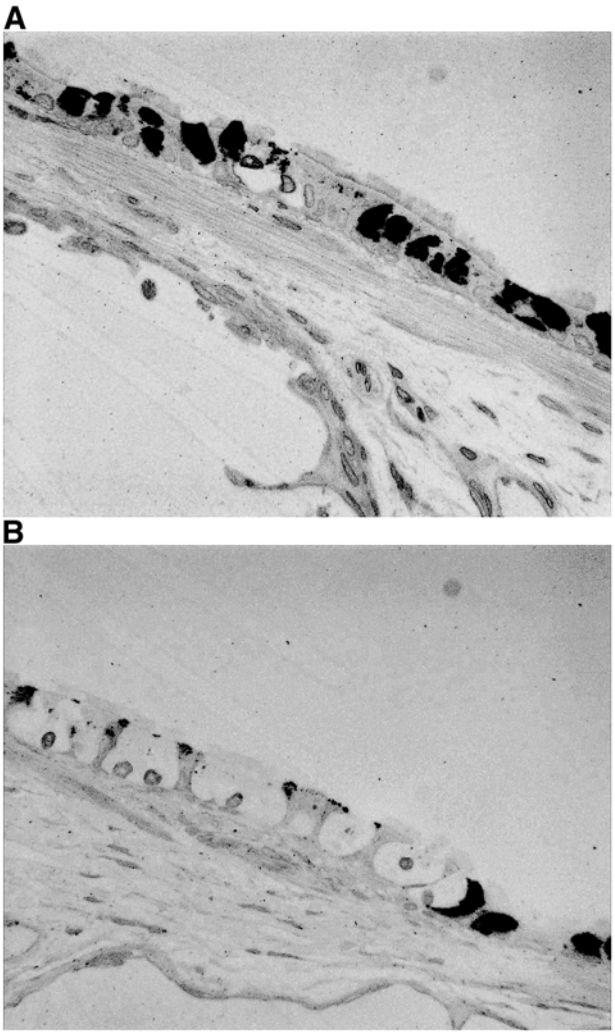

Figure 11. Experiment 1: airway mucous cell metaplasia in airway generation 12. (*A*) Aged control rat instilled with endotoxin; **(*B*)** aged rat exposed to ultrafine V_2O_5 (200 μg/m^3) and instilled with endotoxin. ×40 Magnification (1 cm = 100 μm); AB/PAS stain.

Table 11. Experiment 2: Statistical Analysis of Body and Lung Weights[a]

	Two-Way ANOVA *P* Value		
Variable	Particle Type[b]	Exposure Concentration[c]	Particle Type × Concentration[d]
Change in body weight	0.04	0.54	0.02
Lung weight	<0.01	0.13	0.02
Lung–body weight ratio	<0.01	0.13	<0.01

[a] Data from experiment 2 were compared with data from the aged rats exposed to the same concentrations of ultrafine V_2O_5 in experiment 1.

[b] *P* values indicate the difference between the carbon black and V_2O_5 exposure groups.

[c] *P* values indicate the differences among the 0 μg/m^3 (control), 150+50 μg/m^3, and 200 μg/m^3 exposures.

[d] *P* values reflect the differences in exposure concentrations between carbon black and V_2O_5.

Table 12. Experiment 2: Statistical Analysis of Effect of Ultrafine Particle Type and Exposure Concentration[a]

Mechanism / Variable	Two-Way ANOVA *P* Values		
	Particle Type[b]	Exposure Concentration[c]	Particle Type × Concentration[d]
Cytotoxicity			
LDH (IU/L)	0.02	0.02	0.01
Inflammation			
Neutrophils/mL	<0.01	< 0.01	< 0.01
Cells/mL	0.99	< 0.01	< 0.01
Protein (mg/dL)	< 0.01	0.06	< 0.01
TNF-α (pg/mL)	< 0.01	0.12	< 0.01
Lymphocytes/mL	0.51	< 0.01	0.05
Macrophage Activation			
Macrophages/mL	< 0.01	< 0.01	0.01
β-glucuronidase (IU/L)	< 0.01	0.37	< 0.01
MIP-2 (pg/mL)	< 0.01	< 0.01	< 0.01
Mucous Cell Metaplasia			
Mucus in airway generation 12 (nL/mm^2)	0.01	< 0.01	0.06
Mucus in airway generation 4 (nL/mm^2)	0.83	0.02	0.18
Histologically Evident Inflammation			
Total grade	< 0.01	< 0.01	0.20
Alveolar inflammation	< 0.01	< 0.01	< 0.01
Interstitial reaction	—	—	—
Macrophage hyperplasia	< 0.01	< 0.01	0.01

[a] Data from experiment 2 were compared with data from the aged rats exposed to the same concentrations of ultrafine V_2O_5 in experiment 1.

[b] *P* values indicate the difference between the carbon black and V_2O_5 particle exposure groups.

[c] *P* values reflect differences in the variables for the three exposure concentrations.

[d] *P* values reflect the degree of interaction between particle type and exposure concentration.

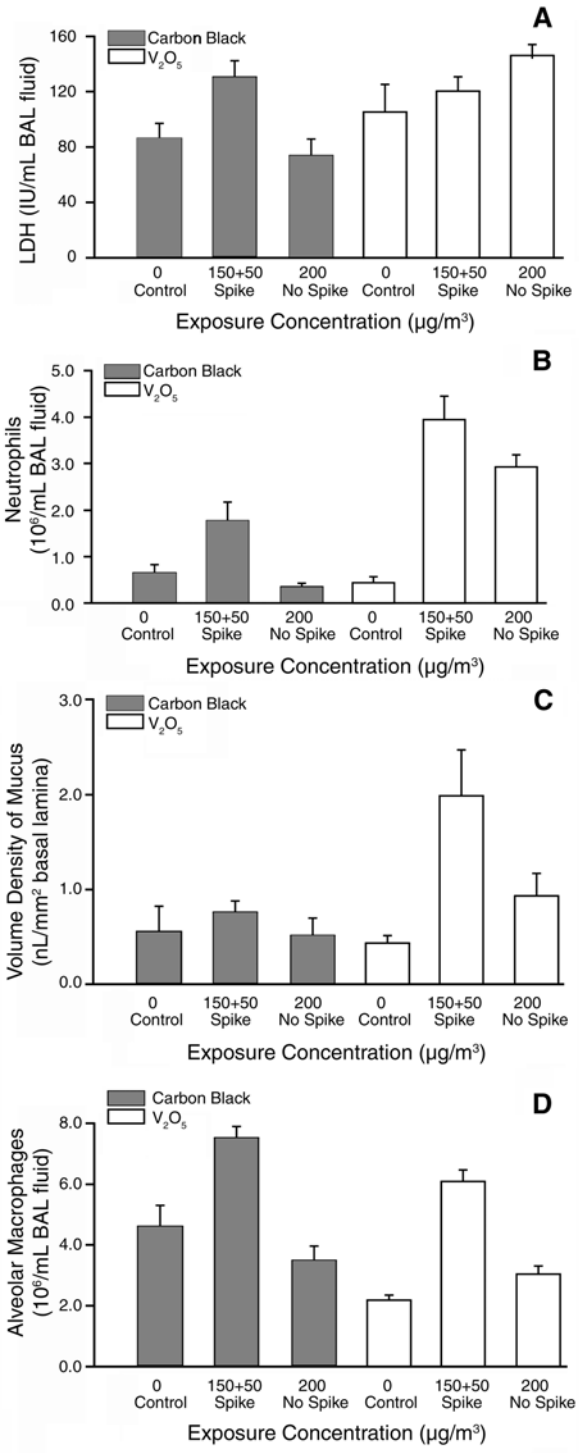

Figure 12. Experiment 2: biological responses of aged rats exposed to ultrafine carbon black or ultrafine V_2O_5 particles (mean ± SE). (*A*) LDH in BAL fluid. **(*B*)** Neutrophils in BAL fluid. **(*C*)** Volume density of mucus in epithelium of airway generation 12. **(*D*)** Macrophages in BAL fluid. Data for ultrafine V_2O_5 exposures are from experiment 1. *Spike* = 150 μg/m^3 for 15 days followed by 150+50 μg/m^3 for 2 days. *No spike* = 200 μg/m^3 for 17 days. Note the different y-axis scales among panels.

The influence of short-term spikes in exposure to carbon black particles compared with ultrafine V_2O_5 particles is summarized in Figure 13. The lines in the diagram indicate any significant change in response and the direction of that change between 0, 150+50, and 200 μg/m^3 of particles. The patterns demonstrate that spikes in concentration of either carbon black or V_2O_5 often caused a significant change in effect.

The primary determination of cytotoxicity of pulmonary cells was LDH concentration in BAL fluid (Figure 12A). For ultrafine carbon black, rats exposed to a spike of 150+50 μg/m^3 had significantly greater LDH concentration than the rats exposed to 0 μg/m^3 or 200 μg/m^3. A similar spike in exposure concentration of V_2O_5 did not cause significant elevation of LDH concentration compared to the other exposures.

The primary determination of inflammation was number of neutrophils in the BAL fluid (Figure 12B). For carbon black and V_2O_5 exposures, rats exposed to a spike of 150+50 μg/m^3 had significantly greater concentrations of neutrophils, as well as total cell and lymphocyte concentrations (key and supporting variables), in BAL fluid than rats exposed to 0 μg/m^3 or 200 μg/m^3. In addition, protein concentrations in BAL fluid, a supporting variable, were greater in rats exposed to 150+50 μg/m^3 ultrafine carbon black, but not ultrafine V_2O_5, than in rats exposed to 0 μg/m^3 or 200 μg/m^3.

The primary determination of alveolar macrophage activation was the number of alveolar macrophages per milliliter of BAL fluid (Figure 12D). For ultrafine carbon black and V_2O_5 particles, rats exposed to a spike of 150+50 μg/m^3 had significantly greater alveolar macrophage numbers than rats exposed to 0 μg/m^3 or 200 μg/m^3. MIP-2 concentrations, a supporting variable, were greater in rats exposed to 150+50 μg/m^3 carbon black but not in rats exposed to 150+50 μg/m^3 V_2O_5, than in rats exposed to 0 μg/m^3 or 200 μg/m^3.

The primary determination of mucous cell metaplasia in the airways was mucus content of the airway generation 12 epithelium (Figure 12C). Rats exposed to a spike of 150+50 μg/m^3 ultrafine V_2O_5 had a significantly greater amount of mucus in airway generations 12 and 4 (not shown) compared with rats exposed to 0 μg/m^3 or 200 μg/m^3. No increase was found in rats exposed to ultrafine carbon black particles.

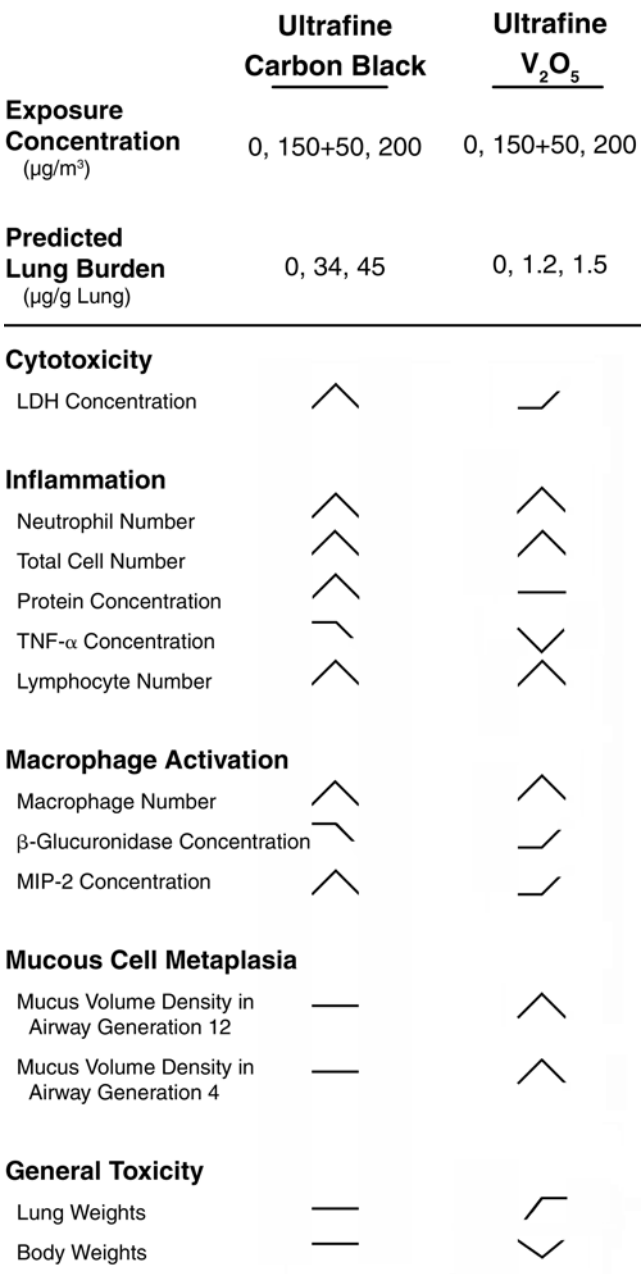

Figure 13. Experiment 2: influence on aged rats of short-term spikes in exposure to ultrafine carbon black or ultrafine V_2O_5 particles. The lines in the diagram indicate any significant change in response and the direction of the change between 0 μg/m^3, 150+50 μg/m^3, and 200 μg/m^3 of particles. Data for ultrafine V_2O_5 exposures are from experiment 1.

EXPERIMENT 3: RESPONSES OF AGED AND YOUNG ADULT RATS TO INHALED FINE V_2O_5

The summary data for experiment 3 are given in Appendix H. The text tables and figures were developed from these data.

Achieved Fine V_2O_5 Exposure Concentration and Size

The aerosol concentration and size measured for the exposure are shown in Table 6. The actual concentration and size were reasonably close to the targets.

Lung Burden Measurements

The amount of vanadium in the lung at the end of the 17-day exposure to fine V_2O_5 is shown for young adult and aged rats in Table 7. Although all rats were exposed at the same time to the same aerosol, significantly more (nearly twice as much) vanadium was retained in the lungs of the aged rats (2.43 µg/g) than in the lungs of the young adult rats (1.33 µg/g).

Biological Responses

Survival and Weights In experiment 3, 10 rats were 8 to 10 weeks of age (young adult) and 10 were between 22 and 24 months of age (aged). One aged rat exposed to fine V_2O_5 and two exposed to filtered air died during the exposure period. Other rats showed no clinical effects during exposures. All rats were instilled with endotoxin 3 days before the end of the exposure period.

Body and lung weights are shown in Table H.1 and the ANOVA of key weight parameters are in listed in Table 13. The body weights of all rats, regardless of whether they were exposed to 0 or 500 µg/m^3 fine V_2O_5, decreased during the 17 days of the experiment. The decrease was much greater in exposed aged rats than in exposed young adult rats (14% vs 4%). This magnitude of weight loss in the aged rats was consistent with that seen in the other exposures to V_2O_5 particles. The lung weights and the lung–body weight ratios increased in both the young adult and aged groups exposed to 500 µg/m^3 of fine V_2O_5 compared with respective control rats. However, the lung weights for the aged rats were significantly greater than those for the young adult rats.

Analysis of Response Data The response data for aged and young adult rats exposed to 0 or 500 µg/m^3 fine V_2O_5 and instilled with endotoxin are shown in Table H.2. Statistical analyses, using a two-way ANOVA, revealed a significant effect of particle exposure concentration on many indicators of cytotoxicity, inflammation, alveolar macrophage activation, and airway mucus volume density (Table 14), as might be expected from the similar findings

Table 13. Experiment 3: Statistical Analysis of Body and Lung Weights

	Two-Way ANOVA *P* Value		
Variable	Age[a]	Exposure Concentration[b]	Age × Concentration[c]
Change in body weight	<0.01	0.02	0.69
Lung weight	<0.01	<0.01	0.01
Lung–body weight ratio	0.28	<0.01	0.28

[a] *P* values indicate the difference between the young adult and aged groups.

[b] *P* values reflect the differences in weights due to differences between the 0 and 500 µg/m^3 concentrations.

[c] *P* values reflect the differences in weights, due to differences in exposure concentrations, according to rat age (young adult or aged).

Table 14. Experiment 3: Statistical Analysis of Effect of Rat Age and Exposure Concentration

	Two-Way ANOVA *P* Value		
Mechanism / Variable	Age[a]	Exposure Concentration[b]	Age × Concentration[c]
Cytotoxicity			
LDH (IU/L)	0.03	< 0.01	0.94
Inflammation			
Neutrophils/mL	0.25	< 0.01	0.07
Cells/mL	0.93	< 0.01	0.91
Protein (mg/dL)	0.11	< 0.01	0.74
TNF-α (pg/mL)	< 0.01	0.08	0.86
Lymphocytes/mL	0.65	0.09	0.62
Macrophage Activation			
Macrophages/mL	0.89	< 0.01	0.40
β-glucuronidase (IU/L)	< 0.01	0.09	0.57
MIP-2 (pg/mL)	0.02	< 0.01	0.04
Mucous Cell Metaplasia			
Mucus in airway generation 12 (nL/mm^2)	0.16	0.01	0.74
Mucus in airway generation 4 (nL/mm^2)	0.21	0.07	0.34
Histologically Evident Inflammation			
Total grade	0.18	< 0.01	0.59
Alveolar inflammation	0.02	< 0.01	0.01
Interstitial inflammation	0.20	0.08	0.08
Macrophage hyperplasia	0.01	< 0.01	0.04

[a] *P* values indicate the difference between the young adult and aged groups.

[b] *P* values reflect differences in response due to exposure concentration.

[c] *P* values indicate whether or not there is a difference in response due to an interaction between age and exposure concentration.

in experiment 1. In addition, the cytotoxic response (represented by LDH concentration in BAL fluid) significantly differed between the aged and young adult rats and was significantly greater in rats exposed to 500 µg/m^3 particles in both groups than in respective control rats. Neutrophil concentrations in BAL fluid, which are primary indicators of inflammation, were significantly greater in aged and young adult rats exposed to 500 µg/m^3 particles than in the respective control rats (Figure 14A). The magnitude of the increase did not significantly differ between the two age groups.

Alveolar macrophage concentrations in the BAL fluid, which are primary indicators of alveolar macrophage activation, were significantly greater in aged and young adult rats exposed to 500 µg/m^3 particles than in the respective control rats. The magnitude of the increase did not significantly differ between the two age groups. β-Glucuronidase activity and MIP-2 concentration were significantly greater in aged rats exposed to 500 µg/m^3 particles than in the aged control rats. Mucus volume density in airway generation 12, which is a primary indicator of mucous cell metaplasia, was significantly greater in aged and young adult rats exposed to 500 µg/m^3 particles than in the respective control rats (Figure 14B).

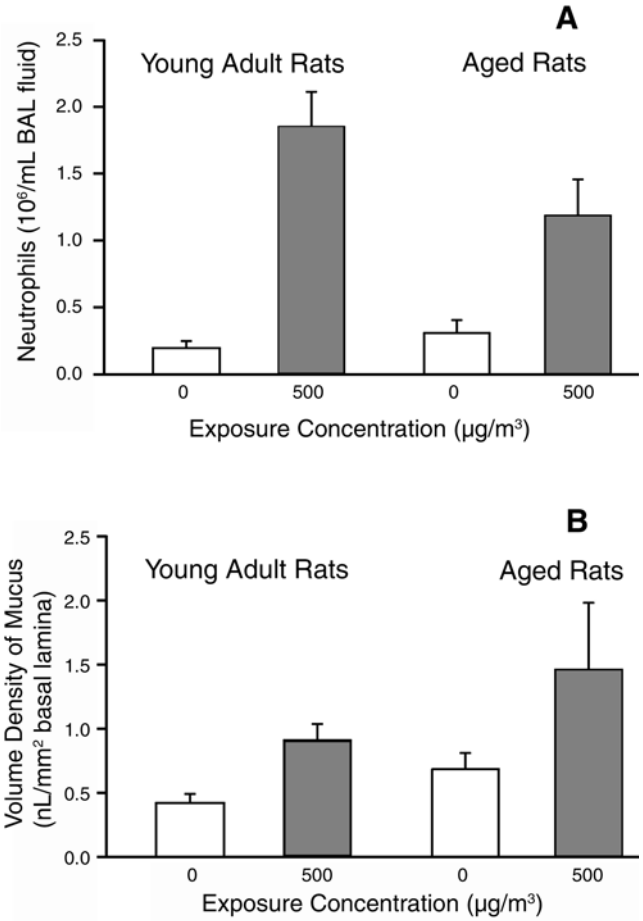

Figure 14. Experiment 3: biological responses of young adult or aged rats exposed to fine V_2O_5 (mean ± SE). (*A*) Number of neutrophils in BAL fluid. **(*B*)** Volume density of mucus in epithelium of airway generation 12.

DISCUSSION

Four basic conclusions can be made from the three main experiments in this study.

1. Ultrafine particles of V_2O_5 had a higher mass retained in the lung of rats than fine particles of V_2O_5 at the end of 17 days of exposure to aerosols with a similar mass concentration.

Ultrafine particles deposit with a higher efficiency in the lung than do fine particles (Swift et al 1994; Yeh et al 1997). In addition, Ferin and colleagues (1992) have shown that, in rats exposed to equal airborne mass concentrations of fine or ultrafine TiO_2 particles for 12 weeks, clearance is slower (half-life = 501 days) for ultrafine particles than fine particles (half-life = 174 days). The greater deposition and slower clearance of ultrafine particles would logically lead to a higher lung burden of TiO_2 ultrafine particles. However, the high exposure concentration of 23 mg/m^3 complicated the interpretation of that study; the exposure was well above an overload exposure concentration and above a concentration that has caused fibrosis and tumors in rats after long-term exposure.

Our result in rats exposed to relatively low ultrafine or fine particle concentrations (150 or 200 µg/m^3) indicates that the lung burden of ultrafine particles is indeed greater at particle concentrations close to those found in ambient air. The modeling in this study suggests that the greater burden is strongly influenced by greater deposition of ultrafine particles rather than by substantial differences in particle clearance.

2. Inhaled ultrafine particles of V_2O_5 caused significantly higher levels of neutrophils (indicative of inflammation) in BAL fluid from aged, endotoxin-treated rats than did inhaled fine particles of V_2O_5 when compared on a basis of equal predicted lung burden of particles.

This finding is consistent with the findings of others who have compared the inflammatory response to fine and ultrafine particles deposited in the lungs of rats. However, our study extends the findings into lower exposure concentration ranges.

Increased toxicity has been shown with instillation of various types of fine and ultrafine particles into the lung:

TiO_2 in rats (Donaldson et al 1998), polystyrene particles in rats (Brown et al 2001), carbon black particles in rats (Oberdörster et al 1998; Li et al 1999), and insoluble manganese dioxide dusts in mice (Lison et al 1997). One study involved intratracheal inhalation of TiO_2 (Osier and Oberdörster 1997). The inflammatory reaction to the particles correlated more closely with the total surface area of the particles than with the mass of the particles. Free radical activity on the surfaces of these chemically disparate particles has been suggested as the common factor in their toxicity (Gilmour et al 1997).

Only a few studies have used the inhalation route of exposure to compare pulmonary effects of fine and ultrafine particles. The pulmonary response to a single inhaled dose differs from that to a single instilled dose (Osier and Oberdörster 1997). Deposition of inhaled TiO_2 particles in the lungs decreases pulmonary response both in persistence and in severity. Different patterns of injury occurs in rats after instillation or inhalation of α quartz, with pleural granulomas present only in those exposed by inhalation (Henderson et al 1995). These results show the importance of inhalation studies in verifying principles developed from studies of instilled materials.

The seminal inhalation study of ultrafine particles (Ferin et al 1992) showed that when rats were exposed to equal aerosol mass concentrations of fine and ultrafine TiO_2 particles (about 23 mg/m^3 for 12 weeks), far more inflammatory cells were found in the BAL fluid of rats exposed to the ultrafine particles. The exposure mass concentration used was several orders of magnitude greater than the concentrations used in our inhalation experiments of fine and ultrafine particles. In addition, the length of the exposure was not comparable. Regardless of these differences, our study suggests that, at a relatively low lung burden, ultrafine V_2O_5 is more toxic than fine V_2O_5, as shown by the increased number of neutrophils in BAL fluid (although many other inflammatory parameters did not differ between fine and ultrafine V_2O_5). Our study also extends this principle to the low exposure concentration of 100 $\mu g/m^3$.

