11. TOXICOLOGICAL STUDIES OF PARTICULATE MATTER

11.1 INTRODUCTION

This chapter assesses results of exposure to particulate matter (PM) in controlled human clinical studies, selected occupational studies, and animal toxicology studies. It focuses mainly on those studies published since the 1982 Air Quality Criteria Document for Particulate Matter and Sulfur Oxides (U.S. Environmental Protection Agency, 1982), emphasizing coverage of selected constituents of ambient air PM that may contribute to those types of health effects found by epidemiological studies discussed in Chapter 12 of this document. The data discussed in Chapter 12 indicate that increased levels of PM in the ambient atmosphere are associated with increased mortality risk, especially among the elderly (aged 65+ years) and individuals with preexisting cardiopulmonary diseases, such as chronic obstructive pulmonary disease (COPD), pneumonia, and chronic heart disease. The epidemiology studies also provide evidence for associations of ambient PM exposures with increased risk of respiratory and cardiovascular morbidity effects (e.g., increased hospital admissions or emergency room visits for asthma or other respiratory problems, increased incidence of respiratory symptoms, or alterations in pulmonary function).

Chronic obstructive pulmonary disease is defined as a disease state characterized by the presence of airflow obstruction due to chronic bronchitis or emphysema; the airflow obstruction is generally progressive, may be accompanied by airway hyperreactivity, and may be partially reversible (American Thoracic Society, 1995). The biological responses occurring in the respiratory tract following controlled PM inhalation encompass a continuum of changes, including changes in pulmonary function, respiratory symptoms (i.e., wheeze, coughing, etc.), inflammation, and tumor formation. The responses observed are dependent on the physicochemical characteristics of the particulate matter, the total exposure and the health status of the host. However, many of the responses are usually seen only at distinctly higher level exposures characteristic of occupational and laboratory animal studies but not at typically much lower ambient particle concentrations.

Particulate matter is a broad term that encompasses thousands of chemical species, many of which have not been investigated in controlled laboratory animal or human studies. However, a full discussion of all the types of particles that have been studied is well beyond the scope of this chapter. Thus, criteria were used to select topics for presentation. High priority was placed on studies that: (1) may elucidate health effects of major common constituents of ambient PM (e.g., sulfates, carbon, silica) and/or (2) contribute to enhanced understanding of the epidemiological studies (e.g., real-world particles, "surrogate" particles; or particles with low inherent toxicity that may cause effects due to their generic nature as a particle, such as their ultrafine size). Based on these criteria, full summaries of acid aerosols, ultrafine particles, real-world particles, and "surrogate" particles are provided.

Diesel exhaust particles generally fit the criteria; but, because they are described in great detail elsewhere (U.S. Environmental Protection Agency, 1994; Health Effects Institute, 1995), they are only summarized briefly here. Diesel particles also differ from other particles in this classification because they are regulated pursuant to mobile source sections of the Clean Air Act (g/mi emission standards), although there is still a relationship of these regulations to the PM_{10} standard. Medium priority was placed on particles with high inherent toxicity that are of concern primarily because of point source emissions and more local exposures (as contrasted to ubiquitous pollutants). Metals having air concentrations greater than 0.5 $\mu g/m^3$ were placed in this class. The health effects of particles in this prioritization class are summarized far more briefly here. It must be emphasized that this prioritization is not related to a judgement or decision about potency or health risk. For example, it should not be inferred that on an individual exposure basis, a "high priority" particle is of more inherent health concern than a "medium priority" particle. The split is primarily related to regulatory issues. The Clean Air Act requires a criteria document for criteria pollutants. Except for lead, individual metals are not criteria pollutants. Rather, they are regulated as hazardous air pollutants under the Clean Air Act. Thus, their inclusion here is only intended to be generally instructive because they can be part of the complex mixture of PM in the ambient air.

As noted above, lead is a criteria air pollutant that, like particulate matter, is also regulated under Sections 108 and 109 of the Clean Air Act. Earlier extensive evaluations in Air Quality Criteria for Lead (U.S. Environmental Protection Agency, 1977) led to setting of

the current National Ambient Air Quality Standard (primary as well as secondary) for lead at 1.5 $\mu g/m^3$ on a quarterly average basis (Federal Register, 1978 [51594]). Subsequent to promulgation of that standard, the U.S. Environmental Protection Agency issued a revised Air Quality Criteria for Lead (1986a) and a Supplement (U.S. Environmental Protection Agency, 1990). These and other such assessments found blood lead levels of 10 $\mu g/dl$ in young children and women of child bearing age (due to risk to the fetus in utero) to be associated with unacceptable risk of slowed prenatal and postnatal growth and neuropsychological development. Air levels below 0.50 to 0.75 $\mu g/m^3$ lead have been proposed as adequate to protect against such risk (World Health Organization, 1987). Typical ambient air levels of lead in U.S. urban areas almost invariably now fall below 0.10 to 0.25 $\mu g/m^3$. The reader is referred to the above-noted air quality criteria documents/supplement and Federal Register notices concerning the lead National Ambient Air Quality Standard for detailed information on particulate lead health effects.

In some widespread geographic areas of the United States, silica can be among major ambient PM constituents and is discussed briefly here. The reader is referred to more extensive evaluation of silica elsewhere (U.S. Environmental Protection Agency, 1996). Asbestos fibers are also well established as a fibrogenic pollutant and they are known to cause mesothelial tumors following chronic exposures in laboratory animals. However, asbestos is not discussed as a separate entity in the present document, but reviews on asbestos effects can be found elsewhere (U.S. Environmental Protection Agency, 1986b; Mossman and Gee, 1989; Rom, et al., 1991; Health Effects Institute, 1991).

The effects of exposure to combinations of particles or particles and gases are important to understand because people are not exposed to single ambient air pollutants. The responses to pollutant mixtures may be different from those of the individual chemical constituents. Effects can be additive, antagonistic, or synergistic. Controlled exposure studies of humans or animals rarely involve more than two pollutants simultaneously or sequentially. Significant exceptions to this are the bodies of work on diesel and gasoline engine emissions, where the exposure has been to the specific mixture. In studies involving more complex mixtures (e.g., ambient air) it is difficult, if not impossible, to assess the relative contributions of individual specific components.

The different nature of the data bases also influences the structure of the chapter. For example, community epidemiology studies that sought associations between health effects and some type of ambient PM metric are described in Chapter 12 to permit full portrayal and integrated evaluation of the results. For the metals and diesel particles, discussed to reach a different goal, epidemiological studies are included here in Chapter 11 to facilitate a full hazard identification, and as appropriate, exposure-response information. Besides the summary of the effects portion of the literature, this chapter also attempts to identify and characterize key factors that may have significant influences on the health effects of PM.

Most of the investigations reported herein were conducted with laboratory animals, raising the question of their quantitative extrapolation to humans. Of the dosimetric and species sensitivity aspects of extrapolation, most is known about the former, which is presented in Chapter 10. Both Chapters 10 and 11 must be jointly considered for interpretation. For example, was one aerosol more toxic than another because it had a greater deposition in a sensitive lung target site or because it had higher potency?

Similarly, most particles considered in the laboratory animal toxicology and occupational studies are mainly in the fine and coarse mode size range. However, the enormous numbers and huge surface area of the ultrafine particles demonstrate the importance of considering the size of the particle. Ultrafine particles with a diameter of 20 nm when inhaled at the same mass concentration have an approximately 6 orders of magnitude higher number concentration than a 2.5 μm diameter particle; particle surface area is also greatly increased (Table 11-1).

TABLE 11-1. NUMBERS AND SURFACE AREAS OF MONODISPERSE PARTICLES OF UNIT DENSITY OF DIFFERENT SIZES AT A MASS CONCENTRATION OF 10 μg/m^3

Particle Diameter μm	Particle Number per cm^3 Air	Particle Surface Area μm^2 per cm^3 Air
0.02	2,400,000	3,016
0.1	19,100	600
0.5	153	120
1.0	19	60
2.5	1.2	24

Source: Oberdörster et al. (1995a).

Most of the laboratory animal and occupational epidemiological studies summarized here used high particulate mass concentrations, relative to ambient, even when laboratory animal-to-human dosimetric differences are considered. This raises a question about the relevance of, for example, a rat study at 5,000 $\mu g/m^3$ in terms of direct extrapolation to humans in ambient exposure scenarios.

In spite of these difficulties, the array of laboratory animal studies does illustrate certain toxicological principles for particles. To identify but a few here, the data base clearly shows that the site of respiratory tract deposition (and hence particle size) influences the health outcome and that toxicity is dependent on the chemical species (e.g., cadmium is different from sulfuric acid, and cadmium chloride is different from cadmium oxide).

11.2 ACID AEROSOLS

The ubiquitous presence of acidic aerosols in the ambient air and concern about their potential health effects led to considerable research over the past 15 years on the response of humans and laboratory animals to exposure to acid aerosols. In Section 11.2.1, responses of both healthy and sensitive humans to acid aerosols and acidic aerosol mixtures with other pollutants are reviewed. Human studies primarily consider brief exposures, whereas the laboratory animal toxicology studies discussed in Section 11.2.2 also consider the effects of chronic exposure to acid aerosols and acidic aerosol mixtures.

Section 11.2 focuses mainly on sulfate-related species (e.g., sulfuric acid [H_2SO_4]). Information on certain other aerosol species (e.g., nitrates) was reviewed in the previous PM/SO_x CD (U.S. Environmental Protection Agency, 1982), the EPA Acid Aerosols Issue Paper (U.S. Environmental Protection Agency, 1989), and the Oxides of Nitrogen Criteria Document (U.S. Environmental Protection Agency, 1993). Those earlier assessments yielded only very limited information indicative of health effects being associated with exposures to aerosol species such as sodium nitrate ($NaNO_3$) or ammonium nitrate (NH_4NO_3) at levels very much in excess of ambient (i.e., at three orders of magnitude [about 1000 times] above nitrate concentrations typically found in ambient air). Ambient levels of airborne nitrate salts are typically less than 5 $\mu g/m^3$ and rarely exceed 50 $\mu g/m^3$ (Sackner et al., 1979). Given that little, if any, important new information on nitrate-related health

effects has appeared in the past few years since the above noted assessments were completed, they are not treated further here, except as components of some particle mixtures discussed later in the chapter.

11.2.1 Controlled Human Exposure Studies

11.2.1.1 Introduction

Human clinical exposure studies utilize controlled laboratory conditions to test responses to atmospheric pollutants. Advantages include the opportunity to study the species of interest (i.e., humans), and the ability to carefully control the atmosphere with regard to pollutant concentration, aerosol characteristics, temperature, and relative humidity. Concentrations can be varied while other conditions are held constant to determine exposure-response relationships. Mixtures of pollutants or sequential exposures to different pollutants can be used to elucidate interactions.

Methods of inhalation used in clinical studies include mouthpiece, face mask, head-dome, and environmental chamber. Breathing through a mouthpiece alters breathing patterns, and bypasses the normal filtering and humidifying role of the nasal passages, thereby increasing delivery of particles to the lower airways. Environmental chamber and head-dome exposures allow the assessment of shifts between nasal and oral-nasal breathing that normally occur with exercise.

Several factors limit the utility of human clinical studies. To meet legal and ethical requirements, exposures must be without significant harm. Studies are typically limited to short-term exposures, since long-term exposures are impractical, and may be more likely to cause harm. Sample sizes are small, and therefore may not be representative of populations at risk. Finally, individuals likely to be at greatest risk (i.e., the very young and very old, those with severe obstructive lung disease, or combined heart and lung disease) have not been studied. The data from human clinical studies should therefore be used together with information from laboratory animal exposure studies, epidemiologic studies, and *in vitro* exposure studies, in the process of health assessment.

The endpoints most commonly measured in human clinical studies are symptoms and pulmonary function tests. The latter are well standardized, and their use in these studies has been reviewed (Utell et al., 1993). Effects in clinical studies can be directly compared to

acute changes in field studies, as has been done extensively in studies of ozone health effects (U.S. Environmental Protection Agency, 1995).

Airway responsiveness is another endpoint commonly measured in human clinical studies. This test measures changes in lung function in response to pharmacologic bronchoconstricting agents, typically methacholine, carbachol, or histamine (see also Section 11.2.1.4). A dose-response curve is obtained for the agent, and airway responsiveness is expressed as the dose of the bronchoconstricting agent resulting in a specific change in lung function: e.g., the PD_{20} is the provocative dose resulting in a 20% fall in forced expiratory volume in 1 sec (FEV_1). Individuals with asthma almost always have hyperresponsive airways, with a PD_{20} well below the normal range. Increase in airway reactivity in response to pollutant exposure could reflect airway inflammation or edema. However, smaller airway caliber as a consequence of the exposure will also increase measured responsiveness because of factors related to airways geometry. It is therefore important to measure responsiveness at a time when spirometric function has returned to baseline. Likewise, performing airway challenge testing prior to pollutant exposure may alter subsequent lung function responses to the pollutant by changing the baseline airways caliber. Differences among laboratories in the protocols and provocative agents used for airway challenge make comparison of experimental results problematic.

Endpoints in human clinical studies have extended beyond measures of air flow and lung volume. Mucociliary clearance is measured using inhaled radio-labelled aerosols. As reviewed in the Acid Aerosols Issue Paper (U.S. Environmental Protection Agency, 1989), exposure to acid aerosols alters mucociliary clearance in humans as well as in several laboratory animal species. Within the past decade, fiberoptic bronchoscopy has been used to examine the lower respiratory tract in healthy volunteers exposed to pollutants. Cells that populate the alveolar space, including alveolar macrophages (AM), lymphocytes, and polymorphonuclear leukocytes (PMN), can be recovered by bronchoalveolar lavage (BAL); bronchial epithelial cells can be sampled using bronchial brushing and endobronchial biopsies. Nasal lavage can be used to quantitate inflammation in the nose.

Features of experimental design of particular importance with regard to human clinical studies are method of exposure, exercise, and selection of control exposures. Exposure by mouthpiece reduces humidification of inhaled air that normally occurs in the nasal passages;

inhalation of dry cold air into the airways may cause bronchoconstriction in asthmatic subjects. Exercise plays an important role in enhancing pollutant effects by causing a change from nasal to oral-nasal breathing, hence decreasing upper airways deposition, and by increasing pollutant dose through increased minute ventilation ($\dot{V}_E$).

Selection of control exposures is of particular importance. Typically, each subject serves as his/her own control to eliminate intersubject variability. The control atmosphere depends on the study objectives and may consist of clean air, or, when acidic aerosols are being tested, a pH neutral aerosol, such as sodium chloride (NaCl), to distinguish non-specific effects of the aerosol from pollutant or hydrogen ion (H^+) effects. It is important that control exposures be performed under similar conditions of temperature, relative humidity, $\dot{V}_E$, and time of day; that control and pollutant exposure be separated by sufficient time to avoid carry-over effects; and that the order of the exposures be randomized among the study group. Double blind procedures (by which neither the investigators collecting data nor the subjects know the contents of exposure atmospheres) should be used to the extent possible.

Human exposure studies of the effects of acid aerosols were reviewed in the *Acid Aerosols Issue Paper* (U.S. Environmental Protection Agency, 1989). That review reached the following conclusions:

(1) In healthy subjects, no effects on spirometry have been observed after exposure to concentrations of H_2SO_4 less than 500 $\mu g/m^3$, and no consistent effects have been observed at levels up to 1,000 $\mu g/m^3$ with exposure durations up to 4 h. Studies of a variety of other sulfate and nitrate aerosols have similarly demonstrated an absence of spirometric effects on healthy subjects.

(2) Combinations of sulfates with ozone or SO_2 have not demonstrated synergistic or interactive effects.

(3) Asthmatic subjects experience modest bronchoconstriction after exposure to $\approx$400 to 1000 $\mu g/m^3$ H_2SO_4, and small decrements in spirometry have been observed in adolescent asthmatics at concentrations as low as 68 $\mu g/m^3$ for 30 min.

(4) Some studies suggest that delayed effects may occur in healthy and asthmatic subjects following exposure to H_2SO_4.

(5) Effects of sulfate aerosols are related to their acidity, and neutralization by oral ammonia tends to mitigate these effects.

(6) Exposure to H_2SO_4 at concentrations as low as 100 $\mu g/m^3$ for 60 min alters mucociliary clearance.

(7) Airway reactivity increases in healthy and asthmatic subjects following exposure to 1,000 $\mu g/m^3$ H_2SO_4 for 16 min.

(8) Differences in estimated respiratory intake explain only a portion of the differences in responses among studies.

In the five years since the publication of the *Acid Aerosol Issue Paper*, several of these summary statements have been further confirmed. For example, recent studies confirm the absence of spirometric effects following acute exposure to H_2SO_4 and other acid aerosols in healthy subjects, at or below 1,000 $\mu g/m^3$. The observation of effects on adolescent asthmatics at levels as low as 68 $\mu g/m^3$ has not been confirmed, and studies utilizing longer exposures have raised further questions about the relationship between dosimetry and health effects. However, additional evidence supports the conclusion that lung function effects in asthmatic subjects are related to hydrogen ion exposure, which is in part determined by the degree of neutralization by oral ammonia. Two recent studies examining sequential exposure to H_2SO_4 and ozone (Linn et al., 1994; Frampton et al., 1995) suggest that acid aerosols may potentiate the response to ozone in some asthmatic subjects. Finally, clinical studies of acid aerosols have been expanded to include endpoints associated with fiberoptic bronchoscopy and BAL.

Table 11-2 summarizes, in alphabetical order by author, controlled human exposure studies of particle exposure published since 1988. The majority of the human clinical studies have focused on the pulmonary function effects of exposure to acid aerosols. These studies are therefore summarized separately below, first reviewing studies of effects on healthy subjects, followed by subjects with asthma. Subsequent sections deal with effects other than lung function, and with studies of particulate pollutants other than acid aerosols.

11.2.1.2 Pulmonary Function Effects of Sulfuric Acid in Healthy Subjects

Since 1988, ten studies have examined the effects of H_2SO_4 exposure on pulmonary function in healthy subjects. Exposure levels ranged from 100 $\mu g/m^3$ to 2,000 $\mu g/m^3$, with exposure durations ranging from 16 min to 6.5 h on two successive days. All of these studies confirmed the findings from previous studies of an absence of spirometric effects on

TABLE 11-2. CONTROLLED HUMAN EXPOSURES TO ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subjects	Exposures	MMAD[2] (μm)[1]	GSD[3] (μm)	Duration	Exercise	Temp (°C)	RH[4] (%)	Symptoms	Lung Function	Other Effects	Comments
Anderson et al. (1992)	15 healthy 15 asthmatic 18 to 45 years	(1): air (2): H_2SO_4 ≈100 $\mu g/m^3$ (3): carbon black ≈200 $\mu g/m^3$ (4): acid-coated carbon with ≈100 $\mu g/m^3$ H_2SO_4	1.0	2	1 h	$\dot{V}_E$ ≈50 L/min	22	50	Healthy subjects more symptomatic in air.	Largest decrements in FVC with air exposure.	No change in airway responsiveness	Smoking status of subjects not stated.
Aris et al. (1990)	19 asthmatic 20 to 40 years	Mouthpiece study: HMSA[5] 0 to 1000 μM + H_2SO_4 50 μM vs H_2SO_4 50 μM	6.1		3 min.	100 W on cycle		100	HMSA did not increase symptoms in comparison with H_2SO_4 alone.	No effects on SRaw[6]		
		Chamber study: HMSA 1 Mm + H_2SO_4 5 Mm vs H_2SO_4 5 Mm	≈7		1 h		≈25					
Aris et al. (1991a)	10 healthy nonsmokers 21 to 31 years ozone sensitive	HNO_3 500 $\mu g/m^3$ or H_2O, or air followed by ozone 0.2 ppm	≈6		2 h 3 h	50 min of each h 40 L/min	22	100	No effects of fog exposure	No direct effects of fog exposures. Greatest decrements when ozone preceded by air.	No change in airway responsiveness	Fog may have reduced ozone effects on lung function.
							22	50				
Aris et al. (1991b)	18 asthmatics 23 to 37 years	Mouthpiece study: H_2SO_4 vs NaCl, ≈3000 $\mu g/m^3$ with varying particle size, osmolarity, relative humidity	0.4 vs ≈6		16 min	With and without exercise.	≈24	<10 vs 100	No effects	Increases in Sraw with low RH conditions; no pollutant-related effects		Postulated that effects seen in other studies due to secretions or effects on larynx
		Chamber study: H_2SO_4 vs NaCl, 960 to 1400 $\mu g/m^3$ with varying water content	6		1 h	100 W on cycle	≈27					

TABLE 11-2 (cont'd). CONTROLLED HUMAN EXPOSURES TO ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subjects	Exposures	MMAD[2] (μm)	GSD[3] (μm)	Duration	Exercise	Temp (°C)	RH[4] (%)	Symptoms	Lung Function	Other Effects	Comments
Avol et al. (1988a)	21 healthy 21 asthmatic 18 to 45 years	Air H_2SO_4: Healthy: 363, 1128, 1578 $\mu g/m^3$ Asthmatic: 396, 999, 1,460 $\mu g/m^3$	0.85 to 0.91	2.4 to 2.5	1 h	10 min × 3 47 to 49 L/min	21	50	Healthy: Slight increase in cough with highest concentrations. Asthma: dose-related increase in lower resp. symptoms.	Healthy: No effects on lung function or airway reactivity. Asthma: ↓FEV_1 0.26 L with H_2SO_4 1,460 $\mu g/m^3$		
Avol et al. (1988b)	22 healthy 22 asthmatic 18 to 45 years	H_2O H_2SO_4: Healthy: 647, 1,100, 2,193 $\mu g/m^3$ Asthmatic: 516, 1,085, 2,034 $\mu g/m_3$	9.7 to 10.7		1 h	10 min × 3 41 to 46 L/min	9	100	Dose-related increase in lower resp. symp. in both groups.	Healthy: No effects on lung function. Asthma: ↓peak flow 16% at 2,034 $\mu g/m^3$ H_2SO_4.	No effects on airway responsivenes s	Half the subjects received acidic gargle; no difference in effects.
Avol et al. (1990)	32 asthmatics 8 to 16 years	Air H_2SO_4 46, 127, and 134 $\mu g/m^3$	0.5	1.9	40 min	30 min rest, 10 min exercise 20L/min/m^2	21	48	No pollutant effect	No pollutant effect. One subject increased Sraw 14.2% with acid exposure.		Did not reproduce findings of Koenig et al., 1983.
Balmes et al. (1988)	12 asthmatics responsive to hypoosmolar saline aerosol 25 to 41 years	Mouthpiece, 5,900 to 87,100 μ/m^3: NaCl 30 mOsm H_2SO_4 30 mOsm HNO_3 30 mOsm H_2SO_4+HNO_3 30 mOsm H_2SO_4 300 mOsm	≈5 to 6	1.5		At rest	≈23			Concentration of acid aerosol required to increase Sraw by 100% lower than for NaCl. No difference between acid species.		Exposures did not mimic environmental conditions. No mitigation by oral ammonia.
Culp et al. (1995)	16 healthy 20 to 39 yrs	NaCl 1000 $\mu g/m^3$ H_2SO_4 1,000 $\mu g/m^3$	0.9	1.9	2 h	10 min × 4 ≈40 L/min	22	40			Mucins from bronchoscopy: no effects on mucin recovery or changes in glycoproteins	

TABLE 11-2 (cont'd). CONTROLLED HUMAN EXPOSURES TO ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subjects	Exposures	MMAD[2] (μm)	GSD[3] (μm)	Duration	Exercise	Temp (°C)	RH[4] (%)	Symptoms	Lung Function	Other Effects	Comments
Fine et al. (1987b)	8 asthmatics 22 to 29 yrs	Mouthpiece: Buffered and unbuffered HCl and H_2SO_4 at varying pH	5.3 to 6.2	1.6 to 1.8	3 min.	At rest			Cough with inhalation of unbuffered pH 2 aerosols	≈50% increase in airway resistance with buffered acid aerosols at pH 2. Little response to unbuffered acids.		Titratable acidity important determinant of response to acid aerosols.
Fine et al. (1987a)	10 asthmatics 22 to 34 yrs	Mouthpiece: Na_2SO_3 0 to 10 mg/ml, pH 9, 6.6, 4; buffered acetic acid pH 4; SO_2 0.25 to 8 ppm	5.6 to 6.1	1.6 to 1.7	1 min.	At rest				For Na SO , bronchoconstriction greater at lower pH; no response to acetic acid.	2 3	Suggests effects related to release of SO_2 or bisulfite, but not sulfite.
Frampton et al. (1992)	12 healthy 20 to 39 yrs	NaCl 1,000 $\mu g/m^3$ H_2SO_4 1,000 $\mu g/m^3$	0.9	1.9	2 h	10 min × 4 ≈40 L/min	22	40	4/12 subjects: throat irritation with acid exposure.	No pollutant effects	BAL findings: No effects on cell recovery, lymphocyte subsets, AM function, fluid proteins.	
Frampton et al. (1995)	30 healthy 30 asthmatics 20 to 42 yrs	NaCl or H_2SO_4 100 $\mu g/m^3$ followed by ozone 0.08, 0.12, or 0.18 ppm	0.45 0.64	4.05 2.50	3 h 3 h	10 min × 6. Healthy: 33 to 40 L/min; asthmatics: 31 to 36 L/min	21	40	No pollutant effects	Healthy subjects: no significant effects. Asthmatics: ozone dose-response following H_2SO_4 pre-exposure, but not NaCl		
Hanley et al. (1992)	22 asthmatics 12 to 19 yrs	Mouthpiece: (1): Air; H_2SO_4 70, 130 $\mu g/m^3$ (2): Air; H_2SO_4 70 $\mu g/m^3$ with and without lemonade	0.72	1.5	40 min. 45 min.	10 min 30 min ≈30 L./min	22	65	No effects	Significant decreases in FEV_1 (≈37 ml/μmol H^+) and FVC at 2 to 3 min but not 20 min after exposure.	Significant correlation between baseline airways responsiveness and $\Delta FEV_1/H^+$ (R^2=0.3).	Large variability in oral NH_3 levels.