The health effects of particulate matter air pollution are influenced by the size of the particles in the atmosphere, but other factors may be involved as well (Utell and Frampton 2000). PM_{10} is more strongly correlated with air pollution health effects than are airborne particles up to 40 µm (TSP). A number of epidemiology studies have shown that $PM_{2.5}$ in the air is associated with increased effects. For example, a meta-analysis of 21 published studies on the correlation of PM and mortality showed greater effects when the $PM_{2.5}$/PM ratio was greater (Levy et al 2000). Schwartz and colleagues (1996) found that daily mortality was more strongly associated with $PM_{2.5}$ than with PM_{10} in six eastern US cities over an 8-year period. Increased respiratory symptoms in people with asthma were more closely correlated with ultrafine particles (those smaller than 0.1 µm) than with fine particles (0.1–2.5 µm) or with PM_{10} in a study by Peters and coworkers (1997). However, one epidemiology study found that daily mortality correlated most strongly with PM_{10} concentration, with a lesser, similar correlation with ultrafine particles and fine particles (Wichmann et al 2000). In addition, studies of pulmonary symptoms in patients with asthma (Pekkanen et al 1997) or COPD (Osunsanya et al 2001) have shown an association of the diseases with PM_{10} but not fine or ultrafine particles.

3. Two-day spikes in particle exposure concentration of 150+50 $\mu g/m^3$ after a constant 150 $\mu g/m^3$ exposure for 15 days resulted in significantly greater responses in the lungs of aged, endotoxin-treated rats than in rats not exposed to a spike.

This result was found for a relatively toxic transition metal compound, V_2O_5, and a relatively inert compound, carbon black. It is the first laboratory demonstration of an effect in the lung of a 2-day spike in exposure aerosol concentration. Notable features of the experimental design include the use of aged rats, the instillation of an inflammatory agent (endotoxin) just before the spike in aerosol concentration, and a 15-day exposure to a constant baseline concentration before the 150+50 $\mu g/m^3$ spike exposure.

The effect was not seen at the high (500 $\mu g/m^3$) or ambient (50 $\mu g/m^3$) baseline concentrations, but only in the high-ambient range (150 $\mu g/m^3$). The cause for the increase at these lower but not the higher exposure concentrations is speculative. The 2-day spike in exposure concentration did not cause a significant increase in lung burdens of V_2O_5 regardless of the baseline exposure or the size of the spike in exposure concentration (Table 7).

We observed several differences in responses to inhaled ultrafine carbon black and V_2O_5. The most striking was the much larger predicted lung burden of carbon black than V_2O_5 at the end of the 17-day exposure to the same aerosol concentrations. The lung burden predicted for carbon black was 28 to 30 times greater than that for V_2O_5 (although we could not compare predicted and measured carbon black lung burden). This difference was related to the relative insolubility of carbon black relative to the V_2O_5. Because the predicted lung burdens for carbon black were so different from those for V_2O_5, it is difficult to assess how substantial the differences in biological responses associated with exposure concentration actually are. Similar response patterns were observed for many variables, however (Figure 13).

The outstanding exception to the similarity of responses to inhaled ultrafine carbon black and V_2O_5 was the marked stimulation of mucous cell metaplasia in the airways of rats exposed to V_2O_5. Ghio and colleagues (2002) have shown the ability of residual oil fly ash and the vanadium therein to stimulate lung mucin production. Of the soluble transition metals in residual oil fly ash (nickel, iron and vanadium), only vanadium individually, or combinations of the metals that contain vanadium, provoked mucin secretion when placed on guinea pig tracheal epithelial cells growing in vitro (Jiang et al 2000). Exposure of a human lung carcinoma cell line to residual oil fly ash or V_2O_5 also resulted in increased mucin production and an increase in mucin messenger RNA. A screen of signaling inhibitors showed that mucin induction is mediated by a phosphotyrosine-dependent pathway. Vanadium, being a potent inhibitor of tyrosine phosphatase, may trigger pulmonary responses by unmasking phosphorylation-dependent resistance pathways (Longphre et al 2000).

Cellular responses in the lung to inhaled carbon black and V_2O_5 were similar despite their large difference in predicted lung burdens. This similarity can be explained by previous work. Particles of carbon black have been shown to induce a cellular response in the lungs of mice that aggravates endotoxin-induced lung injury (Yanagisawa et al 2003). And vanadium instilled in the lung initiates inflammation of an intensity similar to that induced by zinc or iron(II) but much less than that induced by copper, manganese, and nickel (Rice et al 2001).

The exposure concentrations used in our study were within the range found in various communities around the country. Epidemiologic studies have shown that relatively low concentrations of particles in community air could be associated with adverse health effects. For example, a number of studies have reported associations between daily mortality and PM_{10} in Birmingham, Alabama (Schwartz 1993), Chicago, Illinois, and Los Angeles, California (Ito et al 1995), and the Utah valley (Pope et al 1992). The mean ambient air PM_{10} concentrations were 30 to 58 $\mu g/m^3$, ranging from 10 to 365 $\mu g/m^3$. Daily mortality was estimated to increase by 0.5% to 1.6% for each 10 $\mu g/m^3$ increase in PM_{10} concentration.

Morbidity also increases with increased concentrations of PM_{10}. A higher rate of hospital admissions of people older than 65 years for pneumonia, COPD, or cardiovascular disease in 10 US cities was associated with increased PM_{10} concentrations (Zanobetti et al 2000a). The rate was higher when the PM_{10} concentrations were less than 50 $\mu g/m^3$, well below the 24-hour ambient air quality standard. In addition, one study estimated PM_{10}–mortality dose–response curves in 20 large US cities (Daniels et al 2000). The findings indicate that linear models without thresholds are appropriate for assessing effects of particulate air pollution on daily cardiopulmonary deaths.

In our study, we used aged rats that were instilled with endotoxin to simulate a susceptible population. Older people, young people, and people with chronic cardiopulmonary disease and asthma are most likely to be susceptible to the effects of air pollution (Pope 2000). Patients with COPD have an increased risk of death associated with exposure to increased PM_{10} (Sunyer et al 2000). A specific analysis of hospital admissions for heart disease, COPD, or pneumonia associated with air pollution suggested that preexisting respiratory infections or cardiac electrical conduction defects increased the risk of admission (Zanobetti et al 2000b). The PM_{10}-associated increase in hospital admissions for cardiovascular diseases was almost double in people with concurrent respiratory infections or with conduction disorders. Individuals with asthma had twice the risk of a PM_{10}-associated pneumonia admission and those with heart failure had twice the risk of a PM_{10}-associated COPD admission. The PM_{10} effect did not vary by sex or age, indicating that the increased susceptibility in older people is associated with specific disease states. People with diabetes are also susceptible: they have increased PM_{10}-associated admissions for heart disease but not for lung disease (Zanobetti and Schwartz 2001).

Inhalation or intratracheal instillation of endotoxin has been used to initiate inflammation in the lung and airways in many laboratory studies (eg, Nikula and Green 2000). The response is determined by the dose of endotoxin and the mode of exposure. For example, high doses (> 5 mg/kg) administered once to rats by intratracheal aerosolization or instillation resulted in marked acute inflammation, similar to adult respiratory distress syndrome, within 24 hours (Domenici-Lombardo et al 1995; van Helden et al 1997). Exposures to lower doses resulted in less extensive lesions. For example, rats instilled intranasally with endotoxin (2.5 mg/kg, once a day for 3 days) developed a bronchitis characterized by neutrophilic infiltrates, serous cell hyperplasia, and mucous cell hyperplasia. The amount of mucosubstances increased most markedly 4 days after initiation of treatment and persisted for more than 1 week (Harkema and Hotchkiss 1993). Similarly, inhaled endotoxin (0.3 $\mu g/m^3$, 3 times a day for 3 days) increased the volume density of mucosubstances in the airways of rats (Gordon and Harkema 1994).

4. Aged, endotoxin-treated rats did not differ from young adult, endotoxin-treated rats in their responses to relatively high concentrations of V_2O_5.

The aged rats in our study had significantly greater measured lung burdens of vanadium than the young adult rats. The predicted burdens for the young adult rats were less than those for the aged rats (Table 7). Young adult and aged rats

had a similar response to exposure to 500 μg/m^3 of fine V_2O_5 in spite of the difference in measured lung burden. This result may indicate that younger rats are more susceptible to V_2O_5 than aged rats.

The difference in predicted lung burden between young adult and aged rats may relate to uncertainties in the minute volumes for the two ages (Appendix C). However, no reasonable estimates of minute volumes predicted greater deposition in the aged rats. Furthermore, the young adult and aged predicted lung burdens were consistent with other predicted burdens in that they tended to be within about 10% to 15% of the corresponding measured lung burdens. We could not determine the reason for the difference with the data available.

Our experiment comparing the responses of young adult and aged rats was limited because further studies with young adult and aged rats were planned but could not be initiated. In the summer of 2001, a failure of the air conditioning in the animal rooms of the National Institute of Aging rat colony (Harlan Sprague Dawley) resulted in the deaths of many rats, including a large fraction of the older rats. The Institute was able to provide enough rats for the first part of experiment 3 but could not supply additional aged rats for 1 to 1.5 years after the incident, well past the end of the contract.

CONCLUSIONS

The results of this study add further evidence that inhaled ultrafine particles may cause a greater response in the lung than fine particles of the same total mass under these conditions. In addition, spikes in exposure with high-ambient concentrations of particles may be a significant factor in understanding the health effects of particulate air pollution in communities. Further work is needed to confirm this result and to understand the mechanisms that may cause it.

ACKNOWLEDGMENTS

The authors acknowledge the initial principal investigator on the project, Kristen Nikula, DVM, PhD. She was primarily responsible for the initial study design and early work on the study. We thank the following technical staff for their good work in their respective areas: Jessica Costanza (BS) and Mitchell Irwin (BS), aerosol science; Katie Bennett (BS) and Jennifer Berger (BS), pathology; Rhonda Garlick, necropsy; and Yone Knighton and Pat Cossey, histology. Bonne Cleveland did the technical editing and Sylvia Perez, the word processing.

REFERENCES

American Conference of Governmental Industrial Hygienists. 2001. 2001 Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices, pp 59 and 92. ACGIH, Cincinnati OH.

Blackwell TS, Christman JW. 1997. The role of nuclear factor-κB in cytokine gene regulation. Am J Respir Cell Mol Biol 17:3–9.

Bonner JC, Rice AB, Lindroos PM, O'Brien PO, Dreher KL, Rosas I, Alfaro-Moreno E, Osornio-Vargas AR. 1998. Induction of the lung myofibroblast PDGF receptor system by urban ambient particles from Mexico City. Am J Respir Cell Mol Biol 19:672–680.

Brain JD. 1970. Free cells in the lungs. Some aspects of their role, quantitation, and regulation. Arch Intern Med 126:477–487.

Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. 2001. Size-dependent proinflammatory effects of ultrafine polystyrene particles: A role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol Appl Pharmacol 175:191–199.

Burge PS, Hedge A, Wilson S, Bass JH, Robertson A. 1987. Sick building syndrome: A study of 4373 office workers. Ann Occup Hyg 31:493–504.

Cassee FR, Muijser H, Duistermaat E, Freijer JJ, Geerse KB, Marijnissen JC, Arts JH. 2002. Particle size-dependent total mass deposition in lungs determines inhalation toxicity of cadmium chloride aerosols in rats. Application of a multiple path dosimetry model. Arch Toxicol 76:277–286.

Chang L-Y, Stockstill BL, Ménache MG, Mercer RR, Crapo JD. 1995. Morphometric analysis of structural alterations in alveolar regions. Part VIII in: Consequences of Prolonged Inhalation of Ozone on F344/N Rats: Collaborative Studies. Research Report 65. Health Effects Institute, Cambridge MA.

Daniels MJ, Dominici F, Samet JM, Zeger SL. 2000. Estimating particulate matter-mortality dose-response curves and threshold levels: An analysis of daily time-series for the 20 largest US cities. Am J Epidemiol 152:397–406.

Domenici-Lombardo L, Adembri C, Consalvo M, Forzini R, Meucci M, Romagnoli P, Novelli GP. 1995. Evolution of endotoxin induced acute lung injury in the rat. Int J Exp Pathol 76:381–390.

Donaldson K, Li SY, MacNee W. 1998. Ultrafine (nanometre) particle mediated lung injury. J Aerosol Sci 29:553–560.

Donaldson K, Stone V, Clouter A, Renwick L, MacNee W. 2001. Ultrafine particles. Occup Environ Med 58:211–216.

Driscoll KE. 1990. Role of alveolar macrophage-derived inflammatory mediators in pulmonary responses to dust. J Aerosol Med 3(Suppl 1):61–62.

Driscoll KE. 1995. Role of cytokines in pulmonary inflammation and fibrosis. In: Concepts in Inhalation Toxicology (McClellan RO, Henderson RF, eds), pp 471–504. Taylor & Francis, Washington DC.

Driscoll KE, Carter JM, Hassenbein DG, Howard B. 1997. Cytokines and particle-induced inflammatory cell recruitment. Environ Health Perspect 105(Suppl 5):1159–1164.

Driscoll KE, Carter JM, Howard BW, Hassenbein DG, Pepelko W, Baggs RB, Oberdörster G. 1996a. Pulmonary inflammatory, chemokine, and mutagenic responses in rats after subchronic inhalation of carbon black. Toxicol Appl Pharmacol 136:372–380.

Driscoll KE, Howard BW, Carter JM, Asquith T, Johnston C, Detilleux P, Kunkel SL, Isfort R. 1996b. Alpha-quartz-induced chemokine expression by rat lung epithelial cells: Effects of in vivo and in vitro particle exposure. Am J Pathol 149(Suppl 5):1627–1637.

Dye JA, Adler KA, Richards JH, Dreher KL. 1997. Epithelial injury induced by exposure to residual oil fly ash particles: Role of reactive oxygen species. Am J Respir Cell Mol Biol 17:625–633.

Elder ACP, Gelein R, Finkelstein JN, Cox C, Oberdörster G. 2000. Endotoxin priming affects the lung response to ultrafine particles and ozone in young and old rats. Inhalation Toxicol 12(Suppl 1):85–98.

Environmental Protection Agency (US). 1988. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. EPA-600/6-87-008. Office of Health and Environmental Assessment, Cincinnati OH. Available as PB88-179874 from National Technical Information Service, Springfield VA.

Environmental Protection Agency (US). 1994a. Methods for the Determination of Metals in Environmental Samples—Supplement I. EPA-600/R-94-111. EPA, Research Triangle Park NC. Available as PB 94-184942 at *www.ntis.gov*.

Environmental Protection Agency (US). 1994b. Methods for Development of Inhalation Reference Concentrations. EPA/600/8-88/066F. EPA, Research Triangle Park NC.

Environmental Protection Agency (US). 1996a. Air Quality Criteria for Particulate Matter, Vol 1–3: EPA/600/P-95/001a-cf. National Center for Environmental Assessment, Research Triangle Park NC.

Environmental Protection Agency (US). 1996b. National Ambient Air Quality Standards for particulate matter: Proposed rule. Fed Regist 61:65638–65713.

Environmental Protections Agency (US). 2003. 2001 Toxics Release Inventory (TRI) Public Data Release Report. EPA 260-R-03-001. Office of Information Analysis and Access, Washington DC. Available from *www.epa.gov /tri/tridata/tri01*.

Ferin J, Oberdörster G, Penney DP. 1992. Pulmonary retention of ultrafine and fine particles in rats. Am J Respir Cell Mol Biol 6:535–542.

Flaherty DK, Deck FH, Cooper J, Bishop K, Winzenburgen PA, Smith LR, Bynum L, Witmer WB. 1984. Bacterial endotoxin isolated from a water spray air humidification system as a putative agent of occupation-related lung disease. Infect Immun 43:206–212.

Foster JE, Goot F, Schuyler MR, Kozak W, Tesfaigzi Y. 2003. LPS-induced neutrophilic inflammation and Bcl-2 expression in metaplastic mucous cells. Am J Physiol Lung Mol Physiol 285:L405–L414.

Ghio AJ, Stonehuerner J, Pritchard RJ, Piantadosi CA, Quigley DR, Dreher KL, Costa DL. 1996. Humic-like substances in air pollution particulate correlate with concentrations of transition metals and oxidant generation. Inhalation Toxicol 8:479–494.

Ghio SJ, Silbajoris R, Carson JL, Samet JM. 2002. Biologic effects of oil fly ash. Environ Health Perspect 110(Suppl 1):89–94.

Gielden MH, van der Zee SC, van Wijnen JH, van Steen CJ, Brunekreef B. 1997. Acute effects of summer air pollution on respiratory health of asthmatic children. Am J Respir Crit Care Med 155:2105–2108.

Gilmour PS, Brown DM, Beswick PH, MacNee W, Rahman I, Donaldson K. 1997. Free radical activity of industrial fibers: Role of iron in oxidative stress and activation of transcription factors. Environ Health Perspect 105(Suppl 5):1313–1317.

Gomi F, Dooley MM, Matsuo M. 1995. Effects of oxygen inhalation on the antioxidant capacity of lungs, livers, and

brains in normal and vitamin E-deficient rats at various ages. J Nutr Sci Vitaminol 41:139–149.

Gordon T, Harkema JR. 1994. Effect of inhaled endotoxin on intraepithelial mucosubstances in F344 rat nasal and tracheobronchial airways. Am J Respir Cell Mol Biol 10:177–183.

Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ. 1988. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96:379–394.

Gurgueira SA, Lawrence J, Coull B, Krishna Murthy GG, Gonzàlez-Flecha B. 2002. Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. Environ Health Perspect 110:749–755.

Harkema JR, Hotchkiss JA. 1991. In vivo effects of endotoxin on nasal epithelial mucosubstances: Quantitative histochemistry. Exp Lung Res 17:743–761.

Harkema JR, Hotchkiss JA. 1992. In vivo effects of endotoxin on intraepithelial mucosubstances in rat pulmonary airways quantitative histochemistry. Am J Pathol 141:307–317.

Harkema JR, Hotchkiss JA. 1993. Ozone- and endotoxin-induced mucous cell metaplasias in rat airway epithelium: Novel animal models to study toxicant-induced epithelial transformation in airways. Toxicol Lett 68:251–263.

Harkema JR, Plopper CG, Hyde DM, St George JA. 1987. Regional differences in quantities of histochemically detectable mucosubstances in nasal, paranasal, and nasopharyngeal epithelium of the bonnet monkey. J Histochem Cytochem 35:279–286.

Harrison J, Pickering CAC, Faragher EB, Austwick PKC, Little SA, Lowton L. 1992. An investigation of the relationship between microbial and particle indoor air pollution and the sick building syndrome. Respir Med 86:225–235.

Henderson RF. 1989. Bronchoalveolar lavage: A tool for assessing the health status of the lung. Chapter 15 in: Concepts in Inhalation Toxicology (McClellan RO, Henderson RF, eds), pp 415–444. Hemisphere Publishing, New York NY.

Henderson RF, Benson JM, Hahn FF, Hobbs CH, Jones RK, Mauderly JL, McClellan RO, Pickrell JA. 1985. New approaches for the evaluation of pulmonary toxicity: Bronchoalveolar lavage fluid analysis. Fundam Appl Toxicol 5:451–458.

Henderson RF, Driscoll KE, Harkema JR, Lindenschmidt RC, Chang IY, Maples KR, Barr EB. 1995. Comparison of the inflammatory response of the lung to inhaled versus instilled particles in F344 rats. Fundam Appl Toxicol 24:183–197.

Hinds WC. 1999. Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles (2nd ed), p 99. John Wiley & Sons, New York NY.

Hoover MD, Mewhinney CJ. 1985. Evaluation of a 96-port nose-only exposure chamber. In: Inhalation Toxicology Research Institute Annual Report 1984–1985, LMF-114 (Medinsky M, Muggenburg B, eds), pp 41–44. National Technical Information Service, Springfield VA.

Ibald-Mulli A, Wichmann HE, Kreyling W, Peters A. 2002. Epidemiological evidence on health effects of ultrafine particles. J Aerosol Med 15:189–201.

Ito K, Kinney PL, Thurston GD. 1995. Variations in PM-10 concentrations within two metropolitan areas and their implications for health effects analyses. Inhalation Toxicol 7:735–745.

Jiang N, Dreher KL, Dye JA, Li Y, Richards JH, Martin LD, Adler KB. 2000. Residual oil fly ash induces cytotoxicity and mucin secretion by guinea pig tracheal epithelial cells via an oxidant-mediated mechanism. Toxicol Appl Pharmacol 163:221–230.

Johnson WJ, Sung CP. 1987. Rat macrophage treatment with lipopolysaccharide leads to a reduction in respiratory burst product secretion and a decrease in NADPH oxidase affinity. Cell Immunol 108:109–119.

Kadiiska MB, Mason RP, Dreher KL, Costa DL, Ghio AJ. 1997. In vivo evidence of free radical formation in the rat lung after exposure to an emission source air pollution particle. Chem Res Toxicol 10:1104–1108.

Killingsworth CR, Alessandrini F, Krishna Murthy GG, Catalano PJ, Paulauskis JD, Godleski JJ. 1997. Inflammation, chemokine expression, and death in monocrotaline-treated rats following fuel oil fly ash inhalation. Inhalation Toxicol 9:541–565.

Kiviluoto M, Rasanen O, Rinne A, Rissanen M. 1979. Effects of vanadium on the upper respiratory tract of workers in a vanadium factory. Scan J Work Environ Health 5:50–58.

Knecht EA, Moorman WJ, Clark JC, Lynch DW, Lewis TR. 1985. Pulmonary effects of acute vanadium pentoxide inhalation in monkeys. Am Rev Respir Dis 132:1181–1185.

Knox RB, Suphioglu C, Taylor P, Desai R, Watson HC, Peng JL, Bursill LA. 1997. Major grass pollen allergen Lo1 p1 binds to diesel exhaust particles: Implications for asthma and air pollution. Clin Exp Allergy 27:246–251.

Kodavanti UP, Hauser R, Christiani DC, Meng ZH, McGee J, Ledbetter A, Richards J, Costa DL. 1998. Pulmonary responses of oil fly ash particles in the rat differ by virtue of their specific soluble metals. Toxicol Sci 43:204–212.

Laden F, Neas LM, Dockery DW, Schwartz J. 2000. Association of fine particulate matter from different sources with daily mortality in six US cities. Environ Health Perspect 108:941–947.

Lantzy RJ, MacKenzie FT. 1979. Atmospheric trace metals: Global cycles and assessment of man's impact. Geochim Cosmochim Acta 43:511–525.

Lavie L, Weinreb O, Gerson D. 1992. Age-related alterations in superoxide anion generation in mouse peritoneal macrophages studied by repeated stimulations and heat shock treatment. J Cell Physiol 152:382–388.

Levy BS, Hoffman L, Gottsegen S. 1984. Boilermaker's bronchitis. JOM 26:567–570.

Levy JI, Hammitt JK, Spengler JD. 2000. Estimating the mortality impacts of particulate matter: What can be learned from between-study variability? Environ Health Perspect 108:109–117.

Li XY, Brown D, Smith S, MacNee W, Donaldson K. 1999. Short-term inflammatory responses following intratracheal instillation of fine and ultrafine carbon black in rats. Inhalation Toxicol 11:709–731.

Lincoln JA, Lefkowitz DL, Cain T, Castro A, Mills KC, Lefkowitz SS, Moguilevsky N, Bollen A. 1995. Exogenous myeloperoxidase enhances bacterial phagocytosis and intracellular killing by macrophages. Infect Immunol 63:3042–3047.

Linder J, Rennard S. 1988. Bronchoalveolar Lavage. American Society of Clinical Pathologists Press, Chicago IL.

Lison D, Lardot C, Huaux F, Zanetti G, Fubini B. 1997. Influence of particle surface area on the toxicity of insoluble manganese dioxide dusts. Arch Toxicol 71:725–729.

Longphre M, Li D, Li J, Matovinovic E, Gallup M, Samet JM, Basbaum CB. 2000. Lung mucin production is stimulated by the air pollutant residual oil fly ash. Toxicol Appl Pharmacol 162:86–92.

Mamane Y, Pirrone N. 1998. Vanadium in the atmosphere. In: Vanadium in the Environment, Part 1: Chemistry and Biochemistry (Nriagu J, ed), pp 37–71. John Wiley & Sons, New York NY.

Mauderly JL, Gillett NA, Henderson RF, Jones RK, McClellan RO. 1988. Relationships of lung structure and functional changes to accumulation of diesel exhaust particles. Ann Occup Hyg 32(Suppl 1):659–669.

McDonald JD, Powell QH, Bowen LE, Mauderly JL. 2001. Organic carbon content of aerosols produced from a Palas carbon generation system [abstract]. Toxicologist 60:430.

Ménache MG, Miller FJ, Raabe OG. 1995. Particle inhalability curves for humans and small laboratory animals. Ann Occup Hyg 39:317–328.

Ménache MG, Raabe OG, Miller FJ. 1996. An empirical dosimetry model of aerodynamic particle deposition in the rat respiratory tract. Inhalation Toxicol 8:539–578.

Nasrallah VN Jr, Shirley PS, Myrvik Q, Waite M. 1983. The use of acetylated cytochrome c in detecting superoxide anion production in rabbit alveolar macrophages. J Immunol 131:2104–2106.

Nikula KJ, Green FHY. 2000. Animal models of chronic bronchitis and their relevance to studies in particle-induced disease. Inhalation Toxicol 12(Suppl 4):123–153.

Nikula KJ, Snipes MB, Barr EB, Griffith WC, Henderson RF, Mauderly JL. 1995. Comparative pulmonary toxicities and carcinogenicities of chronically inhaled diesel exhaust and carbon black in F344 rats. Fundam Appl Toxicol 25:80–94.