TABLE 11-2 (cont'd). CONTROLLED HUMAN EXPOSURES TO ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subjects	Exposures	MMAD[2] (μm)[1]	GSD[3] (μm)	Duration	Exercise	Temp (°C)	RH[4] (%)	Symptoms	Lung Function	Other Effects	Comments
Koenig et al. (1989)	9 asthmatics with exercise-induced bronchospasm 12 to 18 yrs	Mouthpiece: Air; H_2SO_4 68 $\mu g/m^3$; SO_2 0.1 ppm; H_2SO_4+SO_2; HNO_3 0.05 ppm	0.6	1.5	40 min	10 min	25	65	No effects	↓FEV 6% after H_2SO_4 compared with 2% after air.		
Koenig et al. (1992)	14 asthmatics with exercise-induced bronchospasm 13 to 18 yrs	Mouthpiece: Air; H_2SO_4 35 or 70 $\mu g/m^3$	0.6	1.5	45 or 90 min	≈23 L/min	22	65		↓FEV 6% after H_2SO_4 35 $\mu g/m^3$ for 45 min, 3% after 70 $\mu g/m^3$ (NS). Smaller changes after 90 min exposures.		Responses unrelated to $C \times T \times \dot{V}_E$
Koenig et al. (1993)	8 healthy 9 asthmatic 60 to 76 yrs	Mouthpiece: Air; $(NH_4)_2SO_4$ ≈70 $\mu g/m^3$; H_2SO_4 ≈74 to 82 $\mu g/m^3$ with and without lemonade	0.6	1.5	40 min	10 min 17.5 L/min for asthmatics, 19.7 for healthy	22	65		No significant effects. Correlation between increase in resistance and oral ammonia levels in asthmatics (R^2 = 0.575).		
Koenig et al. (1994)	28 asthmatics 12 to 19 yrs	Mouthpiece: Air; ozone 0.12 ppm+NO_2 0.3 ppm; ozone 0.12 ppm+NO_2 0.3 ppm+H_2SO_4 68 $\mu g/m^3$; ozone 0.12 ppm+NO_2 0.3 ppm+HNO_3 0.05 ppm	0.6	1.5	90 min × 2 days	$\dot{V}_E$ 3 × resting	22	65	No pollutant effects	No pollutant effects	No effects on airway responsiveness	6 subjects with moderate or severe asthma did not complete protocol
Laube et al. (1993)	7 healthy 20 to 31 yrs	Head dome: NaCl ≈500 $\mu g/m^3$ H_2SO_4 ≈500 $\mu g/m^3$	10.3 10.9		1 h	20 min	22 to 25	99	No pollutant effects	No pollutant effects	Tracheal clearance increased (4/4 subjects). Outer zone clearance increased (6/7 subjects). No effects on airway responsiveness	

TABLE 11-2 (cont'd). CONTROLLED HUMAN EXPOSURES TO ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subjects	Exposures	MMAD[2] (μm) [1]	GSD[3] (μm)	Duration	Exercise	Temp (°C)	RH[4] (%)	Symptoms	Lung Function	Other Effects	Comments
Linn et al. (1989)	22 healthy 19 asthmatic 18 to 48 yrs	H_2O H_2SO_4 ≈2,000 $\mu g/m^3$	20 10 1		1 h	40 to 45 L/min	≈10	74 to 100	Increased total score with larger acid particles.	No pollutant effects	No effects on airway reactivity	4 asthmatic subjects unable to complete exposures because of symptoms.
Linn et al. (1994)	15 healthy 30 asthmatic 18 to 50 yrs	Air; ozone 0.12 ppm; H_2SO_4 100 $\mu g/m^3$; ozone+H_2SO_4	≈0.5	~2	6.5 h/d × 2d	50 min × 6 29 L/min	21	50	Symptoms unrelated to atmosphere	↓FEV_1 & FVC in ozone, similar for healthy & asthmatic subjects. Greater fall in FEV_1 for acid+ozone than ozone alone, marginally significant interaction.	Increased airway responsiveness with ozone, marginal further increase with ozone+acid	Average subject lost 100 ml FEV_1 with ozone, 189 ml with ozone+acid Original findings replicated in 13 subjects
Morrow et al. (1994)	17 asthmatic 20 to 57 yrs 17 COPD 52 to 70 yrs	NaCl ≈100 $\mu g/m^3$ H_2SO_4 ≈90 $\mu g/m^3$			2 h	Asthmatics: 10 min × 4 COPD: 7 min × 1	21	30	No pollutant effects.	Asthmatics: ↓FEV_1 slightly greater after acid than after NaCl. COPD: No effects.		
Utell et al. (1989)	15 asthmatic 19 to 50 yrs	Mouthpiece: NaCl 350 $\mu g/m^3$; H_2SO_4 350 $\mu g/m^3$, high NH_3; H_2SO_4, low NH_3	0.80	1.7	30 min	10 min $\dot{V}_E$ 3 × resting		20 to 25		Greater fall in FEV_1 with low NH_3 (19%) than with high NH_3 (8%).		

[1]Exposures in environmental chamber unless otherwise stated.
[2]Mass median aerodynamic diameter. In some studies expressed as volume median diameter; see text.
[3]Geometric standard deviation.
[4]Relative humidity.
[5]Hydroxymethanesulfonic acid.
[6]Specific airways resistance.
BAL=Bronchoalveolar lavage.
AM=Alveolar macrophage.

healthy subjects. Exposures at the highest concentrations (i.e. 1,000 $\mu g/m^3$ or greater) were associated with mild increases in respiratory symptoms (cough, substernal discomfort, throat irritation), especially those exposures with particle sizes in the 10 to 20 μm range.

Two studies reported by Avol and colleagues (Avol et al., 1988a,b) examined effects of 1-h H_2SO_4 aerosol exposures in an environmental chamber. In the first study (Avol et al., 1988b), 22 healthy nonsmoking subjects between the ages of 18 and 45 years, some reporting allergies, were exposed for 1 h to large particle aerosols (volume median diameter (VMD) 9.7 to 10.3 μm, GSD not stated) consisting of H_2O (control) or H_2SO_4 at 647, 1,100, and 2,193 $\mu g/m^3$. Three 10-min periods of moderate exercise (46 L/min) were included. All subjects were exposed to each atmosphere, separated by one week. Half the subjects received an acidic gargle to reduce oral ammonia levels prior to exposure; no difference in effects was observed with or without the gargle, so data were combined in the analysis. Healthy subjects experienced a slight concentration-related increase in lower respiratory symptoms (cough, sputum, dyspnea, wheeze, chest tightness, substernal irritation), but no effect was found on spirometry or on airway reactivity to methacholine measured 1 h after exposure.

A second study (Avol et al., 1988a) essentially duplicated this protocol for H_2SO_4 aerosols with a smaller particle size (MMAD = 0.85 to 0.91 μm, geometric standard deviation [GSD = 2.4 to 2.5]). Twenty-one healthy subjects, 12 with allergies by skin testing, were exposed on separate occasions to air and H_2SO_4 aerosol at each of three concentrations: 363, 1128, 1578 $\mu g/m^3$. A slight increase in cough was found at the two highest concentrations of H_2SO_4, but no effects were found on spirometry, specific airway resistance (Sraw), or airway reactivity to methacholine.

Linn et al. (1989) examined the effects of droplet size on 22 healthy subjects exposed to nominally 2,000 $\mu g/m^3$ H_2SO_4 for 1 h, with three, 10-min exercise periods. Distilled H_2O was used for control aerosols. Aerosol VMDs were 1, 10, and 20 μm. Actual exposure concentrations were 1,496, 2,170, and 2,503 $\mu g/m^3$. Results were similar to the previous fog studies by this group, with no significant effects on lung function or airway reactivity to methacholine. Total symptom scores were increased with exposure to 10 μm and 20 μm H_2SO_4 particles, but not to 1 μm.

Frampton et al. (1992) exposed 12 healthy nonsmokers to aerosols of NaCl (control) or H_2SO_4 (MMAD = 0.9μm, GSD = 1.9) at 1,175 $\mu g/m^3$ for 2 h in an environmental chamber. Four 10-min exercise periods at $\dot{V}_E$ of $\approx$40 L/min were included. Subjects brushed their teeth and rinsed with mouthwash prior to and once during each exposure to reduce oral ammonia levels. Mild throat irritation was described by 4 of 12 subjects after acid exposure and 3 of 12 subjects after NaCl exposure. No effects on lung function were found.

Five other recent studies (Anderson et al., 1992; Koenig et al., 1993; Laube et al., 1993; Linn et al., 1994; Frampton et al., 1995) have included healthy subjects in exposures to H_2SO_4 aerosols at levels below 1000 $\mu g/m^3$; none have shown meaningful effects on lung function. Anderson et al., (1992) studied the responses of 15 healthy subjects exposed for 1 h in a chamber to air, 100 $\mu g/m^3$ H_2SO_4, 200 $\mu g/m^3$ carbon black, and carbon black coated with H_2SO_4, (MMAD $\approx$1 μm). Lemonade or citrus juice gargles were used to reduce oral ammonia levels. Exposures containing acid were without effects on symptoms, lung function, or airway reactivity. Healthy subjects were actually more symptomatic and demonstrated greater increases in Sraw after air than after pollutant exposure, contrary to expectation. In a study designed to examine effects of acid fog on pulmonary clearance, Laube et al., (1993) exposed seven healthy volunteers to NaCl or H_2SO_4 at 470 $\mu g/m^3$, MMAD $\approx$11 μm, for 1 h with 20 min of exercise. Acid exposure did not alter symptoms or lung function. Two chamber studies designed to examine the effects of combined or sequential exposure to acid aerosols and ozone found no direct effects of exposure to $\approx$100 $\mu g/m^3$ H_2SO_4 on lung function of healthy subjects, using exposure durations of 3 h (Frampton et al., 1995) or 6.5 h for two successive days (Linn et al., 1994). Both studies included exercise and acidic mouthwash to minimize oral ammonia. Also of particular interest, Koenig et al, (1993) studied eight elderly subjects age 60 to 76 years exposed to air, H_2SO_4, or ammonium sulfate at approximately 82 $\mu g/m^3$ H_2SO_4 for 40 min, delivered by mouthpiece. No effects were found on spirometry or total respiratory resistance.

Thus, for young healthy adults, brief exposures to H_2SO_4 at mass concentrations more than an order of magnitude above ambient levels do not alter lung function. Some subjects report increased lower respiratory symptoms, including cough, at 1000 $\mu g/m^3$ and higher

levels, particularly with larger particle sizes (> 5 μm). One small study suggests that the elderly do not demonstrate decrements in lung function at low H_2SO_4 exposure levels of (approximately 82 $\mu g/m^3$). There are no data on the responses to particle exposure for healthy adolescents or children.

11.2.1.3 Pulmonary Function Effects of Sulfuric Acid in Asthmatic Subjects

Individuals with asthma often experience bronchoconstriction in response to a variety of stimuli, including exercise, cold dry air, or exposure to strong odors, smoke, and dusts. Considerable individual variability exists in the nature of stimuli that provoke a response, and in the degree of responsiveness. Thus, for clinical studies involving asthmatic subjects, subject selection and sample size deserve particular consideration. Differences among subjects may explain, in part, the widely differing results between laboratories studying effects of acid aerosols. For example, in some studies described below, asthmatic subjects were specifically selected to have exercise-induced bronchoconstriction (Koenig et al., 1989, 1992, 1994; Hanley et al., 1992), or responsiveness to hypo-osmolar aerosols (Balmes et al., 1988). The interval for withholding medications prior to exposure differed among various laboratories and different studies. In addition, the severity of asthma differed among studies; severity is often difficult to compare because published information describing clinical severity and baseline lung function is often incomplete. Table 11-3 lists the characteristics of asthmatic subjects exposed to acid aerosols and other particles.

Several studies have suggested that asthmatics are more sensitive than healthy subjects to effects of acid aerosols on lung function. Utell et al., (1982) found significant decrements in specific airway conductance (SGaw) in asthmatic subjects exposed by mouthpiece for 16 min to 450 and 1,000 $\mu g/m^3$ H_2SO_4 (MMAD 0.5 to 1.0 μm). Moreover, exposure to neutralization products of H_2SO_4 produced smaller decrements in function, roughly in proportion to their acidity (H_2SO_4 > NH_4HSO_4 > $NaHSO_4$).

The role of H^+ in the responsiveness of asthmatics to acid aerosols was explored by Fine et al. (1987b), who found that titratable acidity and chemical composition, rather than pH alone, are key determinants of response in asthmatics. Eight asthmatic subjects were challenged by mouthpiece for 3 min at rest, with buffered or unbuffered hydrochloric acid (HCl) or H_2SO_4 at varying pH levels, and changes in SRaw were measured. Solutions were

TABLE 11-3. ASTHMA SEVERITY IN STUDIES OF ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subject # (F/M)	Age Range (mean)	Exposures [1]	Allergies	Medications	FEV_1 (% pred.)	FEV_1/FVC (%)	Airway Responsiveness	Exercise/ V
Anderson et al. (1992)	15 (6/9)	19 to 45 years (29)	(1): Air (2): H_2SO_4 ≈100 $\mu g/m^3$ (3): carbon black ≈200 $\mu g/m^3$ (4): acid-coated carbon	Not stated	Not stated	Not stated	69±14 (SD)	Methacholine: $PD_{20} \leq 56$ "breath-units"	Intermittent at ≈50 L/min
Aris et al. (1990)	19 (8/11)	20 to 40 years	Mouthpiece study: HMSA 0 to 1,000 mM + H_2SO_4 50 mM vs H_2SO_4 50 mM Chamber study: HMSA 1 mM + H_2SO_4 5 mM vs H_2SO_4 5 mM	Not stated	All but one on albuterol. 3 on inhaled steroids. No meds 24 h before study.	82±20 (SD)	Not stated	Methacholine: All responded to <2 mg/ml	Intermittent , 100 W on cycle ergometer
Aris et al. (1991b)	18	23 to 37 years	Mouthpiece study: H_2SO_4 vs NaCl to test changes in particle size, osmolarity (30 to 300 mOsm), relative humidity Chamber study: H_2SO_4 vs NaCl with varying water content	Not stated	Most subjects on albuterol. Several on inhaled steroids. No meds 24 h before study.	79±23 (SD)	Not stated	Methacholine: All responded to <1 mg/ml	Mouthpiece study: with and without exercise. Chamber study: intermittent exercise at 100 W on cycle ergometer.
Avol et al. (1988a)	21 (9/12)	18 to 45 years (30)	Air H_2SO_4 396, 999, 1,460 $\mu g/m^3$	Positive skin tests in 20	11 on no regular meds; 10 on regular meds. 3 unable to hold meds prior to exposure.	Not stated	73±14 (SD)	Hyperresponsive by methacholine challenge, not further specified	10 min × 3 47 to 49 L/min
Avol et al. (1988b)	22 (9/13)	18 to 45 years (26)	H_2O H_2SO_4 516, 1,085, 2,034 $\mu g/m^3$	Positive skin tests in 18	"Majority had mild extrinsic disease". 9 on regular meds.	Not stated	45 to 98	Methacholine: $PD_{20} \leq 295$ "dose units"	10 min × 3 41 to 46 L/min

TABLE 11-3 (cont'd). ASTHMA SEVERITY IN STUDIES OF ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subject # (F/M)	Age Range (mean)	Exposures [1]	Allergies	Medications	FEV_1 (% pred.)	FEV_1/ FVC (%)	Airway Responsiveness	Exercise/ V
Avol et al. (1990)	32 (12/20)	8 to 16 years	Air H_2SO_4 46 ,127, and 134 $\mu g/m^3$	All had history of allergy	18 on regular meds, 2 on no meds, rest intermittent. None on steroids.	Less than 70 in 25 subject s	Not stated	Hyperresponsive by exercise, cold air, or methacholine.	30 min rest, 10 min exercise 20L/min/m^2
Balmes et al. (1988)	12 (6/6)	25 to 41 years	Mouthpiece, doubling outputs, 5,900 to 87,100 $\mu g/m^3$: NaCl 30 mOsm H_2SO_4 30 mOsm HNO_3 30 mOsm H_2SO_4+HNO_3 30 mOsm H_2SO_4 300 mOsm	Not stated	All on inhaled meds, 3 on inhaled steroids. No meds 24 h before study.	94±15 (SD)	61 to 89	Responsive to hypoosmolar saline aerosol, methacholine <2 mg/ml.	At rest
Fine et al. (1987b)	8 (6/2)	22 to 29 years	Mouthpiece: Buffered and unbuffered HCl and H_2SO_4 at varying pH	Not stated	6 on inhaled meds and/or theophylline, no steroids. No meds 12 h before study.	41 to 108	74±11 (SD)	Methacholine: All responded to <3 mg/ml.	At rest
Fine et al. (1987a)	10 (5/5)	22 to 34 years (26.7)	Mouthpiece: Na_2SO_3 0 to 10,000 $\mu g/ml$, pH 9, 6.6, 4; buffered acetic acid pH 4; SO_2 0.25 to 8 ppm	Not stated	7 on inhaled meds, no steroids. No meds 12 h before study.	Not stated	Not stated	9 subjects had bronchoconstriction and greater response to aerosol with lower pH. Response to $NaSO_3$ aerosols may be due to release of SO_2 gas in bisulfiteions.	At rest
Frampton et al. (1995)	30 (20/10)	20 to 42 years	NaCl or H_2SO_4 100 $\mu g/m^3$ followed by ozone 0.08, 0.12, or 0.18 ppm	All had positive skin tests. ↑IgE in 10.	All on intermittent or daily bronchodilators. None on steroids. Meds held 24 h before study.	81±4 (SE)	75±2 (SE)	Positive carbachol challenge if normal spirometry	10 min × 6 for each exposure

TABLE 11-3 (cont'd). ASTHMA SEVERITY IN STUDIES OF ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subject # (F/M)	Age Range (mean)	Exposures [1]	Allergies	Medications	FEV_1 (% pred.)	FEV_1/ FVC(%)	Airway Responsiveness	Exercise/ V_E
Hanley et al. (1992)	22 (7/15)	12 to 19 years	Mouthpiece: (1): Air or H_2SO_4 70, 130 $\mu g/m^3$ (2): Air or H_2SO_4 70 $\mu g/m^3$, with and without lemonade	"All had allergic asthma". ↑IgE in 8.	All but 2 on meds, no steroids. No meds 4 h before study.	Not stated	Not stated	Methacholine: PD_{20} 0.25 to 25 mg/ml; not available for 3 subjects. 18 were responsive to exercise by treadmill test	(1): 10 min (2): 30 min ≈30 L./min
Koenig et al. (1989)	9 (3/6)	12 to 18 years	Mouthpiece: Air H_2SO_4 68 $\mu g/m^3$ SO_2 0.1 ppm H_2SO_4+SO_2 HNO_3 0.05 ppm	5 "allergic asthma"	Not stated	Not stated	Not stated	Methacholine: All responded to <20 mg/ml. All had ↓FEV_1 >15% with treadmill test	"Moderate", on treadmill for 10 min
Koenig et al. (1992)	14 (5/9)	13 to 18 years	Mouthpiece: Air H_2SO_4 35 or 70 $\mu g/m^3$	"Allergic asthma"	Not stated	Not stated	Not stated	Methacholine: PD_{20} 0.25 to 25 mg/ml; not available for 1 subject; 8 had pos. treadmill tests, 4 history of exercise responsiveness, 2 did not meet stated criteria for exercise responsiveness.	Intermitten t ≈23 L/min
Koenig et al. (1993)	9 (7/2)	60 to 76 years	Mouthpiece: (1): Air (2): $(NH_4)_2SO_4$ ≈70 $\mu g/m^3$ (3&4): H_2SO_4 ≈74 $\mu g/m^3$ with and without lemonade	Not stated	All on "bronchodilator and/or anti-inflammatory treatment". Steroids not specified.	75	Not stated	Methacholine: $PD_{20} \leq 10$ mg/ml	10 min 17.5 L/min

TABLE 11-3 (cont'd). ASTHMA SEVERITY IN STUDIES OF ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subject # (F/M)	Age Range (mean)	Exposures [1]	Allergies	Medications	FEV_1 (% pred.)	FEV_1/ FVC (%)	Airway Responsiveness	Exercise/ V_E
Koenig et al. (1994)	28 (9/19)	12 to 19 years	Mouthpiece: (1): Air (2): ozone 0.12 ppm+NO_2 0.3 ppm (3): ozone 0.12 ppm+NO_2 0.3 ppm+H_2SO_4 68 $\mu g/m^3$ (4): ozone 0.12 ppm+NO_2 0.3 ppm+HNO_3 0.05 ppm	"Personal history of allergic asthma"	3 on no meds, rest on regular meds. 4 on inhaled steroids.	87	Not stated	Methacholine: PD_{20}<25 mg/ml. All but 1 responsive to exercise by treadmill test.	Intermittent $\dot{V}_E$ 3 × resting
Linn et al. (1989)	19 (13/6)	18 to 48 years (29)	H_2O H_2SO_4 ≈2,000 $\mu g/m^3$	"Some" subjects had history of allergy	All on bronchodilators at least weekly. No regular steroid use. No meds 12 h before study.	Not stated	70±11 (SD)	Hyperresponsiveness based on methacholine PD_{20}<38 "breath units", exercise responsiveness, or bronchodilator response.	Intermittent 40 to 45 L/min
Linn et al. (1994)	30 (17/13)	18 to 50 years (30)	(1): Air (2): ozone 0.12 ppm (3): H_2SO_4 100 $\mu g/m^3$ (4): ozone+H_2SO_4	Some subjects had positive skin tests.	Wide range of medication usage. Some on inhaled steroids. No meds 4 h before study.	Not stated	72	Responsive to methacholine or exercise, or bronchodilator response	50 min × 6 29 L/min
Morrow et al. (1994)	17	20 to 57 years (35)	NaCl ≈100 $\mu g/m^3$ H_2SO_4 ≈90 $\mu g/m^3$	Positive skin tests	Requirement for bronchodilators	Not stated	65±8 (SD)	Positive carbachol challenge if normal spirometry	10 min × 4

TABLE 11-3 (cont'd). ASTHMA SEVERITY IN STUDIES OF ACID AEROSOLS AND OTHER PARTICLES

Ref.	Subject # (F/M)	Age Range (mean)	Exposures [1]	Allergies	Medications	FEV_1 (% pred.)	FEV_1/ FVC (%)	Airway Responsiveness	Exercise/ $\dot{V}_E$
Utell et al. (1989)	15	19 to 50 years	Mouthpiece: (1): NaCl 350 $\mu g/m^3$ (2): H_2SO_4 350 $\mu g/m^3$ high NH_3 (3): H_2SO_4 low NH_3	Not stated	All on intermittent or daily bronchodilators. None on steroids. Meds held 24 h before study.	88±4 (SE)	70±3 (SE)	Positive carbachol challenge if normal spirometry	10 min $\dot{V}_E$ 3 × resting
Yang and Yang (1994)	25 (15/10)	23 to 48 years	Mouthpiece: Bagged polluted air, TSP = 202 $\mu g/m^3$	All ↑IgE	No steroids. Holding of medications not stated.	Not stated	Not stated	Hyperresponsive to methacholine	Rest

[1]Exposures in environmental chamber unless otherwise stated.

buffered with glycine, which, by itself, was found to have no direct effect on lung function. Aerosol MMAD ranged from 5.3 to 6.2 μm (GSD 1.6 to 1.8), simulating acid fogs. There was no group response to unbuffered acid, even at pH 2. However, SRaw increased in seven of eight subjects after inhalation of H_2SO_4 and glycine at pH 2, suggesting that titratable acidity or available H^+, rather than pH, plays a role in mediating acid fog-induced bronchoconstriction. Nevertheless, the response occurred at H_2SO_4 concentrations estimated in excess of 10,000 $\mu g/m^3$, more than an order of magnitude higher than the concentration producing a response in the study of Utell et al. (1982).

Fine et al. (1987a) further examined the role of pH in sulfite-induced bronchoconstriction in asthmatics. Ten subjects with asthma were challenged with increasing concentrations of sodium sulfite (Na_2SO_3) at three different pH levels. Challenge with buffered acetic acid aerosols at pH 4 was used to control for the airway effects of acid aerosols. Subjects also inhaled increasing concentrations of SO_2 gas during eucapneic hyperpnea. Exposures consisted of 1 min of tidal breathing on a mouthpiece at rest. Particle MMAD ranged from 5.6 to 6.1 μm. Nine of ten subjects experienced bronchoconstriction with Na_2SO_3,with greater responses to aerosols made from solutions with lower pH. No response was seen following acetic acid. The authors concluded that bronchoconstriction in response to Na_2SO_3 aerosols may be caused by the release of SO_2 gas or by bisulfite ions, but not by sulfite ions and not merely by alterations of airway pH. These studies of Fine et al., as pointed out by the authors, addressed potential mechanisms for bronchoconstriction in response to acidic sulfates, but did not attempt to mimic the effects of environmental exposures.

Hypo-osmolar aerosols can induce bronchoconstriction in some asthmatics. To test the effects of varying osmolarity of acidic aerosols, Balmes et al. (1988) administered aerosols of NaCl, H_2SO_4, HNO_3, or H_2SO_4 + HNO_3 to 12 asthmatic subjects via mouthpiece. All solutions were prepared at an osmolarity of 30 mOsm, and delivered at doubling concentrations until SRaw increased by 100%. An additional series of challenges with H_2SO_4 at 300 mOsm was performed. The 12 subjects were selected from a group of 17 asthmatics on the basis of responsiveness to challenge with hypo-osmolar saline aerosol. Aerosol particle size was similar to coastal fogs, with MMAD ranging from 5.3 to 6.1. Delivered nebulizer output during exposure was quite high, ranging from 5,900 to

approximately 87,000 $\mu g/m^3$. All hypo-osmolar aerosols caused bronchoconstriction. Lower concentrations of hypo-osmolar acidic aerosols were required to induce bronchoconstriction than with NaCl, and there was no difference between acidic species. No bronchoconstriction occurred with isosmolar H_2SO_4, even at maximum nebulizer output (estimated H_2SO_4 concentration greater than 40,000 $\mu g/m^3$). The authors concluded that acidity can potentiate bronchoconstriction caused by hypo-osmolar aerosols. As in the studies of Fine et al. (1987a,b), these exposures did not mimic environmental conditions.

Koenig and colleagues have studied the responses of adolescents with allergic asthma to H_2SO_4 aerosols with particle sizes in the respirable range, and concentrations only slightly above peak, worst-case ambient levels. In one study (Koenig et al., 1983), ten adolescents were exposed to 110 $\mu g/m^3$ H_2SO_4 (MMAD = 0.6 μm) by mouthpiece for a total of 40 min, 30 min at rest followed by 10 min of exercise. The FEV_1 decreased 8% after exposure to H_2SO_4, and 3% after a similar exposure to NaCl, a statistically significant difference. In another study (Koenig et al., 1989), nine allergic adolescents were exposed to 68 $\mu g/m^3$ H_2SO_4 (MMAD = 0.6 μm) for 30 min at rest followed by 10 min of exercise ($\dot{V}_E$ = 32 L/min). Although only five subjects were described as having "allergic asthma", all subjects had exercise-induced bronchoconstriction; thus all subjects were asthmatic by generally accepted criteria (Sheffer, 1991). Effects were compared with similar exposures to air, 0.1 ppm SO_2, 68 $\mu g/m^3$ H_2SO_4 + 0.1 ppm SO_2, and 0.05 ppm HNO_3. The FEV_1 decreased 6% after exposure to H_2SO_4 alone, and 4% after exposure to H_2SO_4 + SO_2, compared to a 2% decrease after air. Increases in total respiratory resistance were not significant. These results were presented as preliminary findings, in that a total of 15 subjects were to be studied; formal statistical comparison of H_2SO_4 versus air was not presented. Findings from the full group of 15 subjects have not been published. These studies suggest that allergic asthmatics with exercise-induced bronchoconstriction may be more sensitive to effects of H_2SO_4 than adult asthmatics, and that small changes in lung function may be observed at exposure levels below 100 $\mu g/m^3$.

Two studies reported by Avol et al. (1988a,b) examined effects of H_2SO_4 aerosols and fogs on asthmatic subjects. The results for healthy subjects in these studies were described in Section 11.2.1.2. In the first study, 21 adult asthmatics, 20 of whom had positive skin tests to common allergens, were exposed to air or 396, 999, and 1,460 $\mu g/m^3$ H_2SO_4

(MMAD 0.85 to 0.91 μm) for one hour with intermittent exercise. The asthmatic subjects experienced concentration-related increases in lower respiratory symptoms (most notably, cough), with some persistence of symptoms at 24 h. The FEV_1 decreased by a mean of 0.26 L after exposure to 999 $\mu g/m^3$, and 0.28 L after exposure to 1,460 $\mu g/m^3$. Results using analysis of variance (ANOVA) were significant for concentration effects on change in FEV_1 and FVC. However, decrements at 396 $\mu g/m^3$ were identical to those seen with air exposure. The SRaw approximately doubled following exposure to both air and 396 $\mu g/m^3$ H_2SO_4, and approximately tripled following exposure to 999 and 1,460 $\mu g/m^3$. Although absolute change in SRaw related to concentration was not significant, percent change in SRaw was not analyzed as was done for FEV_1 and FVC; ANOVA of percent change for each of these measures may have proved more sensitive. These findings are similar to those of Utell, et al. (1983b), who found significant effects on SGaw following exposure to 450 and 1,000 $\mu g/m^3$, and significant effects on FEV_1 at 1,000 $\mu g/m^3$ (MMAD = 0.8 μm). However, exposures in the Utell study were performed at rest for a considerably shorter duration (16 minutes).