Oberdörster G. 2001. Pulmonary effects of inhaled ultrafine particles. Int Arch Occup Environ Health 74:1–8.

Oberdörster G, Finkelstein JN, Johnston C, Gelein R, Cox C, Baggs R, Elder ACP. 2000. Acute Pulmonary Effects of Ultrafine Particles in Rats and Mice. Research Report 96. Health Effects Institute, Cambridge, MA.

Oberdörster G, Gelein R, Johnson C, Mercer P, Corson N, Finkelstein JN. 1998. Ambient ultrafine particles: Inducers of acute lung injury? In: Relationships Between Respiratory Disease and Exposure to Air Pollution (Dungworth DL et al, eds), pp 216–229. ILSI Press, Washington DC.

O'Leary EC, Marder P, Zuckerman SH. 1996. Glucocorticoid effects in an endotoxin-induced rat pulmonary inflammation model: Differential effects on neutrophil influx, integrin expression and inflammatory mediators. J Respir Cell Mol Biol 15:97–106.

Osier M, Oberdörster G. 1997. Intratracheal inhalation vs. intratracheal instillation: Differences in particle effects. Fundam Appl Toxicol 40:220–227.

Osunsanya T, Prescott G, Seaton A. 2001. Acute respiratory effects of particles: Mass or number? Occup Environ Med 58:154–1549.

Pekkanen J, Timonen KL, Ruuskanen J, Reponen A, Mirme A. 1997. Effects of ultrafine and fine particles in urban air on peak expiratory flow among children with asthmatic symptoms. Environ Res 74:24–33.

Pérez R, López M, Barja de Quiroga G. 1991. Aging and lung antioxidant enzymes, glutathione, and lipid peroxidation in the rat. Free Radic Biol Med 10:35–39.

Pernis B, Vigliani EC, Cavanga C, Finuli M. 1961. The role of bacterial endotoxins in occupational diseases caused by inhaling vegetable dusts. Br J Ind Med 18:120–129.

Peters A, Wichmann HE, Tuch T, Heinrich J, Heyder J. 1997. Respiratory effects are associated with the number of ultrafine particles. Am J Respir Crit Care Med 155:1376–1383.

Peterson RD, Wicklung PE, Good RA. 1964. Endotoxin activity of house-dust extracts. J Allergy 35:134–142.

Pick E, Mizel D. 1981. Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic enzyme immunoassay reader. J Immunol Methods 46:211–226.

Plopper CG, Mariassy AT, Lollini LO. 1983. Structure as revealed by airway dissection: A comparison of mammalian lungs. Am Rev Respir Dis 128:S4–S7.

Pope CA. 1995. Combustion source particulate air pollution and human health: Causal associations or confounding? In: Proceedings of Particulate Matter: Health and Regulatory Issues, pp 60–77. Air and Waste Management Association, Pittsburgh PA.

Pope III CA. 2000. Epidemiology of fine particulate air pollution and human health: Biologic mechanisms and who's at risk? Environ Health Perspect 108(Suppl 4):713–723.

Pope III CA, Schwartz J, Ransom MR. 1992. Daily mortality and PM_{10} pollution in Utah valley. Arch Environ Health 47:211–217.

Prieditis H, Adamson IYR. 2002. Comparative pulmonary toxicity of various soluble metals found in urban particulate dusts. Exp Lung Res 28:563–576.

Pritchard RJ, Ghio AJ, Lehmann JR, Winsett DW, Tepper JS, Park P, Gilmour MI, Dreher KL, Costa DL. 1996. Oxidant generation and lung injury after particulate air pollutant exposure increase with the concentrations of associated metals. Inhalation Toxicol 8:457–477.

Raabe OG, Al-Bayati MA. 1997. Distribution and retention of inhaled vanadium on inert airborne particles. Ann Occup Hyg 41(Suppl 1):593–600.

Raabe OG, Al-Bayati MA, Teague SV, Rasolt A. 1988. Regional deposition of inhaled monodisperse coarse and fine aerosol particles in small laboratory animals. Ann Occup Hyg 32(Suppl 1):53–63.

Rice TM, Clarke RW, Godleski JJ, Al-Mutairi E, Jiang NF, Hauser R, Paulauskis JD. 2001. Differential ability of transitions metals to induce pulmonary inflammation. Toxicol Appl Pharmacol 177:46–53.

Rikans LE, Moore DR 1988. Effect of aging on aqueous-phase antioxidants in tissues of male Fischer rats. Biochim Biophys Acta 966:269–275.

Rylander R, Haglind P. 1984. Airborne endotoxins and humidifier disease. Clin Allergy 14:109–112.

Rylander R, Nordstrand A. 1974. Pulmonary cell reactions after exposure to cotton dust extract. Br J Ind Med 31:220–223.

Salvaggio J, Bucchner HA, Seabury JA. 1966. Precipitins against extracts of crude bagasse in the serum of patients. Ann Intern Med 61:748–758.

Samet JM, Dominici F, Curriero FC, Coursac I, Zeger SL. 2000a. Fine particulate air pollution and mortality in 20 US cities, 1987–1994. N Engl J Med 343:1742–1749.

Samet JM, Zeger SL, Dominici F, Curriero F, Coursac I, Dockery DW, Schwartz J, Zanobetti A. 2000b. The National Morbidity, Mortality, and Air Pollution Study, Part II: Morbidity and Mortality from Air Pollution in the United States. Research Report 94. Health Effects Institute, Cambridge MA.

Schwartz J. 1993. Air pollution and daily mortality in Birmingham, Alabama. Am J Epidemiol 137:1136–1147.

Schwartz J. 1994. Air pollution and daily mortality: A review and meta-analysis. Environ Res 64:36–52.

Schwartz J, Dockery DW. 1992. Increased mortality in Philadelphia associated with daily air pollution concentrations. Am Rev Respir Dis 145:600–604.

Schwartz J, Dockery DW, Neas LM. 1996. Is daily mortality associated specifically with fine particles? J Air Waste Manage Assoc 46:927–939.

Sprott RL. 1997. Diet and calorie restriction. Exp Gerontol 32:205–214.

Stahlhofen W, Rudolf G, James AC. 1989. Intercomparison of experimental regional aerosol deposition data. J Aerosol Med 2:285–308.

Stromberg PC. 1992. Changes in the hematologic system. In: Pathobiology of the Aging Rat, Vol 1 (Mohr U, Dungworth DL, Capen CC, eds), pp 15–24. ILSI Press, Washington DC.

Sugahara K, Iyama K, Sano K, Kuroki Y, Akino T, Matsumoto M. 1996. Overexpression of surfactant protein SP-A, SP-B, and SP-C mRNA in rat lungs with lipopolysaccharide-induced injury. Lab Invest 74:209–220.

Sunyer J, Castellsague J, Saez M, Tobias A, Anto JM. 1996. Air pollution and mortality in Barcelona. J Epidemiol Community Health 50(Suppl 1):S76–S80.

Sunyer J, Schwartz J, Tobias A, Macfarlane D, Garcia J, Anto JM. 2000. Patients with chronic obstructive pulmonary disease are at increased risk of death associated with urban particle air pollution: A case-crossover analysis. Am J Epidemiol 151:50–56.

Swift DL, Cheng YS, Su YF, Yeh HC. 1994. Ultrafine aerosol deposition in the human nasal and oral passages. Ann Occup Hyg 38(Suppl 1):77–81.

Tesfaigzi J, Wood MB, Johnson NF, Nikula KJ. 1998. Apoptosis is a major pathway responsible for the resolution of endotoxin-induced alveolar type II cell hyperplasia in the rat. Am J Respir Cell Mol Biol 79:303–311.

Tu KW, Kanapilly GM. 1979. Generation and characterization of condensation aerosols of vanadium pentoxide and pyrene. J Am Ind Hyg Assoc 40:763–769.

Utell MJ, Frampton MW. 2000. Acute health effects of ambient air pollution: The ultrafine particle hypothesis. J Aerosol Med 13:355–359.

van Helden HPM, Kuijpers WC, Steenvoorden D, Go C, Bruijnzeel PLB, van Eijk M, Haagsman HP. 1997. Intratracheal aerosolization of endotoxin (LPS) in the rat: A comprehensive animal model to study adult (acute) respiratory distress syndrome. Exp Lung Res 23:297–316.

van Vliet P, Knape M, de Hartog J, Janssen N, Harssema H, Brunekreef B. 1997. Motor vehicle exhaust and chronic respiratory symptoms in children living near freeways. Environ Res 74:122–132.

Vedal S. 1997. Ambient particles and health: Lines that divide. J Air Waste Manage Assoc 47:551–581.

Wichmann H-E, Spix C, Tuch T, Wölke G, Peters A, Heinrich J, Kreyling WG, Heyder J. 2000. Daily Mortality and Fine and Ultrafine Particles in Erfurt, Germany. Part I: Role of Particle Number and Particle Mass. Research Report 98. Health Effects Institute, Cambridge MA.

Wolff RK, Henderson RF, Snipes MB, Griffith WC, Mauderly JL, Cuddihy RG, McClellan RO. 1987. Alterations in particle accumulation and clearance in lungs of rats chronically exposed to diesel exhaust. Fundam Appl Toxicol 9:154–166.

Woodin MA, Youcheng L, Neuberg D, Hauser R, Smith TJ, Christiani DC. 2000. Acute respiratory symptoms in workers exposed to vanadium-rich fuel-oil ash. Am J Ind Med 37:353–363.

Xing A, Jordana M, Kirpalani H, Driscoll KE, Schall HJ, Gauldie J. 1994. Cytokine expression by neutrophils and macrophages in vivo: Endotoxin induces tumor necrosis factor-α, macrophage inflammatory protein-2, interleukin-1β, and interleukin-6 but not RANTES or transforming growth factor-β_1 mRNA expression in acute lung inflammation. Am J Respir Cell Mol Biol 10:148–153.

Yanagisawa R, Takano H, Inoue K, Ichinose T, Sadakane K, Yoshino S, Yamaki K, Kumagai Y, Uchiyama K, Yoshikawa T, Morita M. 2003. Enhancement of acute lung injury related to bacterial endotoxin by components of diesel exhaust particles. Thorax 58:605–612.

Yeh HC, Muggenburg BA, Harkema JR. 1997. In vivo deposition of inhaled ultrafine particles in the respiratory tract of rhesus monkeys. Aerosol Sci Technol 27:465–470.

Yu CP. 1996. Extrapolation modeling of particle deposition and retention from rats to humans. In: Particle Overload in the Rat Lung and Lung Cancer: Implications for Human Risk Assessment (Mauderly JL, McCunney RJ, eds). Taylor & Francis, Washington DC.

Zanobetti A, Schwartz J. 2001. Are diabetics more susceptible to the health effects of airborne particles? Am J Respir Crit Care Med 164:831–833.

Zanobetti A, Schwartz J, Dockery DW. 2000a. Airborne particles are a risk factor for hospital admissions for heart and lung disease. Environ Health Perspect 108:1071–1077.

Zanobetti A, Schwartz J, Gold D. 2000b. Are there sensitive subgroups for the effects of airborne particles? Environ Health Perspect 108:841–845.

Zenz, C, ed. 1994. Occupational Medicine (3rd edition), pp 584–594. Mosby, St Louis MO.

Zhou YM, Zhong CY, Kennedy IM, Leppert VJ, Pinkerton KE. 2003. Oxidative stress and NFκB activation in the lungs of rats: A synergistic interaction between soot and iron particles. Toxicol Appl Pharmacol 190:157–169.

APPENDIX A. Pilot Experiments: Endotoxin Dose and Exposure and Dose Safety

Table A.1. Verification of Endotoxin Dose in Aged Rats: BAL Data

Dose[a] / Variable	1 Day After Instillation			3 Days After Instillation		
	Mean	SE	*N*	Mean	SE	*N*
Endotoxin (0.5 mg)						
Number of cells ($\times 10^6$)	127.0	1.47	3	51.4	25.8	3
Number of cells per mL ($\times 10^6$)	13.4	0.41	3	6.87	2.21	3
% Neutrophils	75.7	1.89	3	37.2	8.63	3
% Alveolar macrophages	24.3	1.89	3	62.8	8.63	3
Endotoxin (0.3 mg)						
Number of cells ($\times 10^6$)	140.0	4.57	3	30.2	5.33	3
Number of cells per mL ($\times 10^6$)	14.0	0.41	3	3.2	0.43	3
% Neutrophils	69	6.88	3	16.1	0.66	3
% Alveolar macrophages	31	6.88	3	83.9	0.66	3
Saline Only						
Number of cells ($\times 10^6$)	19.0	NA[b]	1	10.3	4.25	2
Number of cells per mL ($\times 10^6$)	2.06	NA	1	1.13	0.49	2
% Neutrophils	27.7	NA	1	3.33	1.34	2
% Alveolar macrophages	72.3	NA	1	96.7	1.34	2

[a] All endotoxin doses are per kilogram body weight and were given in 0.5 mL saline. *Saline only* was 0.5 mL saline with no endotoxin.

[b] NA = not applicable.

Table A.2. Safety of Test Exposures in Aged Rats: BAL Data

Dose[a] / Variable	1 Day After Instillation[b,c]			3 Days After Instillation[b]		
	Mean	SE	*N*	Mean	SE	*N*
Endotoxin (0.5 mg)						
Number of cells ($\times 10^6$)	117	3.62	4	29.1	4.24	3
Number of cells per mL ($\times 10^6$)	12.9	0.51	4	3.23	0.38	3
% Neutrophils	87.8	3.64	4	44.2	2.31	3
% Lymphocytes	0	0	4	2.67	NA	3
% Alveolar macrophages	12.3	3.64	4	53.1	1.44	3
Endotoxin (0.2 mg)						
Number of cells ($\times 10^6$)	114	1.15	3	27.9	0.94	3
Number of cells per mL ($\times 10^6$)	10.8	0.54	3	2.76	0.08	3
% Neutrophils	83.1	1.61	3	14	1.53	3
% Lymphocytes	0.2	0.2	3	11.8	3.33	3
% Alveolar macrophages	16.7	1.67	3	74.2	2.15	3
Endotoxin (0.1 mg)						
Number of cells ($\times 10^6$)	88.6	4.58	3	25.6	2.84	3
Number of cells per mL ($\times 10^6$)	8.11	0.59	3	2.39	0.23	3
% Neutrophils	72.6	0.8	3	12.7	2.19	3
% Lymphocytes	0	0	3	8.3	3.2	3
% Alveolar macrophages	27.4	0.8	3	79	2.52	3
Ultrafine V_2O_5 (100 µg/m^3) + Endotoxin (0.5 mg)						
Number of cells ($\times 10^6$)	117	NA	1	27.6	3.52	3
Number of cells per mL ($\times 10^6$)	9.98	NA	1	3.03	0.28	3
% Neutrophils	79	NA	1	43.9	3.8	3
% Lymphocytes	0	NA	1	0	NA	3
% Alveolar macrophages	21	NA	1	56.1	3.8	3
Saline Only						
Number of cells ($\times 10^6$)	ND	ND	ND	7.63	1.31	3
Number of cells per mL ($\times 10^6$)	ND	ND	ND	0.76	0.11	3
% Neutrophils	ND	ND	ND	1.67	0.33	3
% Lymphocytes	ND	ND	ND	1.00	2.67	3
% Alveolar macrophages	ND	ND	ND	97.33	0.88	3

[a] All endotoxin doses are per kilogram body weight and were given in 0.5 mL saline. *Saline only* was 0.5 mL saline with no endotoxin.

[b] NA = not applicable.

[c] ND = not done.

APPENDIX B. Calculation of Effective Ultrafine Aerosol Densities

The actual densities of ultrafine V_2O_5 and carbon black aerosols could not be directly measured. Particle densities were estimated on the basis of mean particle number concentration, filter mass concentrations, and diameter of mean particle mass calculated by using the Hatch-Choate equation (eq 4.53 in Hinds 1999). The effective particle density, ρ_e, is:

$$\rho_e = \frac{[\text{Mean particle mass, g}]}{[\text{Mean particle volume, cm}^3]},$$

or

$$\rho_e = \frac{[6 \times \text{Mean particle mass, g}]}{[\pi \times (\text{diameter of } \textbf{\textit{mean}} \text{ volume, cm})^3]}.$$

ULTRAFINE V_2O_5 DENSITY

Mean particle mass was calculated from selected filter data and SMPS data for nominal 100 µg/m^3 exposure groups. The mean filter mass concentration over 5 days (8/16/99–8/20/99) was 103 µg/m^3 and the mean particle number concentration for the same period was 3.05×10^6 particles/cm^3. The calculated mean particle mass was:

$$\text{Mean particle mass, g} = \frac{[1.03 \times 10^{-4}\ \text{g/m}^3 \times \text{m}^3/10^6\ \text{cm}^3]}{[3.05 \times 10^6\ \text{particles/cm}^3]} = 3.38 \times 10^{-17}\ \text{g/particle.}$$

The mean CMD for the same 5 days was 23.8 nm with σ_g = 1.37. The diameter of ***mean*** volume, d_m, was estimated by using the Hatch-Choate equation:

d_m = CMD exp(1.5 × ln^2 σ_g) = 23.8 nm × 1.16 = 27.6 nm.

The effective particle density was therefore:

$\rho_e = 6 \times (3.38 \times 10^{-17}\ \text{g/particle}) / [\pi \times (27.6 \times 10^{-7}\ \text{cm})^3]$
= **3.07 g/cm^3.**

ULTRAFINE CARBON BLACK DENSITY

The mean particle mass was calculated from selected filter data and SMPS data for nominal 200 µg carbon black/m^3 exposure groups. The mean filter mass concentration over 5 days (3/28/00–4/1/00) was 195 µg/m^3 and the mean particle number concentration for the same period was 2.75×10^6 particles/cm^3. The calculated mean particle mass was:

$$\text{Mean particle mass, g} = \frac{[1.95 \times 10^{-4}\ \text{g/m}^3 \times \text{m}^3/10^6\ \text{cm}^3]}{[2.75 \times 10^6\ \text{particles/cm}^3]} = 7.09 \times 10^{-17}\ \text{g/particle.}$$

The mean CMD for the same 5 days was 36.9 nm with σ_g = 1.48. The diameter of the ***mean*** volume, d_m, was estimated by using the Hatch-Choate equation:

d_m = CMD exp(1.5 × ln^2 σ_g) = 36.9 nm × 1.26 = 46.5 nm.

The effective particle density was therefore:

$\rho_e = 6 \times (7.09 \times 10^{-17}\ \text{g/particle}) / [\pi \times (46.5 \times 10^{-7}\ \text{cm})^3]$
= **2.70 g/cm^3**.

APPENDIX C. Modeling to Predict Lung Burdens

To establish lung burden, empirical equations for deposition and for clearance and retention were fit to the data for each rat. The development of each component of the lung burden model is described here.

DEPOSITION MODEL

The empirical model to predict particle deposition in animals (Ménache et al 1996) was developed on the basis of experimental measurements of radiolabeled particle deposition in five species of commonly used laboratory animals (Raabe et al 1988). Animals were exposed to radiolabeled particles ranging in aerodynamic size from approximately 1 to 10 µm for about 5 minutes. One particle in the submicron range (with a geometric diameter of approximately 0.08 µm) was also used in the study. Half of the animals were killed immediately and half were killed 24 hours after exposure. The animals were carefully dissected into 17 compartments (including collected urine and feces) for a complete mass balance of radioactivity. The respiratory tract was dissected into the five lobes, the trachea, the larynx, and the upper airways. For the animals killed immediately after exposure, the gastrointestinal tract was assumed to contain particles that had deposited in the upper airways and were swallowed.

The empirical deposition model was then developed in the following stages:

1. Fractional deposition measurements for individual animals were converted to efficiencies for each of three regions: extrathoracic, tracheobronchial (or conducting airway), and alveolar. Efficiency increases from 0 to 1 with aerodynamic particle size and is independent of deposition in other regions. In contrast, deposition fraction is affected by deposition in proximal regions and

does not increase monotonically. For example, deposition in the alveolar region depends on how many particles are not deposited in the extrathoracic and tracheobronchial regions as well as on the efficiency deposition in the alveolar region. As a result, deposition in the alveolar region first increases as a function of particle size but then decreases as fewer particles reach the region because they have already deposited in the extrathoracic and tracheobronchial regions.

2. An equation for inhalability was developed to account for the difference between the concentration of particles in the air and the concentration of particles that the animal can inhale (Ménache et al 1995).
3. Logistic equations (bounded by 0 and 1) were fit to the efficiencies for particles within the range of aerodynamic particle size for each of the three regions (Ménache et al 1996).
4. Although the data of Raabe and associates (1988) had only one submicron particle in the thermodynamic range, nonlinear equations were fit following the parameterization of Stahlhofen and colleagues (1989).
5. A total of three equations were fit simultaneously for each region: one for the aerodynamic particle size range (ae), one for the thermodynamic particle size range (th), and one for a weighted estimate of efficiency for both aerodynamic and thermodynamic particles.

The completed empirical deposition model consisted of:

1. one equation for inhalability efficiency (eff_I) (Ménache et al 1995):

$$\text{eff}_\text{I} = \frac{1}{1 + e^{2.57 + 2.82 \ln(d_{ae})}}$$

where d_{ae} is the particle aerodynamic diameter,

and

2. three equations for efficiencies of the form:

$$\text{eff} = \sqrt{\text{eff}_{ae}^{\,2} + \text{eff}_{th}^{\,2}},$$

where

3. the general form for efficiencies in the aerodynamic particle size range (eff_{ae}) is:

$$\text{eff}_{ae} = \frac{1}{1 + e^{\alpha + \beta \ln(x)}}$$

where x is the impaction parameter (in the extrathoracic region) or the aerodynamic diameter (in the tracheobronchial and alveolar regions) and α and β differ for each of the three regions, and

4. the general form for efficiencies in the thermodynamic particle size range (eff_{th}) is:

$$\text{eff}_{th} = \gamma \times (\text{CD}/q) \qquad \text{(extrathoracic region)}$$

or

$$\text{eff}_{th} = 1 - e^{-\gamma(\text{CD}/q)} \qquad \text{(tracheobronchial and alveolar regions),}$$

where CD is the coefficient of diffusion for the particle, q is the flow rate, and γ differs for each of the three regions. Although the equations of Stahlhofen and colleagues (1989) used residence time rather than flow rate, we had inadequate information to estimate residence time for the animals.

Using the model to calculate the efficiencies requires knowledge of particle mean diameter (MMAD) and its variability (σ_g), particle density, and animal body weight (which is used to estimate the minute volume). If the particle is polydisperse ($\sigma_g > 1$), then the efficiencies are calculated for each particle size and weighted by the fraction in the lognormal distribution.

Fractional deposition (dep) was estimated from the efficiency equations as follows for extrathoracic (et), tracheobronchial (tb), and alveolar (al) regions:

1. $\text{dep}_{et} = \text{eff}_{et}$

2. $\text{dep}_{tb} = (1 - \text{eff}_{et}) \times \text{eff}_{tb}$

3. $\text{dep}_{al} = (1 - \text{eff}_{et}) \times (1 - \text{eff}_{tb}) \times \text{eff}_{al}$.

To adjust for inhalability, the deposition fraction was multiplied by the inhalability equation. The inhalability adjustment provides a more accurate estimate of particle deposition. Not all particles in the air can be inhaled, so calculating deposition fractions on the basis of ambient concentrations may overestimate lung burdens. If a regulatory decision considers the predicted deposition (or burden), the decision will err on the side of presuming that the health consequence is associated with a higher level of deposition (or burden) than is actually occurring. This presumption can have serious consequences if the regulatory decision makes use of information extrapolating from animals to humans, because commonly used laboratory animals are much less able than humans to inhale particles in the size range of regulatory importance. These issues are discussed in more detail elsewhere (Ménache et al 1995).

The particle size parameters we used for the deposition calculations are given in Table 6. The density used for carbon black was 2.70 g/cm^3; for V_2O_5, 3.07 g/cm^3 (Appendix B). The mean body weights, minute volumes, and predicted fractional depositions are shown in Table C.1.

Table C.1. Mean Body Weights, Minute Volumes, and Predicted Deposition Fractions

Particle Size and Type	Body Weight (g)[a]	Minute Volume (mL/min)[b]	Deposition Fraction[c]		Inhalability Fraction[d]
			Alveolar	Thoracic	
Aged Rats					
Ultrafine carbon black	381 ± 3	254 ± 2	0.149 ± 0.000	0.356 ± 0.001	>0.99
Ultrafine V_2O_5	392 ± 2	260 ± 1	0.118 ± 0.004	0.296 ± 0.008	>0.99
Fine V_2O_5	391 ± 2	260 ± 1	0.043 ± 0.000	0.069 ± 0.000	0.95
Young Adult Rats					
Fine V_2O_5	229 ± 3	167 ± 2	0.062 ± 0.001	0.098 ± 0.001	0.95

[a] Body weights from the beginning and end of the exposure, averaged for each animal.

[b] Minute volume was estimated from species-specific allometric scaling equations published by the EPA (1988).