The second study (Avol et al., 1988b) utilized an identical protocol to examine effects of a large particle aerosol (MMAD = 10 μm). Twenty-two asthmatic subjects were exposed to fogs containing 516, 1,085 and 2,034 $\mu g/m^3$ H_2SO4, compared with H_2O. Although concentration-related increases in respiratory symptoms were similar to those in the study of submicron aerosols, no significant effects were found on FEV_1, FVC, or SRaw, even at the highest concentration of greater than 2,000 $\mu g/m^3$. The findings from these two studies suggest that aerosols of submicron particle size may alter lung function to a greater degree than large particle aerosols in asthmatic subjects. Deep breaths of air containing acid aerosol would often provoke cough. However, the concentrations required to produce an effect (> 5000 $\mu g/m^3$) differ strikingly from the studies of adolescent asthmatics of Koenig and colleagues (1983, 1989).

Linn et al. (1989) utilized a similar exposure protocol to specifically examine effects of particle size. Nineteen asthmatic adults were exposed for 1 h to a pure water aerosol or approximately 2,000 $\mu g/m^3$ H_2SO_4 at 3 difference droplet sizes: 1, 10, and 20 μm. Subjects exercised for 3 10-min periods at $\dot{\mathbf{V}}_E$ of 40 to 45 L/min. Grapefruit juice gargles were used to minimize oral ammonia. As in previous studies by this group, symptoms increased in acid

atmospheres with larger particles. Four of the 19 asthmatic subjects were unable to complete one or more exposures because of respiratory symptoms. All but one of the aborted exposures was in an acid aerosol-containing atmosphere: three subjects did not complete the 1 μm acid exposure, one the 10 μm exposure, and three the 20 μm exposure. The authors reported significant decrements in lung function in these subjects, requiring administration of a bronchodilator. As stated by the authors, "the patterns of these appreciable clinical responses by asthmatics suggests a causal relationship to acid exposure, without obvious dependence on droplet size". These more dramatic responses to acid aerosols are not reflected in the mean responses, and suggest the existence of a few particularly susceptible individuals. Mean responses of FEV_1 to acid aerosol exposure were about -21%, with responses to exercise in clean air of about -12%. Some subjects experienced decreases in FEV_1 in excess of 50%, as a result of combined exercise and acid aerosol exposure. Analysis of variance found significant effects of acid × time on SRaw and FEV_1. There was no apparent effect of droplet size.

Utell et al. (1989) examined the influence of oral ammonia levels on responses to H_2SO_4. Fifteen subjects with mild asthma inhaled H_2SO_4 aerosols (350 $\mu g/m^3$, MMAD = 0.8 μm) via mouthpiece for 20 min at rest followed by 10 min of exercise. Sodium chloride aerosol served as control. Low oral ammonia levels were achieved using a lemon juice gargle and toothbrushing prior to exposure, and high levels were achieved by eliminating oral hygiene and food intake for 12 h prior to exposure. These procedures achieved a five-fold difference in oral ammonia levels. The FEV_1 decreased 19% with low ammonia versus 8% with high ammonia ($p<0.001$). The FEV_1 also decreased 8% with NaCl aerosol. These findings extended the authors' previous findings (Utell et al., 1983b) of decrements in SGaw following exposure to 450 $\mu g/m^3$ H_2SO_4, and demonstrated the importance of oral ammonia in mitigating the clinical effects of submicron H_2SO_4 aerosols.

The findings of Koenig et al. (1989) in adolescent asthmatics prompted an attempt by Avol and colleagues (1990) to replicate the study using a larger group of subjects. Thirty-two subjects with mild asthma, aged 8 to 16 years, were exposed to 46 and 127 $\mu g/m^3$ H_2SO_4 (MMAD $\approx 0.5\mu$m) for 30 min at rest followed by 10 min of exercise at 20 L/min/m^2 body surface area. Subjects gargled citrus juice prior to exposure to reduce oral ammonia. Bronchoconstriction occurred after exercise in all atmospheres, with no statistically

significant difference between clean air and acid exposures at any concentration. Because these exposures were undertaken in an environmental chamber with unencumbered oral/nasal breathing, in contrast to mouthpiece exposure in the Koenig et al. studies (1983, 1989), a subsequent study was performed to examine the effects of oral breathing only. Twenty-one of these subjects were therefore exposed to 134 $\mu g/m^3$ H_2SO_4 while breathing chamber air through an open mouthpiece. Again, no acid effect was found. One subject who was "unusually susceptible to exercise-induced bronchospasm" also showed the largest decrements in lung function with both exposures to the highest acid concentrations. It is possible that the subjects in the Koenig et al. (1989) study, all of whom demonstrated exercise-induced bronchoconstriction during a specific exercise challenge test, represented a more responsive subgroup of adolescent asthmatics. Only 15 of the 32 subjects in the Avol et al. (1990) study were known to have exercise-induced bronchoconstriction. Indeed, subsequent data (Hanley et al., 1992) suggest exercise responsiveness is predictive of H_2SO_4 responsiveness (see below).

Aris et al. (1990) examined the effects of hydroxymethanesulfonic acid (HMSA), which has been identified as a component of west coast acidic fogs. They postulated that HMSA might cause bronchoconstriction in asthmatics because, at the pH of airway lining fluid, it dissociates into CH_2O and SO_2 . In the first part of the study, nine asthmatics were serially challenged by mouthpiece with 0, 30, 100, 300 and 1,000 μM HMSA in 50 μM H_2SO_4 (MMAD = 6.1 μm). The SRaw was measured after each challenge. These findings were compared on a separate day to a similar series of exposures to 50 μM H_2SO_4 alone. No effect was found for HMSA on symptoms or airways resistance. An environmental chamber exposure study was then performed in which 10 asthmatic subjects were exposed to 1 mM HMSA + 5 mM H_2SO_4 for 1 h with intermittent exercise. The control was exposure to 5 mM H_2SO_4 alone. Three subjects underwent additional exposures to NaCl aerosol. Particle MMAD was approximately 7 μm. Both acid exposures slightly increased respiratory symptoms, but no significant effects on SRaw were found.

In a subsequent series of studies, Aris et al. (1991b) examined the effects of varying particle size, osmolarity, and relative humidity on airways resistance in response to H_2SO_4 aerosol. To study effects of particle size and osmolarity, 11 asthmatics inhaled five different aerosols for 16 min by mouthpiece at rest: (1) H_2SO_4 at 300 mOsm (VMD approximately 6 μm); (2) H_2SO_4 30 mOsm (VMD approximately

6 μm); (3) sodium chloride 30 mOsm (VMD approximately 6 μm); (4) H_2SO_4 (VMD approximately 0.4 μm); and (5) H_2SO_4, (VMD approximately 0.4 μm). Sulfuric acid concentrations were high, at approximately 3000 $\mu g/m^3$. Airway resistance actually decreased slightly with all aerosol exposures and there were no significant effects on respiratory symptoms.

In a second mouthpiece study, nine subjects were exposed at rest (part 1) to H_2SO_4 at approximately 3000 $\mu g/m^3$, with large (VMD $\approx$6 μm) versus small (0.3 μm) particle size and low (< 10%) versus high (100%) relative humidity. Sodium chloride aerosols under similar conditions served as control. Because these exposures caused no decrements in SRaw, six subjects underwent exposures to small particle, low humidity H_2SO_4 versus sodium chloride while exercising at 40 L/min (part 2). Although SRaw increased significantly with exercise, there was no difference between H_2SO_4 and sodium chloride exposures. These results are shown in Figure 11-1. A significant increase in throat irritation was observed with the low humidity, small particle H_2SO_4 inhalation in part 1 of this study (n=9) but was not replicated in part 2 (n=6).

Finally, an environmental chamber exposure study was undertaken to examine effects of H_2SO_4 fogs (VMD approximately 6 μm) with varying water content on airways resistance. Ten subjects were exposed for 1 h with intermittent exercise to H_2SO_4 and NaCl at low (0.5 $\mu g/m^3$) and high (1.8 $\mu g/m^3$) liquid water content. The mean sulfate concentrations were 960 $\mu g/m^3$ for low water content fogs and 1,400 $\mu g/m^3$ for high liquid water content fog. Surprisingly, SRaw decreased slightly with most exposures, with no significant difference among the 4 atmospheres. The authors speculated that the decrements in pulmonary function following exposure to acid aerosols in previous studies may have been due to increases in airway secretions or effects on the larynx rather than bronchoconstriction.

Responsiveness of adolescent asthmatic subjects to H_2SO_4 aerosols was further explored by Hanley et al. (1992). Fourteen allergic asthmatics aged 12 to 19 years inhaled air or H_2SO_4 at targeted concentrations of 70 and 130 $\mu g/m^3$, for 30 min at rest and 10 min with exercise. In a second protocol, nine subjects were exposed to targeted concentrations of 70 $\mu g/m^3$ H_2SO_4, with and without drinking lemonade to reduce oral ammonia. Actual exposure concentrations ranged from 51 to 176 $\mu g/m^3$ H_2SO_4. Exposures lasted 45 min, including two 15-min exercise periods. Aerosol MMAD was 0.72 μm. For the purposes of

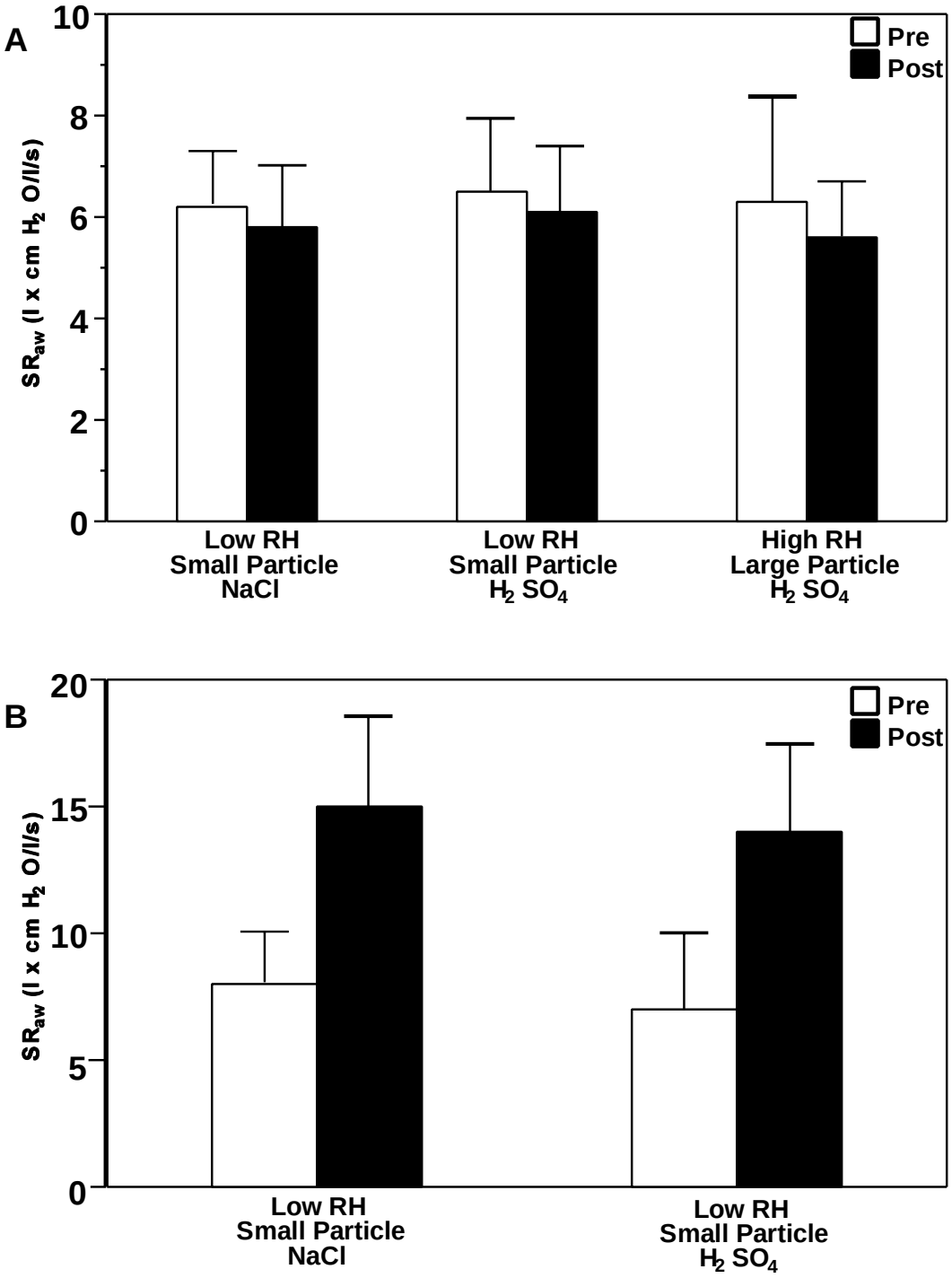

Figure 11-1. Mean plus or minus standard error of the mean specific airway resistance (SR_{aw}) before and after a 16-min exposure for (A) nine subjects who inhaled low relative-humidity (RH) sodium chloride (NaCl), low-RH sulfuric acid (H_2SO_4), and high-RH H_2SO_4 aerosols at rest, and (B) six subjects who inhaled low-RH NaCl and low-RH H_2SO_4 aerosols during exercise.

Source: Aris et al. (1991b).

this document, mean changes in FEV_1 were calculated from individual subject data provided in the published report. In the first protocol, FEV_1 fell 0.05 ± 0.08 L after air and 0.15 ± 0.14 L after nominal 70 $\mu g/m^3$ H_2SO_4. In the second protocol, FEV_1 fell 0.00 ± 0.23 L without lemonade gargle and 0.13± 0.09 L with lemonade gargle. Results from the 22 subjects exposed in the two protocols were combined for the published analyses, and changes in pulmonary function were regressed against H^+ concentration for each subject. Decrements in FEV_1 and FVC were statistically significant at 2 to 3 min after exposure, but not at 20 min after exposure. Changes in $Vmax_{50}$ and total respiratory resistance were not significantly different. The findings corresponded to a fall in FEV_1 of approximately 37 ml/μM H^+. A significant correlation was found between exercise-induced bronchoconstriction, determined prior to exposure using a treadmill test, and the slope of Δ FEV_1/H^+. A similar observation linking baseline airways reactivity to H_2SO_4 responsiveness had been made previously by Utell et al. (1983b).