[c] All deposition fractions were calculated for each animal using that animal's minute volume. Deposition fractions were calculated separately for the initial 15-day exposure concentrations and for the final, spiked 2-day exposure concentrations in the exposures with spikes. We observed slight differences in MMAD or CMD and σ_g between the 15-day and 2-day exposures. These resulted in small differences in estimated fractional deposition but are not reported here. This table shows only the deposition fractions for the 15-day exposure concentrations for exposures with spikes or the full 17-day exposure concentrations for exposures without spikes.

[d] Inhalability fractions are given for the monodisperse particle with the appropriate diameter but with a σ_g of 1. Because inhalability is a function of particle size, if the lognormal distribution of the polydisperse particle includes many large particles, then the inhalability factor may range from 1 (complete inhalability) to nearly 0. This value will be weighted by the proportion of particles in the size range. Because this calculation is embedded in the deposition calculations on a particle size basis, the actual values are not shown. The fractions in the table may be interpreted as follows: half the particles in the distribution being used have inhalability greater and half have inhalability less than the fraction.

V_2O_5 RETENTION

Raabe and Al-Bayati (1997) reported data for a study of the distribution and retention of ^{48}V-radiolabeled V_2O_5 in male Sprague-Dawley rats aged 1 and 3 months. Percentages of initial lung burden were reported 1 hour, 1 day, 3 days, 1 week, 2 weeks, and 3 weeks after exposure. A two-compartment nonlinear model was fit to these data with the following result:

$$V_2O_5 \text{ retention, fraction} = 0.6971e^{(-14.8673 \times \text{time, days})} + 0.3029e^{(-0.0994 \times \text{time,days})}.$$

CARBON BLACK RETENTION

Yu (1996) examined particle disposition for diesel exhaust and other insoluble particles with a carbon core (slowly and quickly cleared organics). On the basis of earlier modeling work, he reported an alveolar clearance rate of 0.0128 per day for these particles. Then:

$$\text{Carbon black retention, fraction} = (1 - 0.0128)^{\text{time, days}} = 0.9872^{\text{time, days}}.$$

PREDICTED LUNG BURDEN

Calculations

Daily deposition fraction was calculated using the following equation:

$$\text{Daily deposition, } \mu g/g \text{ lung} = \text{dep}_i \times 10^{-6} \times \text{actual concentration, } \mu g/m^3 \times \text{minute volume, mL/min} \times 60 \times 5 \text{ hr/lung weight, g,}$$

where dep_i is fractional deposition for the *i*th region (thoracic [the sum of tracheobronchial and alveolar]) in each animal; actual concentration is the exposure concentration reported in Table 6; minute volume is that calculated for each animal on the basis of body weight; and 10^{-6}, 60, and 5 are unit adjustments. Each animal was presumed to receive this amount of deposited mass each day of the exposure.

Concomitantly, clearance was occurring. Cumulative retention was estimated by summing the daily fractional retentions and multiplying that number by the daily deposition mass. Because the spike exposure involved different exposure concentrations as well as possibly different deposition fractions, cumulative retention was calculated for exposure days 1 through 15 and for exposure days 16 and

17 separately. Because of the limited information available to estimate clearance, all animals were assumed to have the same clearance rates, regardless of age or weight.

Calculation Example

Thoracic (tracheobronchial and alveolar) lung burden per gram of lung tissue was calculated for rat J137 as follows. This rat was exposed to fine V_2O_5 at a concentration of 150 μg/m^3 for 15 days with a spike to 150+50 (200) μg/m^3 for 2 days. Table C.1 shows that the actual mean concentrations were 153 and 181 μg/m^3, respectively. The MMAD and σ_g were the same for both exposures. The cumulative fractional retention was 1.84055 μg for the first 15 days of exposure and 0.52257 μg for the last 2 days. This rat had an estimated minute volume of 241.3 mL/min and a lung weight of 1.938 g. Because the MMAD and σ_g were the same for the baseline and spike exposures, a single predicted thoracic deposition fraction was calculated as 0.07353.

Thoracic burden was then calculated as the predicted deposition fraction, adjusted for the inhaled concentration, weighted by the fractional retention, and normalized by the lung weight:

$$\begin{aligned}\text{Thoracic burden, μg/g lung} &= [(0.07353 \times 10^{-6} \times 153 \\ &\quad \times 241.3 \times 60 \times 5) \times 1.84055 \\ &\quad + (0.07353 \times 10^{-6} \times 181 \\ &\quad \times 241.3 \times 60 \times 5) \\ &\quad \times 0.52257]/1.938 \\ &= 1.03 \text{ μg/g.}\end{aligned}$$

The question of the number of significant digits often arises. Using many digits in calculations but dropping them in the final result, as was done here, is appropriate.

CORRELATION OF PREDICTED AND MEASURED LUNG BURDEN

The relation between thoracic burden (calculated using the method described above) and the measured lung burdens is shown in Figure C.1. The illustrated difference between the predicted lung burdens and the measured lung burdens for fine and ultrafine particle exposures was significant (ANCOVA, $P < 0.01$). In both cases, predicted lung burden exceeded measured lung burden. As shown in Figure C.1, the predicted lung burden for ultrafine particles exceeded the observed lung burden by a factor of 5, whereas the predicted lung burden for fine particles exceeded the observed lung burden by a factor of 2.25.

The objective of these calculations was to develop a metric to accurately estimate effective dose. The exposures

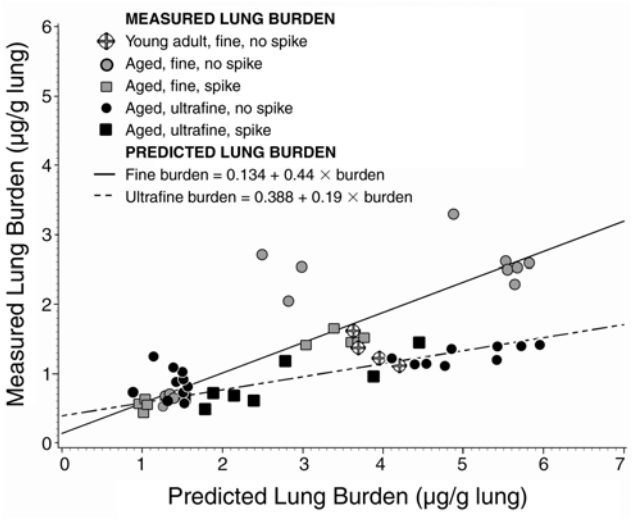

Figure C.1. Predicted lung burden compared to measured lung burden for V_2O_5.

were complex, involving similar concentrations of particles of different sizes, which would result in different delivered doses. Our use of spikes introduced an additional complexity.

Quantification via dosimetry modeling can improve interpretation of results. For example, if a biological response differs between exposures to similar concentrations of different-sized particles, then without knowing the expected lung burden or delivered dose, one cannot determine whether the difference in response is due solely to the difference in delivered dose or (more importantly) to the particle size. Similarly, a spike would be expected to affect a biological response relative to an exposure with the same baseline concentration but no spike. Without knowing the expected increase in delivered dose, however, it is impossible to assess whether increases in biological response are simply the expected result of the increased delivered dose or a response to the nature of the increase.

Individual measured lung burdens are the preferred metric relative to predicted lung burdens, but we could not measure lung burdens in all rats. The dosimetry model reasonably estimates lung burden, although its predictions differ from the measurements by a linear factor. Therefore, for the statistical analyses described in this report, predicted lung burdens for all rats were adjusted as follows:

Fine particles: adjusted burden = 0.45 × predicted burden.

Ultrafine particles: adjusted burden = 0.40 + 0.2 × predicted burden.

APPENDIX D. Measurements of Oxidative Stress

For methods, see Materials and Methods / BAL Fluid Analysis. The results are as follows.

SUPEROXIDE ANION PRODUCTION

Superoxide dismutase in many cases failed to block reduction of cytochrome C, for reasons that are unclear. The results are therefore described in terms of changes in the uninhibited reaction rate. In the first three exposures of rats to ultrafine V_2O_5 particles (150, 150+50, and 200 $\mu g/m^3$), phorbal myristate acetate did not stimulate superoxide anion production by the cells (data not shown). In subsequent exposures, opsonized zymosan was used to stimulate the cells.

In unstimulated cells, superoxide anion production did not differ significantly between any particle exposure and exposure to filtered air (control rats; Figure D.1A). Stimulation of the cells caused a significant increase in superoxide anion production (Figure D.1B) compared with no stimulation. Compared with control rats exposed to filtered air, superoxide anion production by stimulated cells was significantly *less* ($P < 0.05$) in cells from rats exposed to 150+50 $\mu g/m^3$ carbon black and 500+500 $\mu g/m^3$ fine V_2O_5.

H_2O_2 PRODUCTION BY ALVEOLAR MACROPHAGES

Measured H_2O_2 production by unstimulated lavaged cells was lower in rats exposed to any particle concentrations than in rats exposed to filtered air (Figure D.2A). The one apparent exception, greater production in cells from rats exposed to 1000 $\mu g/m^3$ of fine V_2O_5, was not significant ($P > 0.08$). For lavaged cells stimulated by opsonized zymosan, only in rats exposed to 1000 $\mu g/m^3$ of fine V_2O_5 was measured H_2O_2 production significantly greater than in the control rats exposed to filtered air (Figure D.2B; $P < 0.05$). In two ultrafine V_2O_5 exposure groups (50+50 $\mu g/m^3$ spike and 200 $\mu g/m^3$), H_2O_2 production by stimulated BAL cells was significantly *less* than that for control rats exposed to filtered air.

OXIDATION OF PROTEINS IN BAL FLUID

Protein carbonyl levels in the BAL samples were about 40% to 120% of the bovine serum albumin standard

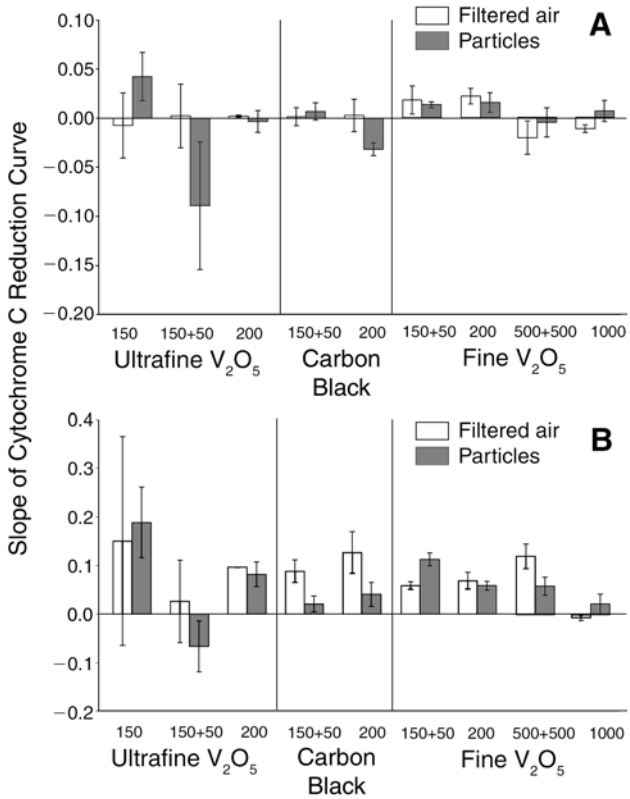

Figure D.1. Adjusted levels (mean ± SEM) of superoxide anion production by adherent BAL cells (*A*) unstimulated or (*B*) stimulated by opsonized zymosan. All exposure concentrations are micrograms per cubic meter. Note the different y-axis scales between the panels.

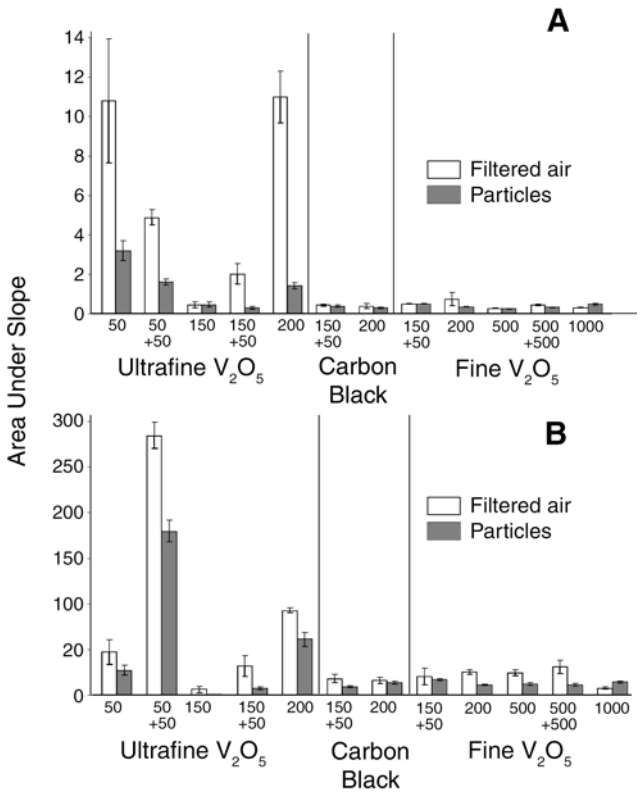

Figure D.2. H_2O_2 production levels (mean ± SEM) in adherent BAL cells (*A*) unstimulated or **(*B*)** stimulated by opsonized zymosan. All exposure concentrations are micrograms per cubic meter. Note the different y-axis scales between the panels.

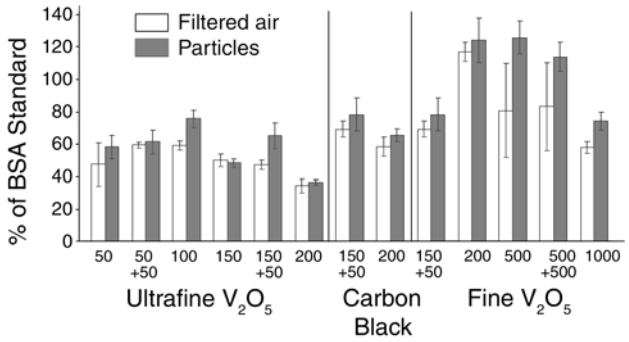

Figure D.3. Protein carbonyl levels (mean ± SEM) in BAL fluid. All exposure concentrations are micrograms per cubic meter. BSA, bovine serum albumin.

(Figure D.3). Only two particle exposures resulted in a significant increase ($P < 0.05$) in protein carbonyl levels compared with exposure to filtered air: 100 μg/m^3 of ultrafine V_2O_5 and 1000 μg/m^3 of fine V_2O_5. None of the particle exposures resulted in significantly lower protein carbonyl levels in the BAL fluids compared with the corresponding filtered air exposure.

DISCUSSION

Few groups of exposed rats showed indications of oxidative stress in lavaged cells. Significant increases in protein carbonyls were noted in rats exposed to 100 μg/m^3 of ultrafine V_2O_5 and 1000 μg/m^3 of fine V_2O_5; significant increases in H_2O_2 production were found in rats exposed to 1000 μg/m^3 of fine V_2O_5, the only group to show evidence of increased oxidative stress by more than one measure.

Short-term inhalation exposure of rats to increased concentrations of particulate air pollution has been shown to promote oxidative stress and mild damage to the lungs and heart. In a previous study, rats exposed to concentrated ambient particles (CAPs) at a concentration of 300 ± 60 μg/m^3 for 5 hours showed significant oxidative stress as measured by an in situ chemiluminescence technique (Gurgueira et al 2002). Increases in oxidant levels were also triggered by inhaled residual oil fly ash (1.7 mg/m^3 for 30 minutes) but not by carbon black (170 μg/m^3 for 5 hours) or filtered air. Increased chemiluminescence of the lung was strongly associated with the iron, manganese, copper, and zinc content of the aerosols but not vanadium content or total particle mass.

Gurgueira and colleagues' study (2002) also showed that increases in oxidative stress may be relatively short lived after exposure. Twenty-four hours after the end of the CAPs exposure, chemiluminescence of the lung had returned to the level of controls exposed to room air. In addition, rats exposed to filtered air for 3 days showed reduced chemiluminescence of the lung that returned to normal after 5 hours, but not 3 hours, of room air exposure.

These findings may explain why we consistently detected oxidative stress only in the rats exposed to 1000 μg/m^3 of fine V_2O_5. Vanadium compounds were not associated with chemiluminescence induced by inhaled CAPs; they may induce only low levels of oxidative stress. Carbon black aerosols did not induce any oxidative stress after CAPs exposure. The relatively short persistence of chemiluminescence in the lung after exposure to CAPs suggests that the superoxide anion, H_2O_2, and protein carbonyl concentrations measured in the current study were below detection levels after either the 17-day exposure period or the 18 hours from the end of the exposure period to the time of analysis. The one exception may be the group exposed to 1000 μg/m^3 of fine V_2O_5, the highest concentration we used and the exposure that resulted in the most indications of toxicity.

In nearly all exposed groups, oxidative stress (measured by superoxide anion or H_2O_2 production) was less than that in control groups, significantly so in several instances. Many phenomena may be responsible for this reduction. The endotoxin instillation 3 days before the rats were killed (and tested for indicators of oxidative stress) may have reduced the capacity of the alveolar macrophages to respond. In vitro studies of rat peritoneal macrophages have shown marked diminution of macrophage capacity to secrete high levels of respiratory burst products after being cultured with lipopolysaccharide (Johnson and Sung 1987). This reduction did not depend on the type of stimulating agent. The reduction of oxidative stress measurements may relate to the diminished capacity of macrophages to recover after repeated stimulation and also to the effect of age on this response. Studies of mouse peritoneal macrophages have shown diminished ability of these cells to generate superoxide anions after repeated stimulation by opsonized zymosan or phorbal myristate acetate (Lavie et al 1992). In addition, a considerably diminished capacity to generate superoxide anions was noted in macrophages from senescent mice.

Table E.1. Time Course for Pulmonary Reactions

Dose[a] / Procedure / Mechanism	Variable	Units	5 Hours After Instillation			1 Day After Instillation			3 Days After Instillation		
			Mean	SD	N^b	Mean	SD	N^b	Mean	SD	N^b
Instilled Endotoxin (0.1 mg)											
BAL											
Cytotoxicity	LDH	IU/L	49.7	10.1	6	175	83.7	5	75.6	16.9	7
Inflammation	Cells	number ($\times 10^6$)	150	45.3	6	146	33.1	5	49.8	13.9	7
Inflammation	Cells	per mL ($\times 10^6$)	14.5	5.06	6	18.6	3.48	5	4.50	1.27	7
Inflammation	Neutrophils	per mL ($\times 10^6$)	13.4	4.04	6	13.4	3.69	5	0.35	0.17	7
Inflammation	Lymphocytes	per mL ($\times 10^6$)	0.203	0.11	6	0.203	0.10	5	0.20	0.23	7
Inflammation	Protein	mg/dL	28.7	20.2	6	21.4	3.0	5	6.3	1.4	7
Inflammation	TNF-α	pg/mL	271	12.7	2	56.5	19.7	3	12.0	2.62	2
Macrophage activation	Alveolar macrophages	per mL ($\times 10^6$)	3.15	4.42	6	5.03	0.83	5	3.95	1.18	7
Macrophage activation	β-Glucuronidase	IU/L	0.142	0.146	6	0.558	0.296	5	0.113	0.057	7
Macrophage activation	MIP-2	pg/mL	600	8.49	2	396	20.6	3	251	30.4	2
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	0.048	0.014	4	0.798	1.464	4	1.670	0.321	3
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.059	0.025	4	0.419	0.690	4	1.448	0.735	3
Histology											
Inflammation	Edema	histologic grade	1.5	0.70	6	0.0	0.0	6	0.0	0.0	7
Inflammation	Alveolar	histologic grade	1.5	0.50	6	2.0	0.58	6	1.3	0.45	7
Inflammation	Alveolar macrophage hyperplasia	histologic grade	1.5	0.50	6	2.0	0.58	6	1.1	0.64	7
Inflammation	Interstitial	histologic grade	0.2	0.37	6	1.7	1.37	6	1.3	1.03	7
Inflammation	Total	histologic grade	4.3	1.25	6	5.7	1.97	6	3.7	1.75	7
Instilled Saline Only											
BAL											
Cytotoxicity	LDH	IU/L	25.3	10.1	8	43.9	29.3	8	22.3	8.80	8
Inflammation	Cells	number ($\times 10^6$)	19.3	6.93	8	18.1	3.86	8	14.8	2.05	8
Inflammation	Cells	per mL ($\times 10^6$)	2.07	0.593	8	1.99	0.504	8	1.38	0.160	8
Inflammation	Neutrophils	per mL ($\times 10^6$)	0.49	0.519	8	0.181	0.138	8	0.011	0.006	8
Inflammation	Lymphocytes	per mL ($\times 10^6$)	0.027	0.031	8	0.027	0.020	8	0.026	0.026	8
Inflammation	Protein	mg/dL	6.0	1.19	8	5.6	1.99	8	4.4	0.92	8
Inflammation	TNF-α	pg/mL	20.7	1.06	2	21.6	2.4	2	25.7	12.4	2
Macrophage activation	Alveolar macrophages	per mL ($\times 10^6$)	1.55	0.442	8	1.78	0.417	8	1.35	0.158	8
Macrophage activation	β-Glucuronidase	IU/L	0.005	0.049	8	0.056	0.083	8	0.028	0.060	8
Macrophage activation	MIP-2	pg/mL	520	77.8	2	320	37.5	2	276	14.8	2

Table continues on next page

[a] All endotoxin doses are per kilogram body weight and were given in 0.5 mL saline. *Saline only* was 0.5 mL saline with no endotoxin. ND = not done.

[b] Sample sizes vary because samples could not be obtained from all rats.

Table E.1 (*continued*). Time Course for Pulmonary Reactions

Dose[a] / Procedure / Mechanism	Variable	Units	5 Hours After Instillation			1 Day After Instillation			3 Days After Instillation		
			Mean	SD	N^b	Mean	SD	N^b	Mean	SD	N^b
Instilled Saline Only (*continued*)											
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	0.065	0.043	4	0.113	0.102	4	0.084	0.046	4
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.105	0.046	4	0.165	0.069	4	0.167	0.059	4
Histology											
Inflammation	Edema	histologic grade	0.13	0.33	8	0.0	0.0	8	0.0	0.0	8
Inflammation	Alveolar	histologic grade	0.3	0.43	8	0.0	0.0	8	0.0	0.0	8
Inflammation	Alveolar macrophage hyperplasia	histologic grade	0.8	0.43	8	0.5	0.5	8	0.5	0.5	8
Inflammation	Interstitial	histologic grade	0.0	0.0	8	0.0	0.0	8	0.3	0.43	8
Inflammation	Total	histologic grade	1.1	0.93	8	0.5	0.5	8	0.8	0.66	8
Sham Instillation											
BAL											
Cytotoxicity	LDH	IU/L	28.4	9.9	8	26.1	8.43	8	21.5	6.80	8
Inflammation	Cells	number ($\times 10^6$)	13.5	3.00	8	22.2	4.45	8	15.3	3.00	8
Inflammation	Cells	per mL ($\times 10^6$)	1.21	0.077	8	2.00	0.488	8	1.47	0.23	8
Inflammation	Neutrophils	per mL ($\times 10^6$)	0.062	0.032	8	0.031	0.026	8	0.015	0.014	8
Inflammation	Lymphocytes	per mL ($\times 10^6$)	0.033	0.002	8	0.024	0.027	8	0.020	0.016	8
Inflammation	Protein	mg/dL	8.9	1.9	8	5.0	1.77	8	4.1	1.36	8
Inflammation	TNF-α	pg/mL	20.7	1.06	2	21.6	2.40	2	25.7	12.4	2
Macrophage activation	Alveolar macrophages	per mL ($\times 10^6$)	1.12	0.210	8	1.94	0.47	8	1.44	0.22	8
Macrophage activation	β-Glucuronidase	IU/L	0.075	0.047	8	0.085	0.041	8	0.014	0.035	8
Macrophage activation	MIP-2	pg/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	0.071	0.017	3	0.063	0.056	3	0.042	0.007	4
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.093	0.006	3	0.099	0.022	3	0.085	0.047	4
Histology											
Inflammation	Edema	histologic grade	0.0	0.0	7	0.0	0.0	8	0.0	0.0	8
Inflammation	Alveolar	histologic grade	0.0	0.0	7	0.0	0.0	8	0.0	0.0	8
Inflammation	Alveolar macrophage hyperplasia	histologic grade	0.3	0.45	7	0.3	0.43	8	0.3	0.43	8
Inflammation	Interstitial	histologic grade	0.0	0.0	7	0.1	0.33	8	0.0	0.0	8
Inflammation	Total	histologic grade	0.3	0.45	7	0.4	0.70	8	0.3	0.43	8

[a] All endotoxin doses are per kilogram body weight and were given in 0.5 mL saline. *Saline only* was 0.5 mL saline with no endotoxin. ND = not done.

[b] Sample sizes vary because samples could not be obtained from all rats.