Koenig et al. (1992) examined the effects of more prolonged mouthpiece exposures to H_2SO_4 (MMAD = 0.6μm). Fourteen allergic asthmatic subjects aged 13 to 18, with exercise-induced bronchoconstriction, were exposed to air or 35 and 70 $\mu g/m^3$ H_2SO_4, for 45 min and 90 min, on separate occasions. Oral ammonia was reduced by drinking lemonade. The exposures included alternate 15-min periods of exercise at three times resting $\dot{V}_E$. The largest decrements in FEV_1 (6%) actually occurred with the shorter exposure to the lower concentration of H_2SO_4 (35 $\mu g/m^3$). Changes following exposure to 70 $\mu g/m^3$ and following 90 min exposures were not significant. The authors concluded that duration of exposure did not play a role in the response to H_2SO_4 aerosols. However, the absence of a concentration response in the studies suggests that the statistical findings may be due to chance. Therefore, the study does not appear to demonstrate a convincing effect of H_2SO_4 at these exposure levels.

Anderson et al. (1992) included 15 asthmatic adults in a study comparing the effects of exposure for 1 h to air, 100 $\mu g/m^3$ H_2SO_4, 200 $\mu g/m^3$ carbon black particles, and acid-coated carbon black (MMAD $\approx$1.0μm). Decrements in FEV_1 were observed for all exposures, averaging about 9%. Analysis of variance for FVC showed a significant interaction of acid, carbon, and time factors ($p = 0.02$), but the largest decrements actually occurred with air exposure.

In the only study of elderly asthmatics, Koenig et al. (1993) exposed nine subjects, 60 to 76 years of age, by mouthpiece to air, $(NH_4)_2SO_4$, or 70 $\mu g/m^3$ H_2SO_4 (MMAD = 0.6μm), with and without lemonade gargle. Exposures were 30 min at rest followed by 10 min of mild exercise ($\dot{V}_E$ = 17.5 L/min). Greater increases in total respiratory resistance occurred following H_2SO_4 without lemonade than following the other atmospheres, but the difference between atmospheres was not significant.

In a study comparing effects of H_2SO_4 exposure in subjects with asthma and COPD, Morrow et al. (1994) exposed 17 allergic asthmatic subjects in an environmental chamber to 90 $\mu g/m^3$ H_2SO_4 or NaCl (MMAD<1 μm) for 2 h with intermittent exercise. Pulmonary function was measured after each of four 10 min exercise periods, and again 24 h after exposure, before and after exercise. Decrements in FEV_1 were consistently greater in H_2SO_4 than NaCl, although the difference was statistically significant only following the second exercise period. FEV_1 decreased $\approx$18% after H_2SO_4 compared with $\approx$14% after NaCl ($p = 0.02$). Reductions in SGaw were significantly different only following the fourth exercise period ($p = 0.009$). No changes were found in symptoms or arterial oxygen saturation, and there were no significant changes in lung function 24 h after exposure.

Finally, two recent studies have examined combined exposures to H_2SO_4 and ozone, one using a combined pollutant atmosphere for 6 h per day over 2 days, (Linn et al., 1994) and the other using sequential 3 h exposures to H_2SO_4 followed 1 day later by ozone (Frampton et al., 1995). These reports will be discussed in detail in section 11.2.1.7. However, neither study found any significant changes in lung function in asthmatics exposed to 100 $\mu g/m^3$ H_2SO_4 alone.

In summary, asthmatic subjects appear to be more sensitive than healthy subjects to the effects of acid aerosols on lung function, but the effective concentrations differ widely among laboratories. Although the reasons for these differences remain largely unclear, subject selection and differences in neutralization of acid by NH_3 may be important factors. Adolescent asthmatics may be more sensitive than adults, and may experience small decrements in lung function in response to acid aerosols at exposure levels only slightly above peak ambient levels. Even in studies reporting an overall absence of effects on lung function, some individual asthmatic subjects appear to demonstrate clinically important effects. Submicron aerosols appear to have greater effects on spirometry and airway

resistance than particles in the 10μ-20 μm range. However, respiratory symptoms (cough, irritation, etc.) are observed with both large and small aerosols.

11.2.1.4 Effects of Acid Aerosols on Airway Responsiveness

Human airways may undergo bronchoconstriction in response to a variety of stimuli. Airway responsiveness can be quantitated by measuring changes in expiratory flow or airways resistance in response to inhalation challenge. Typically, the challenging agent is a non-specific pharmacologic bronchoconstrictor such as methacholine or histamine. Other agents include carbamylcholine (carbachol), cold dry air, sulfur dioxide, hypo-osmolar aerosols, or exercise. In allergic subjects, airway challenge with specific allergens can be performed, although the responses are variable, and late phase reactions can result in bronchoconstriction beginning 4 to 8 h after challenge and lasting 24 h or more. Although many individuals with airway hyperresponsiveness do not have asthma, virtually all asthmatics have airway hyperresponsiveness, possibly reflecting underlying airway inflammation. Changes in clinical status are often accompanied by changes in airway responsiveness. Thus alterations in airway responsiveness may be clinically significant, even in the absence of direct effects on lung function (Godfrey, 1993; Weiss et al., 1993). Molfino et al. (1992) have provided a brief review of air pollution effects on allergic bronchial responsiveness.

As noted in section 11.2.1.3, two studies (Utell et al., 1983b; Hanley et al., 1992) have suggested that the degree of baseline airway responsiveness may predict responsiveness to acid aerosol exposure in asthmatic subjects. This section will deal only with studies examining changes in airway responsiveness with exposure to particles.

Despite the absence of effects on lung function in healthy subjects, Utell et al. (1983a) observed, in healthy nonsmokers, an increase in airway responsiveness to carbachol following exposure to 450 $\mu g/m^3$ H_2SO_4 (MMAD = 0.8). The increase occurred 24 h, but not immediately, after exposure. In addition, some subjects reported throat irritation between 12 and 24 h after exposure to H_2SO_4. These findings suggested the possibility of delayed effects. These investigators also observed increases in airway responsiveness among asthmatic subjects following exposure to 450 and 1000 $\mu g/m^3$, but not 100 $\mu g/m^3$ H_2SO_4. These findings have been reviewed (Utell et al., 1991).

Avol et al. (1988a,b) included airway responsiveness as an outcome measure in their studies of healthy and asthmatic subjects exposed to varying concentrations of H_2SO_4. No effects on responsiveness were reported, with either acidic fogs or submicron aerosols, at H_2SO_4 concentrations as high as 2000 $\mu g/m^3$. However, airway challenge was performed using only two concentrations of methacholine. This limited challenge may have been insufficiently sensitive to detect small changes in airway responsiveness.

Using a similar 2-dose methacholine challenge protocol, Linn et al. (1989) found no change in airway responsiveness of healthy subjects following exposure to 2000 $\mu g/m^3$ H_2SO_4 for 1 h, at particle sizes ranging from 1 to 20 μm. Anderson et al. (1992), in their study of responses to 100 $\mu g/m^3$ H_2SO_4, 200 $\mu g/m^3$ carbon black, and acid coated carbon, found no effects on airway responsiveness in healthy or asthmatic subjects. In this study, a conventional methacholine challenge was used, administering doubling increases in methacholine concentration until FEV_1 decreased more than 20%.

In a study primarily designed to examine effects of acid fog exposure on mucociliary clearance, Laube et al. (1993) examined changes in airway responsiveness to methacholine in 7 asthmatic subjects exposed to 500 $\mu g/m^3$ H_2SO_4 or NaCl (MMAD $\approx$10 μm) for 1 h with 20 min of exercise. Responsiveness was measured at screening and 30 min after each exposure. No difference was observed between H_2SO_4 and NaCl exposures.

A recent study (Linn et al., 1994) has suggested that exposure to ozone with H_2SO_4 may enhance the increase in airway responsiveness seen with ozone exposure alone. Fifteen healthy and 30 asthmatic subjects were exposed to air, 0.12 ppm ozone, 100 $\mu g/m^3$ H_2SO_4 (MMAD $\approx$0.5), and ozone + H_2SO_4 for 6.5 h on 2 successive days, with intermittent exercise. Airway responsiveness was measured after each exposure day using a conventional methacholine incremental challenge, and compared with baseline measured on a separate day. An ANOVA using data from all subjects found an increase in airway responsiveness in association with ozone exposure ($p=0.003$), but showed no significant change following exposure to air or H_2SO_4 alone. Multiple comparisons did not reveal significant differences in airway responsiveness between ozone and ozone + H_2SO_4 in healthy or asthmatic subjects. However, asthmatic subjects showed the greatest increase in airway responsiveness following the first day of ozone + H_2SO_4, and ANOVA revealed a significant interaction of clinical status, ozone, acid, and day ($p=0.03$). Decreases in FEV_1 following methacholine

challenge for healthy subjects were 8% after air, 6% after H_2SO_4, 9% after ozone, and 13% after ozone + H_2SO_4. Changes were smaller following the second exposure day, suggesting attenuation of responsiveness with repeated exposure, as seen in previous studies of ozone alone (U.S. Environmental Protection Agency, 1995). These studies suggest that exposure to low concentrations of H_2SO_4 may enhance ozone-induced increases in airway responsiveness in both healthy and asthmatic subjects.

Koenig et al. (1994) sought to determine whether exposure to H_2SO_4 or HNO_3 enhanced changes in lung function or airway responsiveness seen with exposure to ozone + nitrogen dioxide (NO_2). Adolescent asthmatic subjects were exposed to air, 0.12 ppm ozone + 0.3 ppm NO_2, ozone + NO_2 + 73 $\mu g/m^3$ H_2SO_4 (MMAD = 0.6), and ozone + NO_2 + 0.05 ppm HNO_3. Exposures were by mouthpiece for 90 min, with intermittent exercise, on two consecutive days. Airway responsiveness was measured by methacholine challenge at screening and on the day following the second pollutant exposure. No effects on airway responsiveness were found for any atmosphere. However, challenge following pollutant exposure utilized only doses of methacholine well below the level causing significant reductions in FEV_1 for these subjects at baseline, making it unlikely that small or transient changes in responsiveness would be detected. Six subjects did not complete the protocol because of illness, symptoms, and other factors which may or may not have been related to pollutant exposure; these data were not included in the analysis.

In summary, the data suggest that there is little, if any, effect of low concentration acid aerosol exposure (regardless of particle size) on airway responsiveness in healthy or asthmatic subjects. Observations of possible delayed increases in responsiveness in healthy subjects exposed to 450 $\mu g/m^3$ H_2SO_4 (Utell et al., 1983a), and H_2SO_4 enhancement of ozone effects on airway responsiveness in healthy and asthmatic subjects (Linn et al., 1994) require confirmation in additional studies, utilizing standard challenge protocols.

11.2.1.5 Effects of Acid Aerosols on Lung Clearance Mechanisms

Brief (1- to 2-h) exposures to H_2SO_4 aerosols have shown consistent effects on mucociliary clearance in three species: donkeys, rabbits, and humans. The direction and magnitude of the effect are dependent on the concentration and duration of the acid aerosol

exposure, the size of the acid particle, and the size of the tracer particle. Clearance studies in animals are discussed in Section 11.2.2.5.

Initial studies in healthy nonsmokers by Leikauf et al. (1981) found that exposure to 110 $\mu g/m^3$ H_2SO_4 (MMAD $\approx 0.5 \mu m$) for 1 h at rest accelerated bronchial mucociliary clearance of 7.5 μm tracer particles, while a similar exposure to 980 $\mu g/m^3$ H_2SO_4 slowed clearance. A second study (Leikauf et al., 1984) utilizing a smaller tracer particle (4.2 μm) to assess more peripheral airways, found slowing of clearance with both 108 and 983 $\mu g/m^3$ H_2SO_4, in comparison with distilled water aerosol. Spektor et al. (1989) extended these studies, exposing ten healthy subjects to H_2SO_4 (MMAD = 0.5μm) or distilled water aerosols for up to 2 h. Two different 4.2 μm tracer aerosols were used, one administered before and the other after exposure. Following a 2 h exposure to 100 $\mu g/m^3$ H_2SO_4, clearance halftime tripled compared with control, with reduced clearance rates still evident 3 h after exposure. These findings suggested that brief, resting exposures to H_2SO_4 at ≈ 100 $\mu g/m^3$ accelerate clearance in large bronchi but slow clearance in more peripheral airways in a dose-dependent fashion.

Data from studies in asthmatics are less clear. Spektor et al. (1985) exposed ten asthmatic subjects to 0, 110, 319, and 911 $\mu g/m^3$ H_2SO_4 (MMAD = 0.5μm) for 1 h. The effects were difficult to interpret because of inhomogeneous distribution of the tracer aerosol in the more severe asthmatics. However, clearance was decreased following the highest concentration of acid exposure in the six subjects with the mildest asthma (not dependent on regular medications). These responses were similar to those of healthy subjects reported above.

Laube et al. (1993) recently examined the effects of acid fog on mucociliary clearance in asthmatics. Seven nonsmoking subjects with mild asthma (baseline FEV_1 90 to 118% predicted) were exposed in a head dome to 500 $\mu g/m^3$ H_2SO_4 or NaCl (MMAD ≈ 10 μm) for 1 h with 20 min of exercise. Mucociliary clearance was measured using inhalation of a technetium-99M sulfur colloid aerosol after exposure to the test aerosol. Tracheal clearance was measured in four subjects, and was increased in all four after H_2SO_4 exposure (no statistical analysis was performed because of the small number of subjects). Outer zone lung clearance was increased in six of seven subjects after H_2SO_4 exposure ($p < 0.05$). The

dose of H^+ inhaled orally correlated significantly with the change in outer zone lung clearance ($r = 0.79$, $p = 0.05$).

11.2.1.6 Effects of Acid Aerosols Studied by Bronchoscopy and Airway Lavage

Fiberoptic bronchoscopy with BAL has proved a useful technique for sampling the lower airways of humans in clinical studies of oxidant air pollutants. The type and number of cells recovered in BAL fluid reflect changes in alveolar and distal airway cell populations, providing a relatively sensitive measure of inflammation. Increases in serum proteins recovered in BAL fluid can be a result of increased epithelial permeability, a consequence of injury and/or inflammation. Alveolar macrophages obtained by BAL can be assessed *in vitro* for functional changes important in inflammation and host defense. In addition, proximal airway cells and secretions can be recovered using airway washes or proximal airway lavage (Eschenbacher and Gravelyn, 1987).

Only one study has utilized bronchoscopy to evaluate the effects of exposure to acid aerosols. Frampton et al. (1992) exposed 12 healthy nonsmokers to aerosols of NaCl (control) or H_2SO_4 (MMAD = 0.9, GSD = 1.9) at 1000 $\mu g/m^3$ for 2 h. Four 10-min exercise periods at $\approx$40 L/min were included. Subjects brushed their teeth and rinsed with mouthwash prior to and once during each exposure to reduce oral ammonia levels. Fiberoptic bronchoscopy with BAL was performed 18 h after exposure. No evidence for airway inflammation was found. Markers for changes in host defense, including lymphocyte subset distribution, antibody-dependent cellular cytotoxicity of alveolar macrophages, and alveolar macrophage inactivation of influenza virus, were not significantly different between H_2SO_4 and NaCl exposures.

In an effort to define possible effects of H_2SO_4 exposure on airway mucus, Culp et al. (1995) determined the composition of mucins recovered during bronchoscopy of subjects studied by Frampton et al. (1992), as well as from some subjects not exposed. Secretions were lipid extracted from airway wash samples and analyzed with regard to glycoprotein content, protein staining profiles, and amino acid and carbohydrate composition. Mucin composition was similar when non-exposed subjects were compared with NaCl-exposed subjects, indicating that aerosol exposure *per se* did not alter mucus composition. No differences were found between H_2SO_4 and NaCl exposure with regard to absolute yields

of high-density material, proportion of glycoproteins, presence of glycoprotein degradation products, carbohydrate composition, or protein composition.

In these studies, bronchoscopy was performed 18 h after exposure in order to detect delayed effects. Transient effects of exposure to acid aerosols on alveolar macrophage function or mucous composition have therefore not been excluded.

11.2.1.7 Human Exposure Studies of Acid Aerosol Mixtures

In human subjects, previous studies have suggested that exposure to H_2SO_4 does not potentiate responses to other pollutants. A number of more recent studies have also failed to find interactions in effects of pollutant mixtures that include H_2SO_4. Anderson et al. (1992) found no effects on lung function following exposure to 200 $\mu g/m^3$ carbon black alone, or carbon particles coated with H_2SO_4. Aris et al. (1990) found no effects on airways resistance of exposure to mixtures of hydroxymethanesulfonic acid and H_2SO_4. Balmes et al. (1988) found no differences between the effects of H_2SO_4 and HNO_3 exposure in asthmatics, and no interaction with exposure to both aerosols by mouthpiece. Koenig et al. (1989) found that exposure of adolescent asthmatic subjects to 68 $\mu g/m^3$ H_2SO_4 with 0.1 ppm SO_2 did not increase the responses seen with H_2SO_4 alone.

In one recent study funded by the Health Effects Institute, 28 adolescent asthmatic subjects were exposed to air, 0.12 ppm ozone + 0.3 ppm NO_2, ozone + NO_2 + 68 $\mu g/m^3$ H_2SO_4, and ozone + NO_2 + 0.05 ppm HNO_3 (Koenig et al., 1994). Exposures were by mouthpiece for 90 min, with intermittent exercise, on two consecutive days. No significant effects on lung function were seen for any of the atmospheres. However, six subjects did not complete the study protocol for a variety of reasons; these subjects were characterized by the authors as having moderate to severe asthma, based on results of methacholine challenge. Although the reasons for withdrawal of these subjects were not clearly related to exposures, all discontinued participation following exposure to pollutants rather than to clean air. As noted in the published comments of the Health Effects Institute Health Review Committee accompanying the Koenig et al. report, "...the conclusions of the study may have been based on a group of subjects more tolerant to oxidants, acid aerosols, or both, than those constituting the original study group" (Koenig et al., 1994, page 103).

Two recent studies suggest that exposure to 100 $\mu g/m^3$ H_2SO_4 may enhance airway effects of exposure to ozone. Linn et al. (1994) exposed 15 healthy and 30 asthmatic subjects to air, 0.12 ppm ozone, 100 $\mu g/m^3$ H_2SO_4 (MMAD $\approx$0.5 μm), and ozone + H_2SO_4 for 6.5 h on two consecutive days. Each subject received all 4 pairs of exposures, each separated by one week. Subjects were exposed in small groups in an environmental chamber, with six, 50-min exercise periods each day. Acidic gargles were used to reduce oral ammonia. Lung function and methacholine responsiveness were measured at the end of each exposure day. Reductions in FEV_1 and FVC, and increases in airway responsiveness, were observed in association with ozone exposure in both healthy and asthmatic subjects. Some subjects in both the asthmatic and nonasthmatic group demonstrated greater declines in lung function after the first day of acid + ozone than after ozone alone (Figure 11-2), although the group mean differences were only marginally significant by ANOVA. From these data, a "hypothetical average subject", under the specific conditions of the study, would be expected to lose 100 ml FEV_1 during ozone exposure relative to clean air exposure, and would lose 189 ml FEV_1 during ozone + H_2SO_4 exposure. When the responsive subjects were re-studied months later, increased responsiveness to acid + ozone compared with ozone was again demonstrated, although individual responses to O_3 + H_2SO_4 in the original and repeat studies were not significantly correlated.

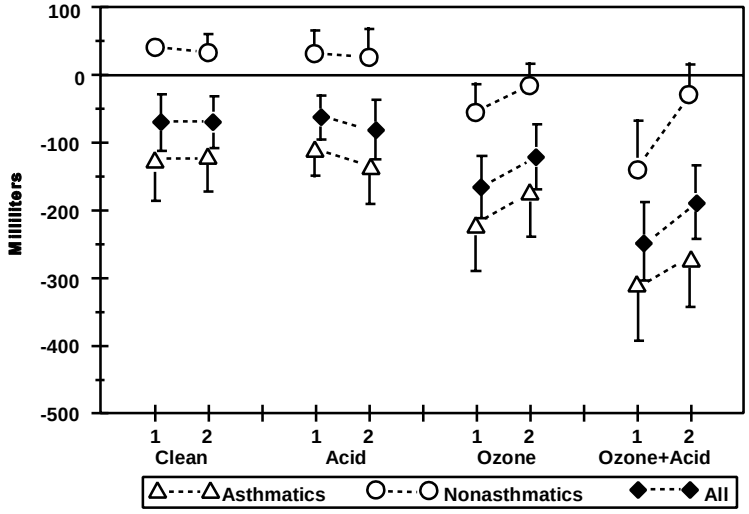

Figure 11-2. Decrements in forced expiratory volume in 1 s (plus or minus standard error) following 6.5-h exposures on 2 successive days.

Source: Linn et al. (1994).

Frampton et al. (1995) exposed 30 healthy and 30 asthmatic subjects to 100 $\mu g/m^3$ H_2SO_4 or NaCl for 3 h followed the next day by 0.08, 0.12, or 0.18 ppm ozone for 3 h. All exposures included intermittent exercise. Each subject received two of the three ozone exposure levels. Exposure to H_2SO_4 or NaCl did not alter lung functions. As shown in Table 11-4, changes in spirometry following exposure to ozone were small, consistent with the relatively low concentrations, short exposure duration, and moderate exercise levels ($\dot{V}_E$ 30.6 to 36.2 L/min for a total of 60 min). Figure 11-3 shows the percentage changes in FVC 4 h after ozone exposure; these changes were similar to those found immediately after exposure. With H_2SO_4 pre-exposure, FVC decreased following ozone in a concentration-response fashion. The ANOVA revealed significant main effects of ozone exposure as well as a significant interaction between aerosol and ozone exposure for effects on FEV_1 and FVC among the asthmatic subjects, but not the healthy subjects. Four-way ANOVA revealed an interaction between ozone and aerosol for the entire group (p=0.0022) and a difference between healthy subjects and subjects with asthma (p=0.0048). Surprisingly, the largest decrements in FVC with the NaCl preexposure were found with 0.08 ppm ozone, whereas no effect was seen at 0.18. With 0.18 ppm ozone preceded by H_2SO_4, the responses were similar to those seen at 0.08 with NaCl. The authors concluded that, for asthmatic subjects, H_2SO_4 alters the response to ozone in comparison with NaCl pre-exposure. Interpretation of these findings would be facilitated by a similar study including air as a further control pre-exposure atmosphere. However, considered together, these two studies (Frampton et al., 1995 and Linn et al., 1994) suggest that H_2SO_4 aerosol exposure may enhance airway responsiveness to ozone.

11.2.1.8 Summary and Conclusions

Controlled human studies offer the opportunity to study the responses of human subjects under carefully controlled conditions, but are limited to short-term exposures to pollutant atmospheres without severe health risks. Outcome measures are limited by safety issues, but have been extended beyond measures of lung function and symptoms to include mucociliary clearance, BAL, and airway biopsies.

Human clinical studies of particle exposure remain almost completely limited to the study of acid aerosols, primarily of H_2SO_4, with the majority of these focussing on

TABLE 11-4. PULMONARY FUNCTION RESPONSES AFTER AEROSOL AND OZONE EXPOSURES IN SUBJECTS WITH ASTHMA[a]

	FVC (L)		FEV_1 (L)		sGaw (cm H_2O/L/sec)	
Time of Measurement	NaCl	H_2SO_4	NaCl	H_2SO_4	NaCl	H_2SO_4
0.08 ppm Ozone						
Baseline	3.80 ± 0.17	3.73 ± 0.17	2.85 ± 0.11	2.79 ± 0.10	0.204 ± 0.021	0.209 ± 0.020
After exercise	3.64 ± 0.17	3.59 ± 0.18	2.84 ± 0.12	2.72 ± 0.12	-	-
Immediately after exposure	3.51 ± 0.18	3.64 ± 0.17	2.73 ± 0.12	2.79 ± 0.11	0.176 ± 0.024	0.177 ± 0.022
2 Hours after exposure	3.67 ± 0.17	3.70 ± 0.16	2.91 ± 0.12	2.89 ± 0.11	-	-
4 Hours after exposure	3.67 ± 0.15	3.74 ± 0.18	2.92 ± 0.10	2.92 ± 0.13	-	-
0.12 ppm Ozone						
Baseline	3.97 ± 0.22	3.95 ± 0.22	2.98 ± 0.17	3.05 ± 0.17	0.220 ± 0.015	0.236 ± 0.020
After exercise	3.72 ± 0.20	3.76 ± 0.19	2.94 ± 0.17	3.01 ± 0.16	-	-
Immediately after exposure	3.72 ± 0.21	3.76 ± 0.20	2.90 ± 0.19	2.97 ± 0.18	0.186 ± 0.019	0.209 ± 0.025
2 Hours after exposure	3.91 ± 0.22	3.85 ± 0.21	3.10 ± 0.18	3.08 ± 0.17	-	-
4 Hours after exposure	3.87 ± 0.22	3.87 ± 0.21	3.07 ± 0.18	3.04 ± 0.18	-	-
0.18 ppm Ozone						
Baseline	3.89 ± 0.23	3.99 ± 0.22	2.92 ± 0.16	3.04 ± 0.17	0.183 ± 0.016	0.207 ± 0.016
After exercise	3.76 ± 0.23	3.71 ± 0.22	2.90 ± 0.19	2.99 ± 0.16	-	-
Immediately after exposure	3.76 ± 0.23	3.74 ± 0.24	2.90 ± 0.19	2.96 ± 0.18	0.170 ± 0.016	0.179 ± 0.018
2 Hours after exposure	3.81 ± 0.25	3.87 ± 0.23	3.03 ± 0.19	3.03 ± 0.17	-	-
4 Hours after exposure	3.90 ± 0.24	3.84 ± 0.25	3.06 ± 0.17	2.99 ± 0.18	-	-

[a] Values are expressed as means ± SEM.

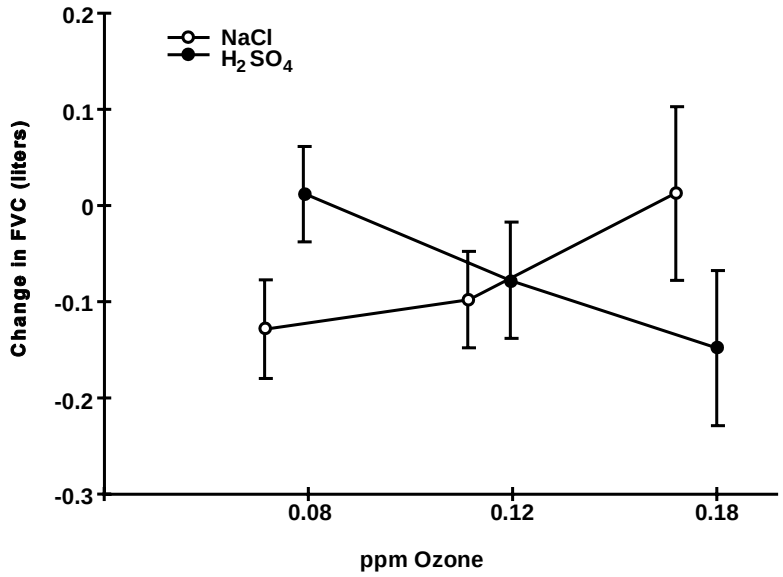

Figure 11-3. Asthmatic subjects. The absolute change in FVC (means ± SE) 4-h after exposure to each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexposure conditions.