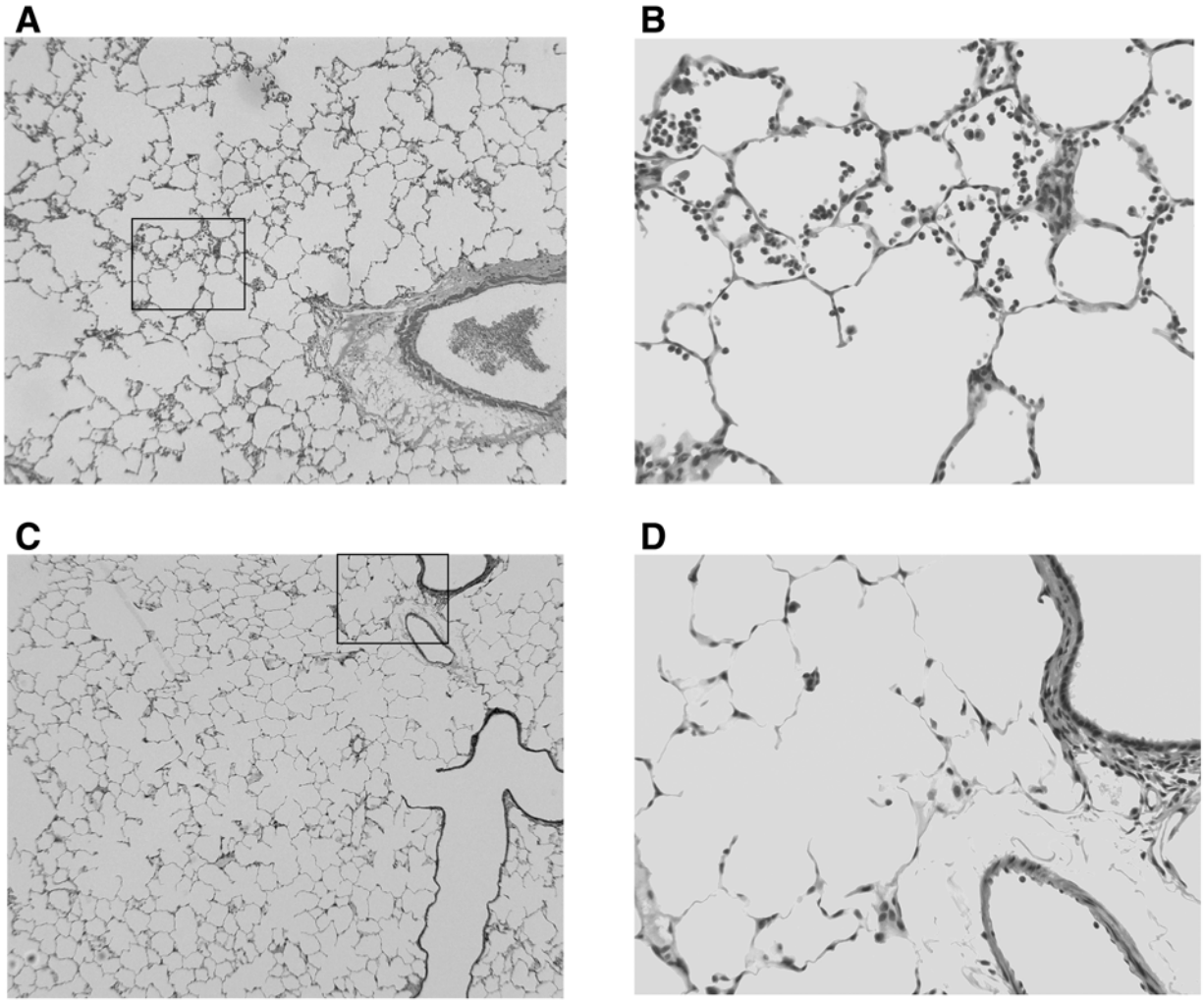

Figure E.1. Morphologic changes with and without endotoxin instillation. An aged rat 5 hours after instillation of endotoxin (0.1 mg/kg body weight; H&E stain) showing **(*A*)** perivascular edema (×4 magnification, 1 cm = 1 mm) and **(*B*)** accumulation of neutrophils in the alveoli (×20 magnification, 1 cm = 200 µm). An aged sham-instilled rat **(*C*)** at low magnification (×4, 1 cm = 1 mm) and **(*D*)** at high magnification (×20, 1 cm = 200 µm).

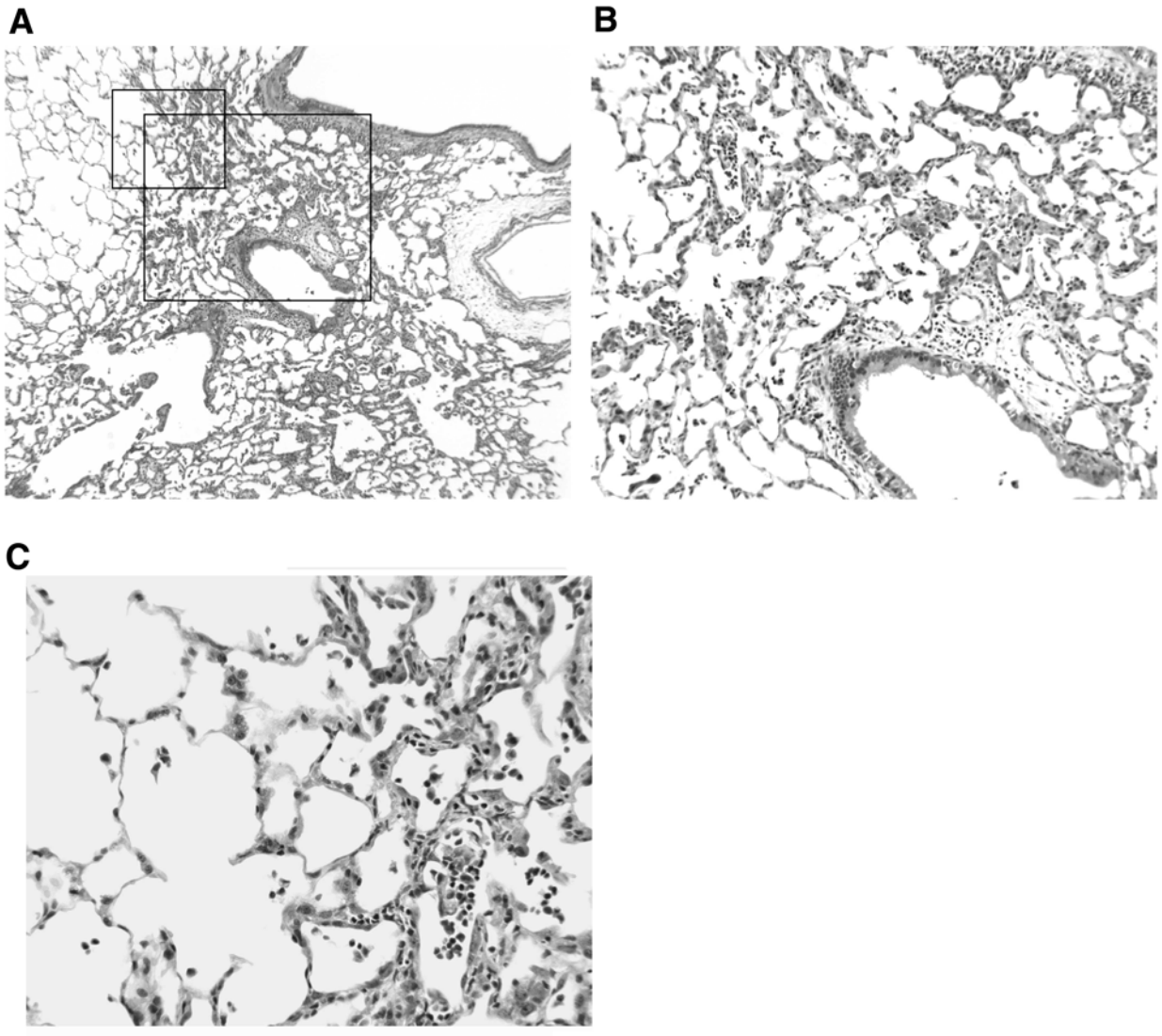

Figure E.2. An aged rat 3 days after instillation of endotoxin (0.1 mg/kg body weight; H&E stain). ***(A)*** Thickening of alveolar septa (×4 magnification, 1 cm = 1 mm). ***(B)*** Hypertrophy of bronchiolar epithelium (×10 magnification, 1 cm = 400 µm). ***(C)*** Accumulation of neutrophils in the alveoli and alveolar septae (×20 magnification, 1 cm = 200 µm).

APPENDIX F. Experiment 1 Data Summary Tables

Table F.1. Body and Lung Weights—V_2O_5 Exposures of Aged Rats

Particle / Weight	Exposure Concentration, μg/m^3 (Predicted Lung Burden [μg/g lung])											
	Mean	SD	N[a]	Mean	SD	N[a]	Mean	SD	N[a]	Mean	SD	N[a]
Ultrafine V_2O_5	**0 (0)**			**50 (0.68)**			**50+50 (0.79)**			**100 (0.67)**		
Body weight–end (g)	359	23.6	31	356	20.9	11	374	23.5	14	362	23.4	14
Body weight–start (g)	425	36.5	31	477	48.8	11	434	21.1	14	431	26.0	14
Body weight–change (fraction)	−0.15	0.08	31	−0.25	0.07	11	−0.14	0.05	14	−0.16	0.09	14
Lung–body weight ratio	0.005	0.001	31	0.006	0.002	11	0.006	0.001	14	0.007	0.001	14
Lung weight (g)	1.95	0.27	31	2.22	0.65	11	2.07	0.31	14	2.39	0.42	14
Lung burden (μg/g lung)	0.094	0.047	5	0.723	0.135	4	0.642	0.097	5	0.970	0.190	6
	150 (1.28)			**150+50 (1.24)**			**200 (1.49)**					
Body weight—end (g)	328	21.6	14	328	25.0	11	364	17.9	12			
Body weight—start (g)	413	23.1	14	416	25.3	11	419	30.0	12			
Body weight—change (fraction)	−0.21	0.05	14	−0.21	0.09	11	−0.13	0.07	12			
Lung–body weight ratio	0.006	0.001	14	0.007	0.001	11	0.006	0.001	12			
Lung weight (g)	2.09	0.22	14	2.18	0.43	11	2.32	0.23	12			
Lung burden (μg/g lung)	1.192	0.097	5	1.205	0.244	3	1.36	0.098	4			
Fine V_2O_5	**0 (0)**[b]			**150+50 (0.45)**			**200 (0.60)**					
Body weight–end (g)	369	16.4	20	361	29.3	11	369	25.7	12			
Body weight–start (g)	416	18.4	20	399	36.0	11	418	41.7	12			
Body weight–change (fraction)	−0.11	0.04	20	−0.09	0.03	11	−0.11	0.14	12			
Lung–body weight ratio	0.005	0.001	20	0.006	0.000	11	0.005	0.000	12			
Lung weight (g)	2.00	0.24	20	2.01	0.11	11	1.99	0.15	12			
Lung burden (μg/g lung)	0.099	0.013	7	0.538	0.086	4	0.636	0.074	4			
	500+500 (1.55)			**1000 (2.48)**								
Body weight–end (g)	370	23.1	13	367	27.5	14						
Body weight–start (g)	417	32.8	13	417	26.2	14						
Body weight–change (fraction)	−0.11	0.08	13	−0.12	0.08	14						
Lung–body weight ratio	0.01	0.00	13	0.006	0.001	14						
Lung weight (g)	2.28	0.15	13	2.20	0.14	14						
Lung burden (μg/g lung)	1.50	0.10	5	2.64	0.339	6						

[a] Sample sizes vary because samples could not be obtained from all rats.

[b] These control rats are from both experiments 1 and 3.

Table F.2. Means and Standard Deviations of All Response Variables—V_2O_5 Exposures of Aged Rats

Particle / Procedure / Mechanism	Variable	Units	Exposure Concentration, µg/m³ (Predicted Lung Burden [µg/g])								
			Mean	SD	N[a]	Mean	SD	N[a]	Mean	SD	N[a]
Ultrafine V_2O_5			**0 (0)**			**50 (0.68)**			**50+50 (0.79)**		
BAL											
Cytotoxicity	LDH	IU/L	105	97.8	25	111	58.9	7	135	34.7	9
Inflammation	Cells	per mL ($\times 10^6$)	2.82	1.11	25	3.05	1.22	7	3.00	0.797	9
Inflammation	Neutrophils	per mL ($\times 10^6$)	0.438	0.628	25	0.462	0.288	7	0.424	0.115	9
Inflammation	Protein	mg/dL	9.84	13.8	25	7.14	4.18	7	11.6	5.13	9
Inflammation	TNF-α	pg/mL	7.04	8.35	31	0.00	0.00	11	11.9	11.4	14
Inflammation	Lymphocytes	per mL ($\times 10^6$)	0.190	0.120	25	0.148	0.070	7	0.310	0.118	9
Inflammation	Cells	number ($\times 10^6$)	29.6	11.8	25	34.3	14.1	7	31.4	10.2	9
Inflammation	Lymphocytes	number ($\times 10^6$)	1.97	1.18	25	1.65	0.798	7	3.21	1.31	9
Inflammation	Lymphocytes	%	6.7	2.5	25	4.8	2.0	7	10.3	2.7	9
Macrophage activation	Alveolar macrophages	number ($\times 10^6$)	23.3	9.34	25	27.4	10.4	7	23.7	8.14	9
Macrophage activation	Alveolar macrophages	%	79.8	11.3	25	81.4	7.3	7	75.3	4.0	9
Inflammation	Neutrophils	number ($\times 10^6$)	4.39	5.17	25	5.22	3.32	7	4.43	1.51	9
Inflammation	Neutrophils	%	13.5	10.1	25	13.9	6.1	7	14.4	3.1	9
Macrophage activation	Alveolar macrophages	per mL ($\times 10^6$)	2.20	0.767	25	2.44	0.897	7	2.27	0.654	9
Macrophage activation	β-Glucuronidase	IU/L	0.221	0.226	25	0.166	0.058	7	0.178	0.037	9
Macrophage activation	MIP-2	pg/mL	140	44.6	25	170	89.6	7	199	47.8	9
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm²)	0.445	0.319	23	0.834	0.463	6	0.768	1.041	8
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm²)	0.916	0.449	23	1.09	0.417	6	0.737	0.386	8
Histology											
Inflammation	Alveolar	histologic grade	0.93	0.47	27	1.29	0.76	7	1.11	0.33	9
Inflammation	Alveolar macrophage hyperplasia	histologic grade	0.41	0.50	27	0.71	0.49	7	0.89	0.33	9
Inflammation	Interstitial	histologic grade	0.37	0.49	27	0.14	0.38	7	0.44	1.01	9
Inflammation	Total	histologic grade	1.70	0.95	27	2.14	1.35	7	2.44	1.33	9
			100 (0.67)			**150 (1.28)**			**150+50 (1.24)**		
BAL											
Cytotoxicity	LDH	IU/L	163	57.9	8	146	53.9	8	120	28.7	8
Inflammation	Cells	per mL ($\times 10^6$)	3.83	0.532	8	8.053	2.32	8	10.6	1.33	8
Inflammation	Neutrophils	per mL ($\times 10^6$)	0.861	0.250	8	3.345	0.901	8	3.94	1.45	8
Inflammation	Protein	mg/dL	11.88	2.90	8	8.75	2.76	8	7.63	1.92	8

Table continues on next page

[a] Sample sizes vary because samples could not be obtained from all rats.

Table F.2 (*continued*). Means and Standard Deviations of All Response Variables—V_2O_5 Exposures of Aged Rats

Particle / Procedure / Mechanism	Variable	Units	Exposure Concentration, µg/m^3 (Predicted Lung Burden [µg/g])								
			Mean	SD	N[a]	Mean	SD	N[a]	Mean	SD	N[a]
Ultrafine V_2O_5 (*continued*)			**100 (0.67)**			**150 (1.28)**			**150+50 (1.24)**		
BAL (*continued*)											
Inflammation	TNF-α	pg/mL	5.48	6.70	14	2.41	3.09	14	3.03	2.67	11
Inflammation	Lymphocytes	per mL (× 10^6)	0.103	0.054	8	0.400	0.213	8	0.576	0.314	8
Inflammation	Cells	number (× 10^6)	37.3	5.62	8	83.3	23.4	8	119	18.0	8
Inflammation	Lymphocytes	number (× 10^6)	1.00	0.513	8	4.10	2.06	8	6.31	3.05	8
Inflammation	Lymphocytes	%	2.7	1.2	8	4.8	2.1	8	5.6	3.2	8
Macrophage activation	Alveolar macrophages	number (× 10^6)	27.9	3.82	8	44.8	15.8	8	68.0	12.5	8
Macrophage activation	Alveolar macrophages	%	75.2	5.6	8	53.0	5.9	8	57.6	8.7	8
Inflammation	Neutrophils	number (× 10^6)	8.38	2.39	8	34.4	7.69	8	44.7	15.5	8
Inflammation	Neutrophils	%	22.2	4.9	8	42.2	5.3	8	36.9	11.1	8
Macrophage activation	Alveolar macrophages	per mL (× 10^6)	2.87	0.352	8	4.31	1.46	8	6.084	1.098	8
Macrophage activation	β-Glucuronidase	IU/L	0.191	0.044	8	0.172	0.074	8	0.208	0.061	8
Macrophage activation	MIP-2	pg/mL	188.6	60.8	8	191.6	39.5	8	164.1	70.3	8
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	1.494	0.787	8	1.355	0.837	8	1.988	1.083	5
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.715	0.425	7	1.138	0.510	8	1.603	0.859	5
Histology											
Inflammation	Alveolar	histologic grade	1.63	0.52	8	2.11	0.33	9	1.73	0.65	11
Inflammation	Alveolar macrophage hyperplasia	histologic grade	0.75	0.46	8	1.11	0.33	9	1.27	0.47	11
Inflammation	Interstitial	histologic grade	0.38	0.52	8	0.44	0.73	9	0.27	0.47	11
Inflammation	Total	histologic grade	2.75	1.16	8	3.67	0.71	9	3.27	0.90	11
			200 (1.49)								
BAL											
Cytotoxicity	LDH	IU/L	147	21.7	8						
Inflammation	Cells	per mL (× 10^6)	6.46	1.05	8						
Inflammation	Neutrophils	per mL (× 10^6)	2.94	0.704	8						
Inflammation	Protein	mg/dL	11.1	5.22	8						
Inflammation	TNF-α	pg/mL	14.2	13.5	12						
Inflammation	Lymphocytes	per mL (× 10^6)	0.477	0.225	8						
Inflammation	Cells	number (× 10^6)	68.5	12.6	8						
Inflammation	Lymphocytes	number (× 10^6)	4.97	2.18	8						

Table continues on next page

[a] Sample sizes vary because samples could not be obtained from all rats.

Table F.2 (*continued*). Means and Standard Deviations of All Response Variables—V_2O_5 Exposures of Aged Rats

Particle / Procedure / Mechanism	Variable	Units	Exposure Concentration, µg/m^3 (Predicted Lung Burden [µg/g])								
			Mean	SD	N^a	Mean	SD	N^a	Mean	SD	N^a
Ultrafine V_2O_5 (*continued*)			**200 (1.49)**								
BAL (*continued*)											
Inflammation	Lymphocytes	%	7.3	2.7	8						
Macrophage activation	Alveolar macrophages	number (× 10^6)	31.9	6.37	8						
Macrophage activation	Alveolar macrophages	%	47.2	8.8	8						
Inflammation	Neutrophils	number (× 10^6)	31.6	10.4	8						
Inflammation	Neutrophils	%	45.5	9.5	8						
Macrophage activation	Alveolar macrophages	per mL (× 10^6)	3.04	0.756	8						
Macrophage activation	β-Glucuronidase	IU/L	0.438	0.223	8						
Macrophage activation	MIP-2	pg/mL	288	95.1	8						
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	0.939	0.629	7						
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.743	0.332	7						
Histology											
Inflammation	Alveolar	histologic grade	1.88	0.35	8						
Inflammation	Alveolar macrophage hyperplasia	histologic grade	1.25	0.46	8						
Inflammation	Interstitial	histologic grade	0.00	0.00	8						
Inflammation	Total	histologic grade	3.13	0.64	8						
Fine V_2O_5			**0 (0)[b]**			**150+50 (0.45)**			**200 (0.60)**		
BAL											
Cytotoxicity	LDH	IU/L	77	35	13	140	27	7	76	18	8
Inflammation	Cells	per mL (× 10^6)	3.5	1.3	13	4.8	1.0	7	3.5	1.4	8
Inflammation	Neutrophils	per mL (× 10^6)	0.31	0.36	13	0.69	0.22	7	0.48	0.26	8
Inflammation	Protein	mg/dL	6.6	4.2	13	7.4	1.1	7	6.3	0.89	8
Inflammation	TNF-α	pg/mL	5.3	5.2	20	7.9	7.4	11	11	11	12
Inflammation	Lymphocytes	per mL (× 10^6)	0.33	0.30	13	0.31	0.097	7	0.31	0.13	8
Inflammation	Cells	number (× 10^6)	37	14	13	46	7.6	7	37	15	8
Inflammation	Lymphocytes	number (× 10^6)	3.4	3.0	13	3.1	0.90	7	3.3	1.5	8
Inflammation	Lymphocytes	%	9.4	7.5	13	6.6	2.0	7	9.4	3.5	8
Macrophage activation	Alveolar macrophages	number (× 10^6)	30	13	13	37	6.0	7	28	12	8
Macrophage activation	Alveolar macrophages	%	82	11	13	79	2.7	7	77	3.7	8
Inflammation	Neutrophils	number (× 10^6)	3.1	3.6	13	6.6	1.7	7	5.1	2.7	8
Inflammation	Neutrophils	%	8.2	7.3	13	14	1.8	7	14	3.8	8

Table continues on next page

[a] Sample sizes vary because samples could not be obtained from all rats.

[b] These control rats are from both experiments 1 and 3.

Table F.2 (*continued*). Means and Standard Deviations of All Response Variables—V_2O_5 Exposures of Aged Rats

Particle / Procedure / Mechanism	Variable	Units	Exposure Concentration, µg/m^3 (Predicted Lung Burden [µg/g])								
			Mean	SD	N[a]	Mean	SD	N[a]	Mean	SD	N[a]
Fine V_2O_5 (*continued*)			**0 (0)**[b]			**150+50 (0.45)**			**200 (0.60)**		
BAL (*continued*)											
Macrophage activation	Alveolar macrophages	per mL (× 10^6)	2.9	1.1	13	3.8	0.78	7	2.7	1.1	8
Macrophage activation	β-Glucuronidase	IU/L	0.12	0.094	13	0.27	0.076	7	0.046	0.036	8
Macrophage activation	MIP-2	pg/mL	160	43	13	240	27	7	200	44	8
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	0.69	0.51	16	0.57	0.25	5	0.47	0.25	6
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.82	0.35	16	0.54	0.20	5	0.53	0.25	6
Histology											
Inflammation	Alveolar	histologic grade	0.8	0.55	20	0.9	0.30	11	1.0	0.00	12
Inflammation	Alveolar macrophage hyperplasia	histologic grade	0.5	0.51	20	0.5	0.52	11	0.2	0.39	12
Inflammation	Interstitial	histologic grade	0.6	0.60	20	0.5	0.82	11	0.4	0.67	12
Inflammation	Total	histologic grade	1.9	1.1	20	1.9	1.2	11	1.6	0.90	12
			500+500 (1.55)			**1000 (2.48)**					
BAL											
Cytotoxicity	LDH	IU/L	110	42	8	150	40	8			
Inflammation	Cells	per mL (× 10^6)	5.0	0.92	8	11	2.9	8			
Inflammation	Neutrophils	per mL (× 10^6)	0.79	0.25	8	2.5	0.77	8			
Inflammation	Protein	mg/dL	11	2.8	8	11	2.4	8			
Inflammation	TNF-α	pg/mL	4.8	4.3	13	5.9	5.8	14			
Inflammation	Lymphocytes	per mL (× 10^6)	0.37	0.22	8	0.78	0.64	8			
Inflammation	Cells	number (× 10^6)	50	6.5	8	116	32	8			
Inflammation	Lymphocytes	number (× 10^6)	3.8	2.3	8	8.2	6.5	8			
Inflammation	Lymphocytes	%	7.6	4.8	8	8.1	7.8	8			
Macrophage activation	Alveolar macrophages	number (× 10^6)	38	6.6	8	81	29	8			
Macrophage activation	Alveolar macrophages	%	77	8.0	8	69	6.5	8			
Inflammation	Neutrophils	number (× 10^6)	8.0	2.2	8	27	8.3	8			
Inflammation	Neutrophils	%	16	3.9	8	23	5.6	8			

Table continues on next page

[a] Sample sizes vary because samples could not be obtained from all rats.

[b] These control rats are from both experiments 1 and 3.

Table F.2 (*continued*). Means and Standard Deviations of All Response Variables—V_2O_5 Exposures of Aged Rats

Particle / Procedure / Mechanism	Variable	Units	Exposure Concentration, µg/m^3 (Predicted Lung Burden [µg/g])								
			Mean	SD	N[a]	Mean	SD	N[a]	Mean	SD	N[a]
Fine V_2O_5 (*continued*)			**500+500 (1.55)**			**1000 (2.48)**					
BAL (*continued*)											
Macrophage activation	Alveolar macrophages	per mL ($\times 10^6$)	3.8	0.86	8	7.7	2.7	8			
Macrophage activation	β-Glucuronidase	IU/L	0.12	0.065	8	0.21	0.058	8			
Macrophage activation	MIP-2	pg/mL	140	41	8	270	49	8			
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	1.1	0.54	7	1.2	0.56	8			
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.73	0.37	7	0.53	0.14	8			
Histology											
Inflammation	Alveolar	histologic grade	1.5	0.52	13	1.5	0.52	14			
Inflammation	Alveolar macrophage hyperplasia	histologic grade	1.00	0.00	13	0.86	0.36	14			
Inflammation	Interstitial	histologic grade	0.46	0.66	13	0.93	0.73	14			
Inflammation	Total	histologic grade	3.0	0.71	13	3.3	1.1	14			

[a] Sample sizes vary because samples could not be obtained from all rats.

APPENDIX G. Experiment 2 Data Summary Tables

Table G.1. Body and Lung Weights—Ultrafine Carbon Black Exposures of Aged Rats[a]

	Exposure Concentration, μg/m^3 (Predicted Lung Burden [μg/g lung])								
	0 (0)			150+50 (34)			200 (45)		
Weights	Mean	SD	*N*[b]	Mean	SD	*N*	Mean	SD	*N*
Body weight–end (g)	345.6	23.4	11	362.8	30.7	10	356.0	27.0	13
Body weight–start (g)	408.4	30.9	12	398.6	23.7	10	410.5	31.1	13
Body weight–change (fraction)	−0.15	0.06	11	−0.09	0.11	10	−0.13	0.10	13
Lung–body weight ratio	0.006	0.001	11	0.005	0.001	10	0.005	0.001	13
Lung weight (g)	1.93	0.27	11	1.95	0.22	10	1.85	0.29	13

[a] See Table F.1 for data for the comparison group: aged rats exposed to the same concentrations of ultrafine V_2O_5.

[b] Sample sizes vary because samples could not be obtained from all rats.

Table G.2. Means and Standard Deviations of All Response Variables—Ultrafine Carbon Black Exposures of Aged Rats[a]

Procedure / Mechanism	Variable	Units	Exposure Concentration, µg/m^3 (Predicted Lung Burden [µg/g lung])								
			0 (0)			150+50 (34)			200 (45)		
			Mean	SD	N^b	Mean	SD	N^b	Mean	SD	N^b
BAL											
Cytotoxicity	LDH	IU/L	86.3	33.0	10	131	29.5	7	73.6	33.2	8
Inflammation	Cells	per mL ($\times 10^6$)	5.69	2.86	10	10.03	0.836	7	4.16	1.59	8
Inflammation	Neutrophils	per mL ($\times 10^6$)	0.654	0.538	10	1.78	1.065	7	0.344	0.196	8
Inflammation	Protein	mg/dL	5.60	2.22	10	8.29	1.98	7	4.00	1.85	8
Inflammation	TNF-α	pg/mL	15.8	8.80	12	14.8	10.9	10	8.34	8.16	13
Inflammation	Lymphocytes	per mL ($\times 10^6$)	0.411	0.438	10	0.752	0.395	7	0.314	0.243	8
Inflammation	Cells	number ($\times 10^6$)	57.4	29.5	10	94.2	8.74	7	40.4	13.4	8
Inflammation	Lymphocytes	number ($\times 10^6$)	4.18	4.71	10	7.05	3.61	7	2.89	2.04	8
Inflammation	Lymphocytes	%	6.9	5.3	10	7.5	3.7	7	7.3	5.3	8
Macrophage activation	Alveolar macrophages	number ($\times 10^6$)	46.6	21.5	10	70.7	10.9	7	34.2	12.0	8
Macrophage activation	Alveolar macrophages	%	82.9	5.3	10	74.9	8.5	7	84.5	8.3	8
Inflammation	Neutrophils	number ($\times 10^6$)	6.61	5.39	10	16.5	9.47	7	3.26	1.67	8
Inflammation	Neutrophils	%	10.3	4.3	10	17.6	10.2	7	8.2	3.7	8
Macrophage activation	Alveolar macrophages	per mL ($\times 10^6$)	4.63	2.07	10	7.51	0.974	7	3.50	1.32	8
Macrophage activation	β-glucuronidase	IU/L	0.174	0.101	10	0.218	0.074	7	0.115	0.081	8
Macrophage activation	MIP-2	pg/mL	117	38.4	10	186	36.5	7	120	26.8	8
Morphology											
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	0.557	0.633	6	0.762	0.266	5	0.514	0.492	7
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.926	0.219	6	1.18	0.235	5	1.05	0.351	7
Histology											
Inflammation	Alveolar	histologic grade	0.7	0.65	12	1.1	0.32	10	0.9	0.49	13
Inflammation	Alveolar macrophage hyperplasia	histologic grade	0.4	0.51	12	0.7	0.48	10	0.5	0.52	13
Inflammation	Interstitial	histologic grade	0.3	0.62	12	0.8	0.79	10	0.5	0.78	13
Inflammation	Total	histologic grade	1.3	0.98	12	2.6	1.07	10	1.9	0.95	13

[a] See Table F.2 for data for the comparison group: aged rats exposed to the same concentrations of ultrafine V_2O_5.

[b] Sample sizes vary because samples could not be obtained from all rats.

APPENDIX H. Experiment 3 Data Summary Tables

Table H.1. Body and Lung Weights—Fine V_2O_5 Exposures of Aged and Young Adult Rats

	Exposure Concentration, μg/m^3 (Predicted Lung Burden [μg/g lung])											
	Aged						Young Adult					
	0 (0)[a]			500 (1.27)			0 (0)			500 (1.77)		
Weights	Mean	SD	*N*[b]	Mean	SD	*N*[b]	Mean	SD	*N*[b]	Mean	SD	*N*[b]
Body weight–end (g)	368.5	16.4	20	367.8	9.2	9	227.4	12.0	10	225.3	19.0	10
Body weight–start (g)	415.8	18.4	20	427.8	9.5	9	231.4	13.0	10	233.5	15.4	10
Body weight–change (fraction)	−0.11	0.04	20	−0.14	0.02	9	−0.02	0.02	10	−0.04	0.02	10
Lung–body weight ratio	0.0054	0.0007	20	0.0063	0.0007	9	0.0054	0.0003	10	0.0067	0.0002	10
Lung weight (g)	2.00	0.24	20	2.32	0.28	9	1.23	0.07	10	1.50	0.09	10
Lung burden (μg/g lung)	0.10	0.01	7	2.43	0.34	3	0.05	0.01	4	1.33	0.21	4

[a] These control rats are from both experiments 1 and 3.

[b] Sample sizes vary because samples could not be obtained from all rats.

Table H.2. Means and Standard Deviations of All Response Variables—Fine V_2O_5 Exposures of Aged and Young Adult Rats[a]

			Exposure Concentration, μg/m^3 (Predicted Lung Burden [μg/g])											
			Aged						Young Adult					
			0 μg/m^3 [b]			500 μg/m^3			0 (0)			500 (1.77)		
Procedure / Mechanism	Variable	Units	Mean	SD	N[c]	Mean	SD	N[c]	Mean	SD	N[c]	Mean	SD	N[c]
BAL														
Cytotoxicity	LDH	IU/L	76.9	35.3	13	146	44.9	6	106	27.5	6	177	30.5	6
Inflammation	Cells	per mL ($\times 10^6$)	3.52	1.26	13	7.08	1.32	6	3.51	1.11	6	7.17	0.630	6
Inflammation	PMN[b]	per mL ($\times 10^6$)	0.309	0.364	13	1.19	0.642	6	0.197	0.129	6	1.86	0.627	6
Inflammation	Protein	mg/dL	6.62	4.21	13	11.8	5.12	6	8.50	1.52	6	14.7	1.75	6
Inflammation	TNF-α	pg/mL	5.25	5.22	20	1.92	3.01	9	11.6	13.3	10	8.12	10.1	10
Inflammation	Lymphocytes	per mL ($\times 10^6$)	0.334	0.304	13	0.119	0.110	6	0.212	0.0557	6	0.157	0.179	6
Inflammation	Cells	number ($\times 10^6$)	36.6	14.0	13	77.1	17.8	6	21.9	6.39	6	45.3	7.07	6
Inflammation	Lymphocytes	number ($\times 10^6$)	3.42	3.00	13	1.28	1.21	6	1.33	0.374	6	0.996	1.17	6
Inflammation	Lymphocytes	%	9.4	7.5	13	1.8	1.7	6	6.3	1.9	6	2.2	2.4	6
Macrophage activation	Alveolar macrophages	number ($\times 10^6$)	30.0	12.7	13	63.0	22.6	6	19.3	5.67	6	32.4	4.33	6
Macrophage activation	Alveolar macrophages	%	82.4	10.5	13	80.1	10.9	6	88.3	4.2	6	72.3	9.0	6
Inflammation	Neutrophils	number ($\times 10^6$)	3.10	3.56	13	12.8	6.82	6	1.24	0.795	6	11.9	4.71	6
Inflammation	Neutrophils	%	8.2	7.3	13	18.1	11.1	6	5.4	2.7	6	25.6	7.3	6
Macrophage activation	Alveolar macrophages	per mL ($\times 10^6$)	2.88	1.09	13	5.76	1.83	6	3.10	0.986	6	5.15	0.559	6
Macrophage activation	β-Glucuronidase	IU/L	0.119	0.094	13	0.158	0.103	6	0.21	0.063	6	0.288	0.077	6
Macrophage activation	MIP-2	pg/mL	158	42.5	13	263	55.3	6	151	37.6	6	178	56.4	6
Morphology														
Mucous cell metaplasia	Airway generation 12 mucus	volume density (nL/mm^2)	0.69	0.51	16	1.46	1.05	4	0.422	0.151	5	0.916	0.264	5
Mucous cell metaplasia	Airway generation 4 mucus	volume density (nL/mm^2)	0.82	0.35	16	0.678	0.069	4	0.763	0.218	5	0.461	0.214	5
Histology														
Inflammation	Alveolar	histologic grade	0.75	0.55	20	1.4	0.73	9	0.7	0.67	10	2.3	0.48	10
Inflammation	Alveolar macrophage hyperplasia	histologic grade	0.5	0.51	20	2.0	0.71	9	1.2	0.42	10	2.1	0.32	10
Inflammation	Interstitial	histologic grade	0.6	0.60	20	1.3	0.87	9	0.7	0.67	10	0.7	0.67	10
Inflammation	Total	histologic grade	1.85	1.09	20	4.8	1.79	9	2.6	1.43	10	5.1	0.99	10

[a] See Table F.2 for data for the comparison group: aged rats exposed to the same concentrations of fine V_2O_5.

[b] These control rats are from both experiments 1 and 3.

[c] Sample sizes vary because samples could not be obtained from all rats.

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ABBREVIATIONS AND OTHER TERMS

σ_g	geometric standard deviation
AB/PAS	Alcian blue (pH 2.5)–periodic acid Schiff sequence
ANOVA	analysis of variance
ANCOVA	analysis of covariance
BAL	bronchoalveolar lavage
CAPs	concentrated ambient particles
CMD	count median diameter
COPD	chronic obstructive pulmonary disease
EPA	Environmental Protection Agency (US)
H&E	hematoxylin and eosin
H_2O_2	hydrogen peroxide
LDH	lactate dehydrogenase
MIP-2	macrophage inflammatory protein-2
MMAD	mass median aerodynamic diameter
PM	particulate matter
PM_{10}	PM less than 10 μm in aerodynamic diameter
$PM_{2.5}$	PM less than 2.5 μm in aerodynamic diameter
SMPS	scanning mobility particle sizer
TiO_2	titanium dioxide
TNF-α	tumor necrosis factor α
TSP	total suspended particulate
V_2O_5	vanadium pentoxide

INTRODUCTION

Epidemiologic studies have established an association between short-term increases in ambient levels of particulate matter (PM*) and increases in morbidity and mortality, especially in older individuals with preexisting cardiovascular or respiratory disease (Dockery and Pope 1994; Goldberg et al 2000; Pope 2000; Anderson 2003). Despite the consistency of the epidemiologic findings, the biological mechanisms underlying these associations are not well understood. One set of research questions has focused on the role of particle attributes (such as size, surface properties, and composition) in causing adverse health effects. Specifically, models predict that a higher proportion of particles in the ultrafine range (< 0.1 μm in aerodynamic diameter) than larger particles reach the air-exchanging, alveolar region of the lung. Smaller particles also provide more surface area for chemical reactions per unit mass (Oberdörster et al 1995). Another set of questions has focused on which individuals, such as children, older people, or people with preexisting disease, are more sensitive to the effects of air pollutants.

In 1998, HEI issued Request for Application (RFA) 98-1, "Characterization of Exposure to and Health Effects of Particulate Matter." The health effects objectives outlined in the RFA invited proposals to: (1) characterize possible pathophysiologic effects caused by PM in sensitive subjects; (2) define the relations between particle characteristics and the dose, distribution, and persistence of particles in the respiratory tract; (3) identify the kinds of particles or particle attributes that may cause toxicity; (4) investigate the diseases or conditions that affect sensitivity; and (5) delineate how copollutants affect or contribute to the physiologic response to particles.

In response to the RFA, Dr Kristen Nikula and colleagues of the Lovelace Respiratory Research Institute proposed to systematically examine lung inflammation in young adult and aged rats after inhalation exposure to fine particles (< 2.5 μm in aerodynamic diameter) and ultrafine particles of different composition: relatively inert carbon and vanadium pentoxide (V_2O_5), which contains the transition metal vanadium, known to cause toxic effects upon inhalation in humans in occupational settings. They proposed to use a model of mild inflammation—induced by intratracheal instillation of endotoxin (components of gram-negative bacterial cell walls)—and examine the role of particle size and composition on the resulting inflammatory response. In addition, they proposed to examine the effect of a short-term increase (spike) in particle exposure concentration on the inflammatory response. The investigators hypothesized that a spike in particle concentration would cause a greater inflammatory response than exposure to a steady concentration at equal particle lung burden would. They also hypothesized that old rats would exhibit greater lung inflammation in response to particle exposure than would young adult rats.

The HEI Research Committee recommended the study for funding because it thought that the systematic comparison of fine and ultrafine particles of different composition would yield important information on the toxicity of different particle attributes. To date, few studies have directly compared the effects of inhaling ultrafine versus fine particles of different composition. The Committee thought that the additional comparison of inflammatory responses between young adult and old rats was a strength of the study design. Committee members were also interested in the proposed exposure regimen of a short-term increase in particle concentration after repeated exposure to a lower concentration, to mimic the pattern of changes in ambient air. During the second year of the study, Dr Nikula left the Lovelace Respiratory Research Institute and her colleague Dr Fletcher Hahn assumed primary responsibility for the study.†

This Commentary is intended to aid the sponsors of HEI and the public by highlighting both the strengths and limitations of the study and by placing the Investigators' Report into scientific and regulatory perspective.

SCIENTIFIC BACKGROUND

The association between increased levels of PM and increased morbidity and mortality, observed in epidemiologic studies, is relatively consistent across different locations. However, more information is needed to understand the nature of the pathobiological mechanisms that might cause such effects, which particles may be most toxic, and which individuals may be most susceptible (Health Effects Institute 2002).

* 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 Hahn's 3-year study, "Mechanisms of Particle Size- and Composition-Related Adverse Health Effects in an Aged, Sensitive Population of Rats," began in September 1998. Total expenditures were $536,010. The draft Investigators' Report from Hahn and colleagues was received for review in February 2002. A revised report, received in May 2004, was accepted for publication in June 2004. 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.

Particle Composition

PM is a complex, heterogeneous mixture of solid and liquid components. Primary particles originating from combustion sources usually consist of a carbonaceous core with many chemicals (such as sulfates, metals, and polycyclic aromatic hydrocarbons) adsorbed onto its surface. Secondary particles form in the atmosphere by way of chemical reactions of primary particles and gases (such as nitric oxides, ozone, and sulfur oxides). The specific composition and size distribution of PM varies by region, time of year, time of day, weather conditions, and other factors.

Because of the heterogeneity of PM, studying its health effects is complicated. In the past decade, many toxicologic studies have investigated which types of particles may cause adverse health effects. One approach is to use particles from different sources, such as residual oil fly ash, diesel soot, coal dust, carbon, or concentrated ambient particles (Becker et al 1996; Costa and Dreher 1997; Nikula et al 1997; Murphy et al 1998). These particles differ substantially in composition, depending on their core compositions, surface characteristics, and whether sulfates, transition metals, organic pollutants, or other toxic and nontoxic compounds have been adsorbed onto their surfaces. Another approach is to use laboratory-generated particles of more homogeneous size and composition, such as metal or metal oxide particles (Oberdörster et al 1995; Leikauf et al 2001).

At the time the current study was funded, evidence suggested that exposure to several kinds of PM triggers inflammatory events. For example, in vitro studies using human or rat macrophages grown in cell cultures showed that cytokine production increased after exposure to ambient urban PM (Becker et al 1996), coal mine dust (Gosset et al 1991), asbestos fibers and silica (Dubois et al 1989), titanium oxide and iron oxide (Driscoll and Maurer 1991; Warheit and Hartsky 1993; Oberdörster et al 1995), diesel particles (Yang et al 1997), and oil fly ash (Killingsworth et al 1997).

Inflammation was also shown in vivo after chronic intratracheal instillation of diesel and carbon particles into the lungs of rodents (Mauderly et al 1994; Nikula et al 1997) or after acute instillation of oil fly ash (Killingsworth et al 1997; Kodavanti et al 1997), diesel particles (Sagai et al 1996), or urban particles collected from filters (Li et al 1997). However, the routes of exposure used and the high concentrations to which animals were exposed in these studies were not representative of humans inhaling ambient PM.

Other particle inhalation studies in rats used lower concentrations that approach high ambient PM levels. For example, Nikula and colleagues (1997) showed that chronic inhalation of urban PM, diesel exhaust, or coal dust caused lung inflammation in rats. Other studies found effects on rat lungs after inhalation of nickel oxide (Benson et al 1995) or urban particles in combination with ozone (Vincent et al 1997). However, questions remained about whether all particles—regardless of size or composition—cause inflammation and about which particle sizes and components are most toxic.

Vanadium Occupational studies have long indicated that inhalation exposure to metal oxides is harmful (Mueller and Seger 1985). In addition, several studies in humans and animals have suggested that transition metals associated with particles play a role in toxicity of ambient particles (Pope 1989; US Environmental Protection Agency [EPA] 1996, 2004; Gavett et al 1997). The focus has been transition metals that are present in the ambient urban atmosphere at concentrations above 0.5 $\mu g/m^3$ (such as arsenic, cadmium, copper, iron, lead, vanadium, and zinc; EPA 1996). Thus, researchers have investigated the toxicity of laboratory-generated particles of those metals and their oxides, as well as nickel, platinum, and titanium. In the study described in this Research Report, Dr Nikula proposed to investigate the health effects of inhaled V_2O_5 in rats and compare V_2O_5 to a relatively inert particle, carbon.

Vanadium has six valence states and can form many different compounds. Although it occurs naturally in continental dust, marine aerosol, and volcanic emissions (Agency for Toxic Substances and Disease Registry 1992), man-made emissions of vanadium—resulting from burning fuel oil or coal—account for approximately two-thirds of vanadium concentrations in ambient air. The general population is exposed to vanadium through ingestion of food—especially food contaminated with soil—and inhalation of ambient particles. Vanadium levels in urban ambient air are 3 to 22 ng/m^3 in most cities across the US and 150 to 1400 ng/m^3 in the northeastern US, owing to greater use of residual fuel oil for heat and electricity generation in winter there (Agency for Toxic Substances and Disease Registry 1992).

Pentavalent vanadium is more acutely toxic than trivalent or divalent vanadium. Toxicologically significant compounds are pentavalent V_2O_5, sodium metavanadate ($NaVO_3$), sodium orthovanadate (Na_3VO_4), ammonium vanadate (NH_4VO_3), and tetravalent vanadyl sulfate ($VOSO_4$) (Agency for Toxic Substances and Disease Registry 1992).

V_2O_5 is the most common form of vanadium associated with particles. In occupational settings (such as in metallurgy and boiler cleaning), inhalation of moderate concentrations (60 $\mu g/m^3$) of V_2O_5 dust causes lung irritation (Agency for Toxic Substances and Disease Registry 1992). The US National Institute for Occupational Safety and Health (2004) recommended a maximum value for a 15-minute period of

50 μg/m^3 total vanadium dust in occupational settings; the World Health Organization (1988) issued a 24-hour air quality guideline for vanadium of 1 μg/m^3.

The respiratory tract is the main target of inhaled vanadium compounds (EPA 1996). For example, intratracheal instillation of different kinds of vanadium particles led to lung inflammation (such as neutrophil influx and cytokine release) in rats (Pierce et al 1996). These effects were greater after exposure to more soluble forms of vanadium (sodium metavanadate [$NaVO_3$] and vanadyl sulfate [$VOSO_4$]) than after exposure to the less soluble form (V_2O_5). Rats exposed for 8 to 10 weeks to 15 μg/m^3 V_2O_5 developed lung congestion and bronchitis (Pazynich 1966). Monkeys that acutely inhaled high concentrations (2.8 mg/m^3) of V_2O_5 had increased pulmonary resistance and significantly increased leukocyte numbers in their lung fluid (Knecht et al 1985). A recent study of rats and mice confirmed chronic lung inflammation after they inhaled V_2O_5 at high concentrations (0.5–4 mg/m^3) over 2 years and reported a significant increase in lung tumors in male and female mice and a marginal increase in lung tumors in male but not female rats (Ress et al 2003).

Additional support for vanadium's effects on the lung has been provided by studies using oil fly ash, which suggest that vanadium on the fly ash particles was responsible for cytokine release in bronchial epithelial cells in vivo (Carter et al 1997) or for lung inflammation and related gene expression in rats after intratracheal instillation (Kodavanti et al 1997; Nadadur and Kodavanti 2002).

Carbon Black carbon represents the carbonaceous core of many combustion-derived particles, without the adsorbed chemicals. Elemental carbon (ie, carbon that has been stripped of hydrogen) may comprise around 25% to 75% of motor-vehicle exhaust from gasoline or heavy-duty diesel engines (EPA 2002). Because carbon particles have low solubility and are thought to have low intrinsic toxicity, they have been used to evaluate nonspecific particle effects. The first inhalation studies with carbon exposed animals to high concentrations (6–10 mg/m^3); these studies demonstrated some changes in lung morphology but few changes in lung-fluid parameters (EPA 1996). Chronic lifetime exposure of rats to high concentrations (2.5 or 6.5 mg/m^3) of carbon—including a substantial level of organic carbon—caused persistent inflammation and cytotoxicity in the lung as well as lung tumors (Mauderly et al 1994; Nikula et al 1995). These results have been attributed to particle overload in the lung (Mauderly et al 1994; Oberdörster 1995) and are therefore not directly relevant to inhalation of carbon particles at lower, ambient concentrations.

Studies using lower concentrations have in general found few effects of carbon, whether fine or ultrafine (effects of particle size will be discussed in detail in the next section). For example, no changes in lung function were observed in healthy volunteers or volunteers with asthma exposed to 200 μg/m^3 carbon (Anderson et al 1992), 510 μg/m^3 activated carbon (Green et al 1989), or 10 μg/m^3 ultrafine carbon particles (Kulle et al 1986; Frampton et al 2004). Other studies found no effects of carbon in rat lung after intratracheal instillation (Li et al 1996; Murphy et al 1998) or inhalation (Vincent et al 2001; Wellenius et al 2002).

Particle Size

In addition to particle composition, size may play an important role in particle toxicity (see sidebar). Some scientists believe that ultrafine particles may be especially toxic, because models predict that they are more likely to reach the air-exchanging, alveolar region of the lung than larger particles are (Utell and Frampton 2000). In general, particles smaller than 20 nm in diameter deposit most efficiently; depending on size and breathing pattern, however, ultrafine particles (which can be up to 100 nm in diameter) may not necessarily deposit more efficiently than fine particles (National Research Council 2004). Ultrafine particles also have a greater total surface area per unit mass for interacting with airway cells.

PARTICLE SIZE

Ambient particles tend to fall into a trimodal distribution with three distinct sizes: ultrafine particles (0.01 to 0.1 μm in diameter); fine particles (0.1 to 1.0 μm in diameter); and coarse particles (greater than 1.0 μm in diameter). To describe particle size, three interrelated parameters are of interest: volume (mass), surface area, and number.

For particles smaller than about 0.5 μm, the relevant parameter is the particle aerodynamic diameter. The **aerodynamic diameter** is the diameter of a sphere of density = 1 μg/cm^3 that has the same gravitational settling velocity as the particle in question, regardless of the particle's size, shape, or density. Particles are usually characterized by the **mass median aerodynamic diameter**, based on the mass distribution, and the degree of dispersion, given as the **geometric standard deviation** (or σ_g).

Ultrafine particles are usually described by their **count median diameter,** based on the number distribution, the diameter at which there are an equal number of particles with larger diameters as there are particles with smaller diameters. Although ultrafine particles contribute only slightly (1% to 3%) to particle mass in the ambient air, they are the major component in terms of particle number.

Some laboratory studies suggest that ultrafine particles are not as effectively phagocytosed (ie, ingested for removal) by lung macrophages as fine particles are and that ultrafine particles more effectively induce airway inflammatory responses after intratracheal instillation (Oberdörster et al 1992; Lundborg et al 2001). Also, ultrafine titanium dioxide particles were found to translocate into the lung interstitium more easily than fine titanium dioxide particles, leading to prolonged retention in the lung (Ferin et al 1992). At the time the current study was funded, some epidemiologic studies had described associations between increases in respiratory effects and exposure to ultrafine particles in ambient air in children with asthma (Pekkanen et al 1997; Peters et al 1997).

Results of toxicity studies using ultrafine particles are mixed, however. Some researchers found that ultrafine carbon particles are more toxic than fine carbon particles, both in vitro (Timblin et al 2002) and in vivo (Li et al 1997). But other researchers found effects of some ultrafine particles but not others (eg, silica but not carbon [Murphy et al 1998]; nickel sulfate but not nickel oxide [Leikauf et al 2001]; titanium dioxide but not carbon or platinum [Oberdörster et al 2000]). Studies of a small number of people found few or no effects on respiratory or cardiac measurements after inhalation of ultrafine magnesium oxide (Kuschner et al 1997) or low concentrations of carbon (Frampton et al 2004).

Because the numbers of ultrafine particles are much greater than numbers of fine particles at the same mass concentration, studies using ultrafine particles run a greater risk of overloading the lung. Therefore, more systematic studies comparing the toxicity of fine and ultrafine particles of the same composition and at number concentrations relevant to ambient exposure levels are needed to determine the relative importance of particle size for particle toxicity.

Drs Nikula and Hahn proposed to evaluate both fine and ultrafine V_2O_5 to investigate effects of particle size. They used a detailed deposition model developed by statistician Dr Margaret Ménache (Ménache et al 1996) in order to determine which exposure concentrations of ultrafine and fine particles would be needed to achieve comparable deposition in the lungs of exposed rats.

Sensitive Populations

Epidemiologic studies have indicated that certain people, such as individuals with respiratory disease or older individuals, are more susceptible than others to the health effects of exposure to ambient particles (Özkaynak and Spengler 1985; Schwartz and Dockery 1992; Lipsett et al 1997). Several observations suggest that older people may have a reduced capacity for repair mechanisms, resulting in oxidative damage and inflammation in the lung being more likely being more likely with increasing age. For example, old rats have lower levels of antioxidants and superoxide dismutase in their lungs than young rats do (Rikans and Moore 1988; Perez et al 1991). In addition, mucociliary clearance rates were found to be lower in older than in younger people (Goodman et al 1978) and rats (Wolff et al 1987).

One study of healthy volunteers showed increased levels of neutrophils and interleukin 8, which indicates low-grade inflammation, in individuals 64 to 83 years of age compared to those 19 to 36 years of age (Meyer et al 1998). Some studies have shown deposition of ultrafine or fine particles to be greater in individuals with obstructive lung disease than in healthy individuals (Anderson et al 1990; Kim and Kang 1997). A recent study by Frampton and colleagues (2004) also found that deposition of ultrafine carbon particles was greater in volunteers with asthma than in healthy volunteers. These results suggest that both older age and disease increase sensitivity to health effects of air pollution.

At the time the Nikula/Hahn study started, few animal studies had systematically investigated whether aged individuals are more susceptible to the effects of air pollutants than young individuals are. One study of ozone did not find differences in lung damage between young and old rats (Pinkerton et al 1998). In addition to their other aims, Drs Nikula and Hahn therefore proposed to evaluate health effects of particle exposure in aged rats compared to young adult rats. They proposed to use an animal model of respiratory disease—aged rats exposed to endotoxin—to mimic some pathogenic features in older individuals with bronchitis or bronchopneumonia. They hypothesized that such a model would increase the likelihood of finding effects associated with particle exposures at concentrations well below the recommended occupational levels.

TECHNICAL EVALUATION

SPECIFIC AIMS

The goal of this study was to compare effects of inhaling particles of varying size and composition in young adult and aged rats with mild lung inflammation. The specific aims were to: (1) determine the relation between size of respirable particles (ultrafine vs fine) and particle-induced lung inflammation and injury and determine the effect of a short-term spike in particle concentration; (2) determine the effect of a short-term spike in particle concentration on lung inflammation and injury, using V_2O_5 and carbon; and (3) determine the effect of age on particle-induced lung inflammation and injury in young adult rats (3 months old) versus aged rats (24 months old).

All rats were intratracheally instilled with endotoxin to represent a model of low-level lung inflammation. Assessed outcomes included cytotoxicity, inflammation, macrophage activation, and airway mucus production.

APPROACH

The investigators used a previously developed deposition model to estimate the relative deposition of fine and ultrafine particles. They had identified predetermined concentrations to which rats would be exposed and used the model to make adjustments according to relative deposition. The model was designed to address several issues: (1) differences in deposition rate between ultrafine and fine particles; (2) differences between carbon and vanadium particle characteristics that may affect particle deposition; (3) differences in lung deposition between young adult and aged rats due to differences in lung morphology; and (4) the need to incorporate a spike in exposure concentration into the model. Revisions to the model were made during the study, to add a clearance component, for example.

The investigators conducted three pilot experiments to establish the dose of endotoxin that produces mild bronchitis, the time course of inflammation after endotoxin instillation, and the safety of the combination of endotoxin and inhaled V_2O_5 for aged rats.

During the three main experiments, F344 rats were exposed for 5 hours/day for 17 consecutive days via nose-only inhalation to ultrafine or fine V_2O_5 or ultrafine carbon particles. Ultrafine particle concentrations were 50, 100, 150, or 200 $\mu g/m^3$ and fine particle concentrations were 150, 200, 500, or 1000 $\mu g/m^3$. Control groups were exposed to filtered air. On day 15, all rats were intratracheally instilled with endotoxin. On days 16 and 17, some groups were exposed to higher concentrations of particles to mimic a short-term increase in air pollution. The other groups remained exposed to the same concentration as on days 1 through 15. All rats were killed 18 hours after the end of exposure on day 17. They were assessed for lung morphology, lung particle burden, and markers of inflammation and oxidative stress in lung tissue and bronchoalveolar lavage (BAL) fluid. In addition, they were evaluated for mononuclear cell leukemia, a relatively common condition in aged F344 rats. Rats with lungs involved with this condition were excluded from data analysis.

METHODS

Aerosol Generation and Characterization

The investigators used three systems to generate particles. Ultrafine V_2O_5 particles were generated by vaporizing V_2O_5 powder in a furnace at 900°C. The vapor was transported using N_2 carrier gas to a condensation chamber, where ultrafine particles formed with a count median diameter of approximately 24 to 40 nm.

Fine V_2O_5 particles were generated by nebulizing a V_2O_5 solution (0.1 g/100 mL distilled water). The resultant droplets were dried to form V_2O_5 particles of 0.7 µm mass median aerodynamic diameter by passing the aerosol through a desiccant chamber and adding additional dry air.

Ultrafine carbon particles were generated with an electric spark discharge generator, which uses high voltage to generate sparks between graphite electrodes. The graphite vapor condenses to ultrafine carbon particles (35 nm count median diameter). Analysis of carbon particles collected on filters showed that they were 70% organic carbon and 30% elemental carbon.

The investigators sampled the room in which rats were housed and one rat cage that housed four rats for background ambient aerosol concentrations. They reported an average background particle mass concentration of about 40 $\mu g/m^3$ inside the cage, indicating that exposing rats to experimental particle concentrations below 50 $\mu g/m^3$ was not feasible.

Exposures occurred in a 96-port nose-only exposure chamber. For each of the three aerosols, concentration was controlled by removing some of the aerosol and diluting the remaining aerosol with clean air. In the exposure chamber, ultrafine particle levels were monitored by using filter samples to determine mass, a scanning mobility particle sizer to determine size distribution, and a condensation nucleus counter to determine particle number concentrations. Fine particle levels were monitored by using filter samples to determine mass and a seven-stage multijet cascade impactor to determine size distribution. The morphologies of both ultrafine and fine particles were examined using transmission electron microscopy. Samples were taken at the beginning, middle, and end of each 5-hour exposure. Densities of the ultrafine V_2O_5 and carbon aerosols were estimated from the particle number concentrations, filter mass, and count median diameter.

Animals

A total of 225 F344 rats was used for the main experiments and about 125 rats for the pilot experiments. The aged rats (23–24 months old) weighed on average 420 g. The young adult rats (8–10 weeks old upon receipt and 12–14 weeks during exposure) weighed on average 230 g. The rats were acclimated for 10 to 14 days upon arrival and were conditioned to the exposure tubes for 3 hours a day for 3 days before the start of the 17-day experiments. Eighteen rats were excluded from data analysis due to aging-related

health problems or problems with experimental manipulations; no deaths were attributed to particle exposures.

Pilot Experiments

The investigators performed three pilot experiments to determine the optimal conditions to produce mild inflammation using endotoxin. In the first pilot experiment, three aged rats per group (15 rats total) were intratracheally instilled with sterile, endotoxin-free saline or with either 0.3 or 0.5 mg endotoxin/kg body weight and were killed 1 or 3 days later. Analysis of differential cell counts in BAL fluid and microscopic examination of lung sections showed substantial inflammation on day 1 for both endotoxin doses. On day 3, the histopathologic changes were less pronounced in rats instilled with 0.3 mg/kg than in rats instilled with 0.5 mg/kg. On the basis of these results, the investigators decided to decrease the endotoxin dose for the second pilot experiment.

In the second pilot experiment, three to four aged rats per group (between 36 and 48 rats total) were exposed to 100 μg/m^3 V_2O_5 or to filtered air for 5 hours/day for 7 consecutive days. Rats were instilled with 0.1, 0.2, or 0.5 mg/kg endotoxin or sterile, endotoxin-free saline on exposure day 5. All rats were killed 1 or 3 days after instillation and assessed for pulmonary inflammation. The results indicated that the 0.1 mg/kg dose of endotoxin induced mild inflammation on day 1 that was resolved by day 3. The 0.2 mg/kg dose produced greater inflammation that was not completely resolved on day 3. Therefore, the 0.1 mg/kg dose was selected for the main experiments. The results also showed that the chosen V_2O_5 concentration was not toxic to endotoxin-treated aged rats.

In the third pilot experiment, 24 aged rats per group (72 rats total) were sham instilled or instilled with 0.1 mg endotoxin/kg body weight or with saline and examined for lung inflammation 5 hours, 1 day, or 3 days after instillation (eight rats per time point) to assess the time course of endotoxin-induced inflammation. This experiment is discussed in Results and Interpretation.

Experimental Design of Main Experiments

An overview of the groups and exposure concentrations is provided in the Commentary Table. Two to three hours before the particle exposure on day 15, all rats were instilled with 0.1 mg endotoxin/kg body weight under light anesthesia. On days 16 and 17, some groups were exposed to a higher (spiked) concentration of particles.

In experiment 1, 10 groups of 12 to 14 aged rats were exposed to ultrafine V_2O_5 (0.03 μm count median diameter; 50–200 μg/m^3) or fine V_2O_5 (0.7 μm mass median aerodynamic diameter; 150–1000 μg/m^3) via nose-only inhalation. Two control groups of aged rats (one for the ultrafine and another for the fine particle exposures) were exposed to filtered air. In experiment 2, two groups of 12 to 14 aged rats were exposed to inhaled ultrafine carbon (0.03 μm count median diameter; 150–200 μg/m^3). A control group of aged rats was simultaneously exposed to filtered air. In experiment 3, groups of 10 young adult and aged rats were exposed to fine V_2O_5 at 500 μg/m^3. Control groups of young adult and aged rats were simultaneously exposed to filtered air. Groups in experiment 2 were compared to groups exposed to the corresponding concentrations in experiment 1 (see Commentary Table).

Commentary Table. Experimental Design[a]

Particles and Exposure Group	Exposure Concentrations[b] (μg/m^3)
Experiment 1	
Ultrafine V_2O_5—aged rats	0[c]
	50
	50 → 100
	100
	150
	150 → 200[c]
	200[c]
Fine V_2O_5—aged rats	0
	150 → 200
	200
	500 → 1000
	1000
Experiment 2[d]	
Ultrafine carbon—aged rats	0
	150 → 200
	200
Experiment 3	
Fine V_2O_5—young adult and aged rats	0
	500

[a] On day 15 of the 17 days of exposure, all rats were intratracheally instilled with endotoxin 2–3 hours before the exposure.

[b] A single number indicates that the exposure concentration was the same on days 1–17. If two numbers are listed, the second number indicates the spike in exposure concentration on days 16 and 17.

[c] Used for comparison to the corresponding groups in experiment 2.

[d] Because lung responses for ultrafine carbon particles were less than for ultrafine V_2O_5 particles—in spite of a higher predicted lung burden—the investigators decided to test neither lower concentrations of ultrafine carbon nor fine carbon particles.

Deposition Calculations

For the preliminary deposition calculations, the investigators used a model developed by Dr Ménache to determine fine and ultrafine particle concentrations that would result in similar predicted depositions (Ménache et al 1996). The model took into account particle diameter and density and allowed for variability. Table 4 of the Investigators' Report provides the predicted pulmonary depositions of fine and ultrafine V_2O_5 particles for various exposure concentrations. A 50 $\mu g/m^3$ ultrafine particle concentration was calculated to yield a mass lung burden roughly equivalent to that yielded by the 150 $\mu g/m^3$ fine particle concentration, and a 150 $\mu g/m^3$ ultrafine particle concentration was calculated to be roughly equivalent to the 500 $\mu g/m^3$ fine particle concentration. Details about the deposition calculations are provided in Appendix C of the Investigators' Report.

Endpoints

The investigators grouped the biological responses into primary, key, and supporting indicators (see Table 3 of the Investigators' Report) to focus the interpretation of results on the most biologically meaningful changes.

Lung Particle Burden The investigators measured the vanadium particle burden in lung tissue by isolating tissue, from a subset of rats, that was processed in acid and heated to 100°C for 4 hours. Vanadium content was measured using inductively coupled plasma mass spectrometry. To determine carbon particle lung burden, the investigators used a spectrophotometric method but reported that it did not yield valid results. The measured lung burdens were used to further adjust the deposition model with the goal of analyzing the endpoints by predicted lung burden rather than by exposure concentration.

BAL Analysis BAL fluid was assessed for cell viability and number, including differential cell counts to determine the numbers and percentages of macrophages, neutrophils, and lymphocytes. BAL fluid was also assessed for total protein content as an indicator of lung damage, lactate dehydrogenase (LDH) activity as an indicator of cytotoxicity, β-glucuronidase activity as an indicator of increased activity or increased lysis of macrophages, and levels of the inflammatory cytokines tumor necrosis factor α (TNF-α) and macrophage inflammatory protein-2 (MIP-2). In addition, adherent cells (primarily macrophages) were isolated from BAL fluid for assessment of superoxide anion and hydrogen peroxide production, indicators of oxidative stress. Further, protein carbonyls were measured in BAL fluid to assess oxidation of proteins in the lung.

Morphologic Analysis Airways were dissected to the 12th generation of branching. Lung sections were stained for mucosubstances and subjected to computerized image analysis of their volume density per unit area of basal lamina. Other lung sections were examined and assigned a score from 0 to 4 for degree of alveolar inflammation, interstitial reaction, alveolar macrophage hyperplasia, and perivascular edema, resulting in a combined score of 0 to 16. Rats showing invasion of lung parenchyma by leukemic cells were excluded from analysis.

Statistics All data were tested for homogeneity of variance and transformed as needed. For experiment 1, the dose level used for analysis was the predicted lung burden determined by the deposition model, rather than the exposure concentration, taking into account the different depositions of fine and ultrafine particles as well as the spiked concentrations. The model was adjusted to incorporate a clearance component and information from the measured lung burden in the small number of animals for which this information was available.

The data were analyzed in two stages. First, the investigators fitted dose–response relations for the nonspiked exposures of fine and ultrafine particles, using linear or logistic regression depending on the outcome variable. They allowed the fitted model to have different intercepts and slopes for fine and ultrafine particles, in the manner of analysis of covariance. Second, they determined whether data from spiked exposures were above or below what would be expected from the nonspiked exposures by comparing them to the fitted curves determined in the first stage. Experiments 2 and 3 were analyzed by two-way analysis of variance. The factors for experiment 2 were particle composition (V_2O_5 vs carbon) and exposure concentration; for experiment 3, particle concentration and rat age.

RESULTS AND INTERPRETATION

Time Course of Endotoxin-Induced Inflammation (Pilot Experiment)

Rats instilled with endotoxin had acute inflammation 5 hours after endotoxin instillation, as shown by increased levels of TNF-α, protein, and neutrophils in BAL fluid compared with controls (sham-instilled or saline-instilled rats). TNF-α and protein levels returned to control levels after 3 days, whereas neutrophil numbers decreased but remained significantly elevated. β-Glucuronidase levels

peaked 1 day after instillation and returned to control levels after 3 days. LDH levels showed the greatest increase compared with control levels 1 day after instillation but remained elevated after 3 days. Alveolar macrophage numbers in BAL fluid were not elevated at any time after endotoxin instillation.

In lung tissue of rats instilled with endotoxin, edema and alveolar inflammatory infiltrates were most prominent 5 hours after instillation. Increased interstitial infiltrates and alveolar macrophage hyperplasia were most prominent 1 and 3 days after instillation. Mucous cell metaplasia was significantly increased 3 days after endotoxin instillation compared with controls.

These results indicate that in the main experiments investigators could reasonably expect to see changes in BAL fluid levels of neutrophils, LDH, and possibly protein and β-glucuronidase, as well as histologic changes in inflammation and mucous cell metaplasia, 3 days after instillation.

Aerosol Characteristics

The ultrafine particle size of 35 nm was achieved as intended. The fine particle size of 0.7 µm was smaller than the target of 2.0 µm but is within the defined range for fine particles. Exposure concentrations of the fine and ultrafine aerosols were within approximately 5% of the target concentration, except for the spiked concentration of fine V_2O_5, which was within 10% (180 instead of 200 µg/m^3).

Summary of Main Results

Actual Versus Predicted Lung Burden In the original deposition model that was used to determine exposure concentrations, predicted lung burden was about 100-fold higher than observed lung burden. After introducing clearance equations into the model, the difference was 2- to 5-fold. Because measuring actual lung burden in all rats was impractical, the investigators adjusted the predicted lung burden for all animals on the basis of the observed lung burden in a subset of animals. They then used this adjusted predicted lung burden to analyze the effect of exposures on biological endpoints (instead of analyzing the data by exposure concentration). The investigators stated that such analyses—taking into account the dose to the lung—better captured differences between fine and ultrafine particles than analyses by exposure concentration would. In the remainder of the Commentary, the term *predicted lung burden* is used to denote the *adjusted predicted lung burden* that was used for data analysis.

Effect of V_2O_5 Particle Size Exposure to fine and ultrafine V_2O_5 caused concentration-dependent increases in several markers of inflammation. On the basis of equal particle mass (150+50 or 200 µg/m^3), ultrafine particle exposures caused a greater lung burden than fine particle exposures did. On the basis of equal predicted lung burden of particles in the lung, a consistently greater number of neutrophils in BAL fluid were found after exposure to ultrafine V_2O_5 than to fine V_2O_5. However, few other differences in markers of inflammation were observed. Also, few changes in markers of oxidative stress were observed in any exposed group.

The investigators observed that for several endpoints a greater effect was observed after a 2-day spike in exposure concentration than was expected on the basis of predicted lung burden (see Table 10 of the Investigators' Report). For example, LDH and β-glucuronidase activity, MIP-2 levels, and numbers of total cells, neutrophils, and macrophages in BAL fluid were greater after exposure to 150 µg/m^3 fine V_2O_5 for 15 days followed by 200 µg/m^3 for 2 days than after exposure to 200 µg/m^3 for 17 days. A similar effect was observed in rats exposed to 150 µg/m^3 ultrafine V_2O_5 for 15 days followed by 200 µg/m^3 for 2 days for numbers of total cells, neutrophils, lymphocytes, and macrophages in BAL fluid as well as mucus production in the airways. Additional evidence was observed in rats exposed to 50 followed by 100 µg/m^3 ultrafine V_2O_5: these animals showed increased TNF-α activity, MIP-2 levels, and numbers of lymphocytes compared with rats exposed to 100 µg/m^3 for 17 days. No effect of a 2-day spike in concentration was observed at the high exposure concentrations for fine V_2O_5 (500 and 1000 µg/m^3), however.

The investigators reported that no animals became ill or died because of particle exposure but that rats exposed to 1000 µg/m^3 fine V_2O_5 were lethargic at the end of each exposure period. Animals exposed to ultrafine V_2O_5 showed greater body weight loss than those exposed to fine particles did, and their lung weights increased more rapidly with increasing exposure concentration. Some weight loss was expected on the basis of normal weight curves for aging rats, but the lack of access to food for 5 hours/day may also have contributed.

Effect of Ultrafine Particle Composition Exposure to ultrafine V_2O_5 caused greater lung responses than exposure to ultrafine carbon did for many important endpoints, such as LDH activity and neutrophil numbers in BAL fluid and mucus production in the small airways. In many cases, the response was greater after exposure to 150 µg/m^3 for 15 days followed by 200 µg/m^3 for 2 days than after exposure to 200 µg/m^3 for 17 days. Such an effect was

observed for LDH activity and protein levels in BAL fluid (for ultrafine carbon only), for neutrophil, total cell, lymphocyte, and macrophage numbers in BAL (for both ultrafine carbon and V_2O_5), and for volume density of mucus in airway generations 12 and 4 (for ultrafine V_2O_5 only). Because lung responses for ultrafine carbon particles were generally less than for ultrafine V_2O_5 particles—in spite of a higher predicted lung burden based on density and solubility—the investigators decided not to expose rats to lower concentrations or fine carbon particles.

Responses in Aged Versus Young Adult Rats Lung burden was considerably higher in aged rats compared to young adult rats, and aged rats showed greater weight loss during the study, especially after V_2O_5 exposures. In both young adult and aged rats, inflammatory responses were observed after exposure to fine V_2O_5, with no major differences in response between the two groups.

Investigators' Conclusions

- A greater mass of ultrafine V_2O_5 particles was retained in the lungs of aged, endotoxin-treated rats compared with fine V_2O_5 particles when the rats were exposed to aerosols of a similar mass concentration.
- Inhaled ultrafine V_2O_5 caused significantly more inflammation (represented by neutrophil numbers in BAL fluid) in aged, endotoxin-treated rats than did inhaled fine V_2O_5 when compared on a basis of equal predicted lung burden.
- An increase in particle exposure concentration for 2 days after constant exposure for 15 days resulted in significantly increased inflammatory responses in the lungs of aged, endotoxin-treated rats compared with aged rats exposed to a constant particle level for 17 days.
- Aged and young adult rats did not differ significantly in their responses to inhaled fine V_2O_5.

DISCUSSION

This multifaceted study was carefully designed to address several important issues related to the toxicity of ambient particles. The investigators found that exposure for 2 weeks to fine and ultrafine particles caused an inflammatory response (ie, increased numbers of neutrophils in BAL fluid) in aged, endotoxin-treated rats. The results indicate that the lung burden of ultrafine V_2O_5 particles was greater than that of fine V_2O_5 particles at an equal inhaled mass concentration. On the basis of equal predicted lung burden, the investigators found more neutrophils in BAL fluid from rats exposed to ultrafine V_2O_5 than from rats exposed to fine V_2O_5, but many other parameters did not differ.

In general, exposure to ultrafine V_2O_5 caused greater lung responses (eg, LDH levels, neutrophil numbers, and production of mucus in small airways) than exposure to ultrafine carbon did, even though predicted lung burden was greater for carbon particles. Because the method used to determine lung burden of carbon particles was ineffective, the predicted difference in lung burdens of carbon and vanadium could not be confirmed experimentally. The investigators did not find major differences in inflammatory responses between aged and young adult rats exposed to fine V_2O_5, although particle lung burden appears to have been greater in aged rats.

An interesting and novel finding was that an increase in exposure concentration for 2 days after 15 days of exposure caused greater inflammatory effects than exposure at a constant level (either at the original lower or the final higher concentration). One possible explanation for this result is that an adaptive response occurred during the 17-day constant exposures, reducing the final inflammatory response. There are some indications that the spike effect may occur regardless of the initial concentration; similar associations have been found in cities with different levels of pollution (Samet et al 2000).

The spike effect was also found in rats exposed to fine or ultrafine particles of different composition. However, the investigators noted that the effects occurred predominantly at 150 $\mu g/m^3$ with a final, spiked concentration of 200 $\mu g/m^3$, but not at 50 $\mu g/m^3$ (final concentration 100) or 500 $\mu g/m^3$ (final concentration 1000). This lack of effect at some concentrations remains unexplained. More research is needed to further investigate and confirm the increased effect of a short-term spike in exposure concentration. Further exploration of the time course of inflammatory responses after constant and spiked particle exposures is also recommended.

ANIMAL MODEL

The investigators conducted three pilot studies to establish optimal conditions for inducing mild inflammation in aged rats by endotoxin treatment. They discuss the limitations of this model in the Investigators' Report. For example, endotoxin treatment causes transient, low-level lung inflammation, which differs from the persistent inflammation that occurs in individuals with chronic lung disease. Also, several other pathologic features occurring in chronic lung diseases are not present in endotoxin-treated rats. Therefore, the results should be interpreted

cautiously with respect to possible effects of particles on inflammation in older people with chronic lung disease.

Endotoxin can be administered in several ways, such as by intratracheal instillation or by inhalation of an aerosol. Although inhalation is preferable because it is a more realistic route of exposure that avoids an acute, high dose to the lungs, the investigators used intratracheal instillation because it causes less variability in exposure levels. They were careful to determine the optimal concentration and the safety of the procedure for the aged rats, and they exposed the control groups to the same endotoxin treatment.

The timing of instillation differed from that in other studies, in which endotoxin was administered before short-term particle exposures (eg, Harkema et al 2004). In the current study, rats were exposed to particles for 15 days without any endotoxin treatment. Administering endotoxin on day 15 and examining the effects 2 days later results in an acute rather than a chronic inflammatory response. What the effect of additional endotoxin treatment before and during the first week of particle exposures would have been is unknown. Such repeated exposures might more closely resemble chronic inflammation in older people than a single exposure would.

Whether the chosen endotoxin concentration (100 µg/kg) was optimal for the younger rats in this study is unclear, because the pilot studies were performed with aged rats only. This endotoxin concentration may or may not have induced similar levels of inflammation with a similar time course in the young adult rats, an uncertainty that complicates interpretation of the results. Another factor that limited the comparison between aged and young adult rats is that some planned experiments were called off because the animal supplier could not provide additional aged rats. Thus, only one particle concentration was tested for aged and young adult rats.

A concern about the animal exposure procedures is that they are stressful, as shown by the substantial weight loss (at least 10%) of all rats over the course of the experiment. Although the aged rats were expected to lose some weight owing to normal body weight changes, their being physically restrained in the tight-fitting tubes used for nose-only exposures and their lack of access to food for 5 hours/day likely contributed to their weight loss.

PARTICLE CHARACTERISTICS

The particle generation and characterization were thorough in this study. However, the particles were generated by different methods: V_2O_5 by vaporizing a powder (ultrafine particles) or by nebulizing a solution (fine particles); ultrafine carbon by a spark generator with graphite electrodes. These differences in particle generation might have introduced additional differences, such as in charge or other surface characteristics. Therefore, the comparability of particles is uncertain, in particular for ultrafine V_2O_5 and carbon particles.

Another consideration is that V_2O_5 and carbon particles differ in density and solubility. Because particles are porous and are likely to form porous chain aggregates, the density of fine V_2O_5 particles is probably less than that reported by the investigators. Calculation of ultrafine particle density depends on the accuracy of measuring particle number and count median diameter. The calculations in Investigators' Report Appendix B indicate that ultrafine V_2O_5 particles were somewhat smaller (27.6 nm) and denser (3.07 g/cm^3) than ultrafine carbon particles (46.5 nm and 2.70 g/cm^3) were. These numbers are probably uncertain because of instrument variability and the fact that they were measured at different dilutions: density was assessed for V_2O_5 at 100 µg/m^3 and for carbon at 200 µg/m^3. In addition, V_2O_5 particles are much more soluble than carbon particles.

Differences in particle generation and particle diameter, density, and solubility are all important for the deposition calculations and may also have influenced the biological effects of the particles. Other studies have shown that solubility and other particle characteristics may be important (Leikauf et al 2001; Donaldson et al 2002). The responses observed in the current study are likely to have been both qualitatively and quantitatively different. For example, compared with filtered air, V_2O_5 caused increased mucus gland hyperplasia, whereas carbon caused increased macrophage recruitment. These differences could have been discussed in more detail in the report.

DEPOSITION MODELING

The deposition model originally developed by Dr Ménache was reevaluated and adjusted for this study. For example, it was adjusted to accommodate the spike in exposure concentration during the last 2 days of exposure. At the beginning of the study, the investigators used the original model—which did not yet include a clearance factor—to determine which mass concentrations of fine and ultrafine particles would yield similar lung burdens. The predicted lung burden followed, on average, the same patterns as the measured lung burden across the exposure concentrations.

The model initially did not perform as well regarding the measured lung burden, however: it showed a 100-fold difference between predicted and measured lung burden. The model was then adjusted by introducing clearance equations for V_2O_5 and carbon; with these adjustments,

the actual lung burden was 2- to 5-fold lower than the predicted lung burden. As a final step, the predicted lung burden was adjusted on the basis of a comparison of actual and predicted lung burdens in the subset of animals for which actual lung burden measurements were available (see Appendix C).

Because of the high solubility of V_2O_5, the lung burden 17 days after exposure does not necessarily reflect deposition in the lung. Vanadium measured in the lung is likely dissolved and bound to proteins and other molecules rather than still being present in particle form. The clearance mechanisms and rates for V_2O_5 and carbon particles probably also differ (which the model accounted for). Unfortunately, actual measurements of carbon particle lung burden could not be completed. Therefore, validating the model predictions with measured carbon burdens was not possible.

The analyses of experiment 1 data (comparison of effects of fine and ultrafine V_2O_5 particles) relied on modeled lung burden values as the exposure variable; predicted lung burden was treated as a quantitative measure of dose in regression analyses. This approach is interesting, if slightly unusual. It was valuable because it aimed to elucidate the relation between exposure concentration and tissue dose. The HEI Health Review Committee thought that performing additional data analyses using actual chamber concentrations as the exposure variable would have added insight to the study because regulation of air pollutant standards is based on exposure concentrations rather than calculated deposition.

SUMMARY AND CONCLUSIONS

In this complex study, the investigators found that exposure for 17 days to fine and ultrafine particles caused an inflammatory response in aged rats with mild inflammation induced by endotoxin treatment. A greater mass of ultrafine V_2O_5 particles than fine V_2O_5 particles was deposited in the lung at the same inhaled mass concentration. On the basis of equal predicted lung burden, more neutrophils were found in BAL fluid from rats exposed to ultrafine V_2O_5 than from rats exposed to fine V_2O_5, indicating that ultrafine particles were more toxic. In general, exposure to ultrafine V_2O_5 caused greater lung responses (eg, LDH levels, neutrophil numbers, and volume of mucus in small airways) than exposure to ultrafine carbon did. No major differences were found between aged and young adult rats exposed to fine V_2O_5.

The results of this study suggest that, under the experimental conditions used, both particle size and composition are important for particle toxicity. However, more research using different kinds of particles and animal models is needed before generalizing these results to the effects of fine and ultrafine particles in the ambient air pollution mixture.

Some uncertainty remains about Hahn and colleagues' comparison of the effects of fine and ultrafine vanadium because of uncertainties in the model. The model accounted well for several aspects of the experimental design, but it may not have accounted for some differences in particle characteristics between V_2O_5 and carbon, such as in solubility, porosity, and tendency to aggregate into larger groups or chains, which would be difficult to incorporate into any model. The investigators used an innovative approach of analyzing data by predicted particle lung burden rather than exposure concentration to evaluate the effects of fine versus ultrafine V_2O_5. Direct validation of the model remained incomplete, however, because lung burden was determined only in a subset of animals exposed to V_2O_5 and because the method used to measure carbon lung burden did not yield valid results (although the predicted lung burdens for carbon exposures were not used for data analysis).

An interesting and novel finding was that an increase in exposure concentration for 2 days after 15 days of exposure caused greater inflammatory effects than exposure at a constant level (either at the original lower or the final higher concentration). The increase in exposure concentration was modeled after epidemiologic observations that a short-term increase in ambient PM concentration is associated with an increase in deaths and hospital admissions. There are some indications that such an association may exist regardless of the initial concentration, because similar short-term associations have been found in cities with different levels of pollution. In the current study, the increased effect of a spiked exposure concentration was found in rats exposed to fine or ultrafine particles of different composition, but not for all concentrations tested. More research is needed to further investigate and confirm the increased effect of a short-term spike in exposure concentration.

ACKNOWLEDGMENTS

The Health Review Committee thanks the ad hoc reviewers for their help in evaluating the scientific merit of the Investigators' Report. The Committee is also grateful to Maria Costantini for her oversight of the study, to Annemoon van Erp for her assistance in preparing its Commentary, to Jenny Lamont and Joann Merriman for science editing of the report and this Commentary, and to Sally Edwards, Melissa R Harke, Fred Howe, Kasey L Oliver, and Ruth E Shaw for their roles in publishing this Research Report.

REFERENCES

Agency for Toxic Substances and Disease Registry. 1992. Toxicological Profile for Vanadium. US Department of Health and Human Services, Public Health Service, Atlanta GA.

Anderson HR, Atkinson RW, Bremner SA, Marston L. 2003. Particulate air pollution and hospital admissions for cardiorespiratory diseases: Are the elderly at greater risk? Eur Respir J Suppl 40:39s–46s.

Anderson KR, Avol EL, Edwards SA, Shamoo DA, Peng R-C, Linn WS, Hackney JD. 1992. Controlled exposures of volunteers to respirable carbon and sulfuric acid aerosols. J Air Waste Manage Assoc 42:770–776.

Anderson PJ, Wilson JD, Hiller FC. 1990. Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease. Chest 97:1115–1120.

Becker S, Soukup JM, Gilmour MI, Devlin RB. 1996. Stimulation of human and rat alveolar macrophages by urban air particulates: Effects on oxidant radical generation and cytokine production. Toxicol Appl Pharmacol 141:637–648.

Benson JM, Chang IY, Cheng YS, Hahn FF, Kennedy CH, Barr EB, Maples KR, Snipes MB. 1995. Particle clearance and histopathology in lungs of F344/N rats and B6C3F1 mice inhaling nickel oxide or nickel sulfate. Fundam Appl Toxicol 28:232–244.

Carter JD, Ghio AJ, Samet JM, Devlin RB. 1997. Cytokine production by human airway epithelial cells after exposure to an air pollution particle is metal-dependent. Toxicol Appl Pharmacol 146:180–188.

Costa DL, Dreher KL. 1997. Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animal models. Environ Health Perspect 105(Suppl 5):1053–1060.

Dockery DW, Pope CA III. 1994. Acute respiratory effects of particulate air pollution. Annu Rev Public Health 15:107–132.

Donaldson K, Brown D, Clouter A, Duffin R, MacNee W, Renwick L, Tran L, Stone V. 2002. The pulmonary toxicology of ultrafine particles. J Aerosol Med 15(2):213–220.

Driscoll KE, Maurer JK. 1991. Cytokine and growth factor release by alveolar macrophages: Potential biomarkers of pulmonary toxicity. Toxicol Pathol 19:398–405.

Dubois CM, Bissonnette E, Rola-Pleszczynski M. 1989. Asbestos fibers and silica particles stimulate rat alveolar macrophages to release tumor necrosis factor. Autoregulatory role of leukotriene B4. Am Rev Respir Dis 139:1257–1264.

Environmental Protection Agency (US). 1996. Air Quality Criteria for Particulate Matter, Vol I. EPA/600/P-95/001aF. Office of Research and Development, Washington DC.

Environmental Protection Agency (US). 2002. Health Assessment Document for Diesel Engine Exhaust. EPA/600/8-90/057F. Office of Research and Development, Washington DC.

Environmental Protection Agency (US). 2004. Air Quality Criteria for Particulate Matter. Volume I. EPA/600/P-99/002aF. National Center for Environmental Assessment, Research Triangle Park NC.

Ferin J, Oberdörster G, Penney DP. 1992. Pulmonary retention of ultrafine and fine particles in rats. Am J Respir Cell Mol Biol 6:535–542.

Frampton MW, Utell MJ, Zareba W, Oberdörster G, Cox C, Huang L-S, Morrow PE, Lee FE-H, Chalupa D, Frasier LM, Speers DM, Stewart J. 2004. Effects of Exposure to Ultrafine Carbon Particles in Healthy Subjects and Subjects with Asthma. Research Report 126. Health Effects Institute, Boston MA.

Gavett SH, Madison SL, Dreher KL, Winsett DW, McGee JK, Costa DL. 1997. Metal and sulfate composition of residual oil fly ash determines airway hyperreactivity and lung injury in rats. Environ Res 72:162–172.

Goldberg MS, Bailar JC III, Burnett RT, Brook JR, Tamblyn R, Bonvalot Y, Ernst P, Flegel KM, Singh RK, Valois M-F. 2000. Identifying Subgroups of the General Population That May Be Susceptible to Short-Term Increases in Particulate Air Pollution: A Time-Series Study in Montreal, Quebec. Research Report 97. Health Effects Institute, Cambridge MA.

Goodman RM, Yergin BM, Landa JF, Golivanux MH, Sackner MA. 1978. Relationship of smoking history and pulmonary function tests to tracheal mucous velocity in nonsmokers, young smokers, ex-smokers, and patients with chronic bronchitis. Am Rev Respir Dis 117:205–214.

Gosset P, Lassalle P, Vanhee D, Wallaert B, Aerts C, Voisin C, Tonnel AB. 1991. Production of tumor necrosis factor-alpha and interleukin-6 by human alveolar macrophages exposed in vitro to coal mine dust. Am J Respir Cell Mol Biol 5:431–436.

Green DJ, Bascom R, Healey EM, Hebel JR, Sauder LR, Kulle TJ. 1989. Acute pulmonary response in healthy, nonsmoking adults to inhalation of formaldehyde and carbon. J Toxicol Environ Health 28:261–275.

Harkema JR, Keeler G, Wagner J, Morishita M, Timm E, Hotchkiss J, Marsik F, Dvonch T, Kaminski N, Barr E. 2004. Effects of Concentrated Ambient Particles on Normal and Hypersecretory Airways in Rats. Research Report 120. Health Effects Institute, Boston MA.

Health Effects Institute. 2002. Understanding the Health Effects of Components of the Particulate Matter Mix: Progress and Next Steps. HEI Perspectives. Health Effects Institute, Boston MA.

Killingsworth CR, Alessandrini F, Murthy GGK, Catalano PJ, Paulauskis JD, Godleski JJ. 1997. Inflammation, chemokine expression, and death in monocrotaline-treated rats following fuel oil fly ash inhalation. Inhalation Toxicol 9:541–565.

Kim CS, Kang TC. 1997. Comparative measurement of lung deposition of inhaled fine particles in normal subjects and patients with obstructive airway disease. Am J Respir Crit Care Med 155:899–905.

Knecht EA, Moorman WJ, Clark JC, Lynch DW, Lewis TR. 1985. Pulmonary effects of acute vanadium pentoxide inhalation in monkeys. Am Rev Respir Dis 132:1181–1185.

Kodavanti UP, Jaskot RH, Costa DL, Dreher KL. 1997. Pulmonary proinflammatory gene induction following acute exposure to residual oil fly ash: Roles of particle-associated metals. Inhalation Toxicol 9:679–701.

Kulle TJ, Sauder LR, Hebel JR, Miller WR, Green DJ, Shanty F. 1986. Pulmonary effects of sulfur dioxide and respirable carbon aerosol. Environ Res 41:239–250.

Kuschner WG, Wong H, D'Alessandro A, Quinlan P, Blanc PD. 1997. Human pulmonary responses to experimental inhalation of high concentration fine and ultrafine magnesium oxide particles. Environ Health Perspect 105:1234–1237.

Leikauf GD, McDowell SA, Wesselkamper SC, Miller CR, Hardie WD, Gammon K, Biswas PP, Korfhagen TR, Bachurski CJ, Wiest JS, Willeke K, Bingham E, Leikauf JE, Aronow BJ, Prows DR. 2001. Pathogenomic Mechanisms for Particulate Matter Induction of Acute Lung Injury and Inflammation in Mice. Research Report 105. Health Effects Institute, Boston MA.

Li XY, Gilmour PS, Donaldson K, MacNee W. 1996. Free radical activity and pro-inflammatory effects of particulate air pollution (PM_{10}) in vivo and in vitro. Thorax 51:1216–1222.

Li XY, Gilmour PS, Donaldson K, MacNee W. 1997. In vivo and in vitro proinflammatory effects of particulate air pollution (PM_{10}). Environ Health Perspect 105(Suppl 5):1279–1283.

Lipsett M, Hurley S, Ostro B. 1997. Air pollution and emergency room visits for asthma in Santa Clara County, California. Environ Health Perspect 105:216–222.

Lundborg M, Johard U, Lastbom L, Gerde P, Camner P. 2001. Human alveolar macrophage phagocytic function is impaired by aggregates of ultrafine carbon particles. Environ Res 86:244–253.

Mauderly JL, Snipes MB, Barr EB, Belinsky SA, Bond JA, Brooks AL, Chang I-Y, Cheng YS, Gillett NA, Griffith WC, Henderson RF, Mitchell CE, Nikula KJ, Thomassen DG. 1994. Part I: Neoplastic and nonneoplastic lung lesions. In: Pulmonary Toxicity of Inhaled Diesel Exhaust and Carbon Black in Chronically Exposed Rats. Research Report 68. Health Effects Institute, Cambridge MA.

Ménache MG, Raabe OG, Miller FJ. 1996. An empirical dosimetry model of aerodynamic particle deposition in the rat respiratory tract. Inhalation Toxicol 8:539–578.

Meyer KC, Rosenthal NS, Soergel P, Peterson K. 1998. Neutrophils and low-grade inflammation in the seemingly normal aging human lung. Mech Ageing Dev 104:169–181.

Mueller EJ, Seger DL. 1985. Metal fume fever: A review. J Emerg Med 2:271–274.

Murphy SA, BéruBé KA, Pooley FD, Richards RJ. 1998. The response of lung epithelium to well characterised fine particles. Life Sci 62:1789–1799.

Nadadur SS, Kodavanti UP. 2002. Altered gene expression profiles of rat lung in response to an emission particulate and its metal constituents. J Toxicol Environ Health A 65:1333–1350.

National Institute for Occupational Safety and Health. 2004. Pocket Guide to Chemical Hazards. NIOSH Publication 97-140. Government Printing Office, Pittsburgh PA. Available from *www.cdc.gov/niosh/npg/npgd0653.html.*

National Research Council. 2004. Research Priorities for Airborne Particulate Matter: IV. Continuing Research Progress. National Academies Press, Washington DC.

Nikula KJ, Avila KJ, Griffith WC, Mauderly JL. 1997. Sites of particle retention and lung tissue responses to chronically inhaled diesel exhaust and coal dust in rats and cynomolgus monkeys. Environ Health Perspect 105(Suppl 5):1231–1234.

Nikula KJ, Snipes MB, Barr EB, Griffith WC, Henderson RF, Mauderly JL. 1995. Comparative pulmonary toxicities and carcinogenicities of chronically inhaled diesel exhaust and carbon black in F344 rats. Fundam Appl Toxicol 25:80–94.

Oberdörster G. 1995. Lung particle overload: Implications for occupational exposures to particles. Regul Toxicol Pharmacol 21:123–135.

Oberdörster G, Ferin J, Gelein R, Soderholm SC, Finkelstein J. 1992. Role of the alveolar macrophage in lung injury: Studies with ultrafine particles. Environ Health Perspect 97:193–199.

Oberdörster G, Finkelstein JN, Johnston C, Gelein R, Cox C, Baggs R, Elder ACP. 2000. Acute Pulmonary Effects of Ultrafine Particles in Rats and Mice. Research Report 96. Health Effects Institute, Cambridge MA.

Oberdörster G, Gelein RM, Ferin J, Weiss B. 1995. Association of particulate air pollution and acute mortality: Involvement of ultrafine particles? Inhalation Toxicol 7:111–124.

Özkaynak H, Spengler JD. 1985. Analysis of health effects resulting from population exposures to acid precipitation precursors. Environ Health Perspect 63:45–55.

Pazynich VM. 1966. Experimental data for hygienic determination of the maximum permissible concentration of vanadium pentoxide in the air. Gig Sanit 31(7):8–12.

Pekkanen J, Timonen KL, Ruuskanen J, Reponen A, Mirme A. 1997. Effects of ultrafine and fine particles in urban air on peak expiratory flow among children with asthmatic symptoms. Environ Res 74:24–33.

Perez R, Lopez M, Barja de Quiroga G. 1991. Aging and lung antioxidant enzymes, glutathione, and lipid peroxidation in the rat. Free Radic Biol Med 10:35–39.

Peters A, Wichmann H-E, Tuch T, Heinrich J, Heyder J. 1997. Respiratory effects are associated with the number of ultrafine particles. Am J Respir Crit Care Med 155:1376–1383.

Pierce LM, Alessandrini F, Godleski JJ, Paulauskis JD. 1996. Vanadium-induced chemokine mRNA expression and pulmonary inflammation. Toxicol Appl Pharmacol 138:1–11.

Pinkerton KE, Weller BL, Ménache MG, Plopper CG. 1998. Part XIII: A comparison of changes in the tracheobronchial epithelium and pulmonary acinus in male rats at 3 and 20 months. In: Consequences of Prolonged Inhalation of Ozone on F344/N Rats: Collaborative Studies. Research Report 65. Health Effects Institute, Cambridge MA.

Pope CA III. 1989. Respiratory disease associated with community air pollution and a steel mill, Utah Valley. Am J Public Health 79:623–628.

Pope CA III. 2000. Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk? Environ Health Perspect 108(Suppl 4):713–723.

Ress NB, Chou BJ, Renne RA, Dill JA, Miller RA, Roycroft JH, Hailey JR, Haseman JK, Bucher JR. 2003. Carcinogenicity of inhaled vanadium pentoxide in F344/N rats and B6C3F_1 mice. Toxicol Sci 74:287–296.

Rikans LE, Moore DR. 1988. Effect of aging on aqueous-phase antioxidants in tissues of male Fischer rats. Biochim Biophys Acta 966:269–275.

Sagai M, Furuyama A, Ichinose T. 1996. Biological effects of diesel exhaust particles (DEP): III. Pathogenesis of asthma like symptoms in mice. Free Radic Biol Med 21:199–209.

Samet JM, Zeger SL, Dominici F, Curriero F, Coursac I, Dockery DW, Schwartz J, Zanobetti A. 2000. The National Morbidity, Mortality, and Air Pollution Study, Part II: Morbidity and Mortality from Air Pollution in the United States. Research Report 94. Health Effects Institute, Cambridge MA.

Schwartz J, Dockery DW. 1992. Increased mortality in Philadelphia associated with daily air pollution concentrations. Am Rev Respir Dis 145:600–604.

Timblin CR, Shukla A, Berlanger I, BéruBé KA, Churg A, Mossman BT. 2002. Ultrafine airborne particles cause increases in protooncogene expression and proliferation in alveolar epithelial cells. Toxicol Appl Pharmacol 179:98–104.

Utell MJ, Frampton MW. 2000. Acute health effects of ambient air pollution: The ultrafine particle hypothesis. J Aerosol Med 13(4):355–359.

Vincent R, Bjarnason SG, Adamson IYR, Hedgecock C, Kumarathasan P, Guénette J, Potvin M, Goegan P, Bouthillier L. 1997. Acute pulmonary toxicity of urban particulate matter and ozone. Am J Pathol 151:1563–1570.

Vincent R, Kumarathasan P, Goegan P, Bjarnason SG, Guénette J, BéruBé D, Adamson IY, Desjardins S, Burnett RT, Miller FJ, Battistini B. 2001. Inhalation Toxicology of Urban Ambient Particulate Matter: Acute Cardiovascular Effects in Rats. Research Report 104. Health Effects Institute, Boston MA.

Warheit DB, Hartsky MA. 1993. Role of alveolar macrophage chemotaxis and phagocytosis in pulmonary clearance responses to inhaled particles: Comparisons among rodent species. Microsc Res Tech 26:412–422.

Wellenius GA, Saldiva PH, Batalha JR, Krishna Murthy GG, Coull BA, Verrier RL, Godleski JJ. 2002. Electrocardiographic changes during exposure to residual oil fly ash (ROFA) particles in a rat model of myocardial infarction. Toxicol Sci 66:327–35.

Wolff RK, Henderson RF, Snipes MB, Griffith WC, Mauderly JL, Cuddihy RG, McClellan RO. 1987. Alterations in particle accumulation and clearance in lungs of rats chronically exposed to diesel exhaust. Fundam Appl Toxicol 9:154–166.

World Health Organization. 1988. Vanadium. Environmental Health Criteria 81. International Programme on Chemical Safety, Geneva, Switzerland.

Yang H-M, Ma JYC, Castranova V, Ma JKH. 1997. Effects of diesel exhaust particles on the release of interleukin-1 and tumor necrosis factor-alpha from rat alveolar macrophages. Exp Lung Res 23:269–284.

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RESEARCH
REPORT

Number 129
September 2005