Source: Frampton et al. (1995).

symptoms and pulmonary function. Only two studies (Frampton et al., 1992; Culp et al., 1995) have utilized BAL to examine effects of particle exposure in humans. No studies have examined effects of particle or acid aerosol exposure on airway inflammation in asthmatic subjects. There are no studies examining the effects of particle exposure on antigen challenge in allergic or asthmatic subjects.

Ten studies since 1988 have confirmed previous findings that healthy subjects do not experience decrements in lung function following single exposures to H_2SO_4 of various particle sizes at levels up to 2,000 $\mu g/m^3$ for 1 h, even with exercise and use of acidic gargles to minimize neutralization by oral ammonia. Mild lower respiratory symptoms occur at exposure concentrations in the mg/m^3 range, particularly with larger particle sizes. Acid aerosols alter mucociliary clearance in healthy subjects at levels as low as 100 $\mu g/m^3$, with effects dependent on exposure concentration, acid aerosol particle size, and the region of the lung being studied.

Asthmatic subjects appear to be more sensitive than healthy subjects to the effects of acid aerosols on lung function, but the effective concentration differs widely among studies.

Adolescent asthmatics may be more sensitive than adults and may experience small decrements in lung function in response to H_2SO_4 at exposure levels only slightly above peak ambient levels. Although the reasons for the inconsistency among studies remain largely unclear, subject selection and acid neutralization by NH_3 may be important factors. Even in studies reporting an overall absence of effects on lung function, occasional asthmatic subjects appear to demonstrate clinically important effects. Two studies from different laboratories have suggested that responsiveness to acid aerosols may correlate with degree of baseline airway hyperresponsiveness. There is a need to identify determinants of responsiveness to H_2SO_4 exposure in asthmatic subjects. In very limited studies, elderly and individuals with chronic obstructive pulmonary disease do not appear to be particularly susceptible to the effects of submicron acid aerosols on lung function.

Two recent studies have examined the effects of exposure to both H_2SO_4 aerosols and ozone on lung function in healthy and asthmatic subjects. Both studies found evidence that 100 $\mu g/m^3$ H_2SO_4 may potentiate the response to ozone, in contrast with previous studies.

Human studies of particles other than acid aerosols provide insufficient data to draw conclusions regarding health effects. However, available data suggest that inhalation of inert particles in the respirable range, including three studies of carbon particles, have little or no effect on symptoms or lung function in healthy subjects at levels above peak ambient concentrations.

11.2.2 Laboratory Animal Studies

11.2.2.1 Introduction

This section reviews the effects of acidic aerosols on laboratory animals. Almost all of the available data have been derived from studies using acidic sulfates, namely ammonium bisulfate (NH_4HSO_4) and sulfuric acid (H_2SO_4).

11.2.2.2 Mortality

The previous CD (U.S. Environmental Protection Agency, 1982) examined animal studies of the acute lethality of acid aerosols (mainly H_2SO_4), and there are few new data to add here. As for other toxicologic endpoints, large interspecies differences occurred, with the guinea pig being the most sensitive, compared to the mouse, rat and rabbit. But high

concentrations of H_2SO_4, generally in excess of 10,000 $\mu g/m^3$, were required for lethality, even in a species as sensitive as the guinea pig. Also, within a particular species of experimental animal, the H_2SO_4 concentration required for lethality was dependent upon particle size, with smaller particles being less effective than larger ones. As noted in the previous CD, the cause of death due to acute, high-level H_2SO_4 exposure was laryngeal or bronchial spasm. Since these are irritant responses, differences in the deposition pattern of smaller and larger acid droplets may account for the aforementioned particle size dependence of lethal concentration; larger particles deposit to a greater extent in the larynx and upper bronchial tree, where the bulk of irritant receptors are located. As acid particle size is reduced, deeper pulmonary damage occurs prior to death. Lesions commonly seen are focal atelectasis, hemorrhage, congestion, pulmonary and perivascular edema, and desquamation of bronchiolar epithelium; hyperinflation is also often evident.

Few data allow assessment of lethality for acid sulfate aerosols other than H_2SO_4. Pattle et al. (1956) noted that if sufficient ammonium carbonate was added into the chamber where guinea pigs were exposed to H_2SO_4 so as to provide excess NH_3, protection was afforded to acid levels which would have produced 50% mortality in the absence of NH_3. This implies that H_2SO_4 is more acutely toxic than its neutralization products [i.e., NH_4HSO_4 and/or $(NH_4)_2SO_4$]. Pepelko et al. (1980a) found no mortality among rats exposed for 8 h/day for 3 days to $(NH_4)_2SO_4$ at 1,000,000 to 1,200,000 $\mu g/m^3$ (2 to 3 μm, MMAD); but 40 and 17% mortality occurred in guinea pigs exposed once for 8 h to 800,000 to 900,000, or 600,000 to 700,000 $\mu g/m^3$, respectively, of similarly sized-particles. Death was ascribed to airway constriction, rather than to extensive lung damage. As with H_2SO_4, guinea pigs were more sensitive than other species.

In summary, very high concentrations of acid sulfates are required to cause mortality in otherwise healthy animals, with variations in effective concentrations depending on acid particle size and the animal species tested.

11.2.2.3 Pulmonary Mechanical Function

Many studies examining the toxicology of inhaled acid aerosols at sublethal levels used changes in pulmonary function as indices of response. A survey of the database since

publication of the previous CD (U.S. Environmental Protection Agency, 1982) is presented in Table 11-5.

One of the major exposure parameters which affects response is particle size. Studies by Amdur (1974) and Amdur et al. (1978a,b), summarized in the previous CD, showed that the irritant potency of H_2SO_4, $(NH_4)_2SO_4$, or NH_4HSO_4, as measured by pulmonary resistance in guinea pigs, increased with decreasing particle size (i.e., the degree of response per unit mass of sulfate [$SO_4^=$] at any specific exposure concentration increased as particle size decreased, at least within the size range of 1 to 0.1 μm). If this is compared to the relationship between particle size and mortality, it is evident that the relative toxicity of different particle sizes also depends upon the exposure concentration. At high concentrations above the threshold for lethality, large particles were more effective in eliciting response, while at lower (sublethal) levels, smaller particles were more effective.

Pulmonary functional responses to H_2SO_4 described previously suggested a major site of action to be the conducting airways, as evidenced by exposure-induced alterations in airflow resistance. However, some earlier data also suggested that high exposure levels may affect more distal lung regions, as evidenced by changes in pulmonary diffusing capacity (DL_{co}) noted in dogs exposed to 889 $\mu g/m^3$ (MMAD = 0.5μm) (Lewis et al., 1973). Deep lung effects of H_2SO_4 are also evident from studies of morphologic and lung defense endpoints, discussed in subsequent sections.

Studies reported in the previous CD (U.S. Environmental Protection Agency, 1982) indicated that the particle size of the acid aerosol affected the temporal pattern of any pulmonary function response. For example, the response to 100 $\mu g/m^3$ H_2SO_4 at 1 μm was slight and rapidly reversible, while that with 0.3 μm droplets was greater and more persistent. At any particular size, however, the degree of change in resistance and compliance in guinea pigs was observed to be concentration related.

Although the earlier studies by Amdur and colleagues appeared to provide a reasonable picture of the relative effects of acid particle size and exposure concentration on the bronchoconstrictive response of guinea pigs at sublethal exposure levels, there is some conflict between these results and reports by others discussed in the previous CD (U.S. Environmental Protection Agency, 1982). Whereas the Amdur work supported a concentration dependence for respiratory mechanics alterations (i.e., animals in each

TABLE 11-5. EFFECTS OF ACIDIC SULFATE PARTICLES ON PULMONARY MECHANICAL FUNCTION

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique (RH)	Particle Characteristics: Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
H_2SO_4	Rat	Whole body	2,370	0.5 (MMD)	14 weeks	NC: V , f, R , Cd, pH, PaCO	Lewkowski et al. (1979)
H_2SO_4	Rat	Whole body	6,350	0.44 (MMD)	6 weeks	↓PaCO	Lewkowski et al. (1979)
H_2SO_4	Rat	Whole body	6,590	0.31 (MMD)	13 weeks	↑pH	Lewkowski et al. (1979)
H_2SO_4	Guinea pig, M Hartley	Whole body	1,000, 3,200	0.54 (MMD); 1.32	24 h/d, 3-30 d	Hypo- to hyperresponsive airways	Kobayashi and Shinozaki (1993)
H_2SO_4	Rabbit, M NZW	Nose-only (50%)	250	0.3 (MMAD); 1.6	1 h/day, 5 days/week, up to 12 mo	NC: R_L Hyperresponsive by 4 mo	Gearhart and Schlesinger (1986)
H_2SO_4	Guinea pig, M Hartley, 260-325 g	Nose-only (50%)	300	0.08 (MMD); 1.3	1 h	NC: VC, IC, VA, TLC; ↓DLco, (3 h post exp)	Chen et al. (1991)
H_2SO_4	Guinea pig, M Hartley, 290-410 g	Head-only (50%)	200	0.06 (MMD); 1.4	1 h	NC: R	Chen et al. (1992b)
$(NH_4)_2SO_4$	Guinea pig, M Hartley, 10 wk	Whole body (50-60%)	1,000	0.4 (MMAD); 2.2	6 h/day, 5 days/week, 1 or 4 weeks	NC: RV; ↑FRC, VC, TLC, DLco, Cd, ΔN_2	Loscutoff et al. (1985)
$(NH_4)_2SO_4$	Rat, M SD, 14 wk	Whole body (50-60%)	1,000	0.4 (MMAD); 2.3	6 h/day, 5 days/week, 1 or 4 weeks	↑RV, ↑FRC, ΔN_2	Loscutoff et al. (1985)

Key to abbreviations:
NC: No significant change
↑: Significant increase
↓: Significant decrease
Cd: Dynamic compliance
DLco: Diffusing capacity, CO
f: Respiratory frequency
FRC: Functional residual capacity
IC: Inspiratory capacity
ΔN_2: Change in distribution of ventilation as measured by nitrogen washout technique
$PaCO_2$: Partial pressure of CO_2 in arterial blood
pH: Arterial pH
R_L: Pulmonary resistance
RV: Residual volume
TLC: Total lung capacity
V_T: Tidal volume
VA: Alveolar volume
VC: Vital capacity

exposure group responded uniformly and the degree of response was related to the exposure concentration), others found that individual guinea pigs exposed to H_2SO_4 at similar sizes showed an "all-or-none" constrictive response (i.e., in atmospheres above a threshold concentration), some animals manifested major changes in pulmonary mechanics ("responders"), while others were not affected at all ("nonresponders") (Silbaugh et al., 1981b). As the exposure concentration was increased further, the percentage of the group which was affected (i.e., the ratio of responders to nonresponders) increased, producing an apparent concentration response relationship. However, the magnitude of the change in pulmonary function was similar for all responders, regardless of exposure concentration. Sensitivity to this all-or-none response may be related to an animal's baseline airway caliber prior to H_2SO_4 exposure, because responders had higher pre-exposure values for resistance and lower values for compliance, compared to nonresponders. In any case, the threshold concentration for the all-or-none response was fairly high (>10,000 $\mu g/m^3$ H_2SO_4). Reasons for the discrepancy with the studies of Amdur and colleagues are not known; they may involve differences in guinea pig strains, ages, or exposure conditions, or differences in techniques used to measure functional parameters. In any case, the dyspneic response of the guinea pig responders is similar to asthma episodes in humans, in both its rapidity of onset and in the associated characteristic obstructive pulmonary function changes.

A more recent approach used to evaluate the acute pulmonary functional response to H_2SO_4 involves co-inhalation of CO_2 (Wong and Alarie, 1982; Matijak-Schaper et al., 1983; Schaper et al., 1984). This procedure assesses the response to irritants by measuring a decrease in tidal volume (V_T) (based upon changes in inspiratory volume and pressure) which is routinely increased above normal by adding 10% CO_2 to the exposure atmosphere. Although the exact mechanism underlying a reduction in response to CO_2 is not clear, the assumption is that the change in ventilatory response after irritant exposure is due to direct stimulation of irritant receptors. A concentration-dependent decrease in CO_2-enhanced ventilation has been found in guinea pigs following 1-h exposures to H_2SO_4 (≈ 1 μm, MMD) at levels $\geq$40,100 $\mu g/m^3$ (Wong and Alarie, 1982). Subsequently, Schaper et al. (1984) exposed guinea pigs for 0.5 h to H_2SO_4 at 1,800 to 54,900 $\mu g/m^3$ (0.6 μm, AED). At concentrations >10,000 $\mu g/m^3$, the level of response (i.e., the maximum decrease in ventilatory response to CO_2) increased as a function of exposure concentration.

At concentrations below 10,000 $\mu g/m^3$, there was no clear relationship between exposure concentration and response; any effects were transient, occurring only at the onset of acid exposure.

The results of the studies with CO_2 differ from those of both Silbaugh et al. (1981b) and Amdur and colleagues, in that there was neither an "all or none" response as seen by the former, nor was there a concentration-response relationship observed at H_2SO_4 concentrations <10,000 $\mu g/m^3$, as reported by the latter. In addition, Amdur and colleagues observed sustained changes in lung function, rather than a fading response, at low concentrations. The reasons for these differences are unknown, but may partly reflect inherent sensitivity differences in the measurement techniques used as noted above.

The specific mechanisms underlying acid sulfate-induced pulmonary functional changes are not known, but may be due to irritant receptor stimulation resulting from direct contact by deposited acid particles or from humoral mediators released as a result of exposure. In terms of the latter, a possible candidate in mediation of the bronchoconstrictive response, at least in guinea pigs, is histamine (Charles and Menzel, 1975). On the other hand, evidence for a direct response to H_2SO_4 in altering pulmonary function was found using the CO_2 co-inhalation procedure. Schaper and Alarie (1985) noted that the responses to histamine and H_2SO_4 differed in both their magnitude and temporal relationship, suggesting direct action of the inhaled acid, or a role of other humoral factors.

Whatever the underlying mechanism, the results of pulmonary function studies indicate that H_2SO_4 is a bronchoactive agent that can alter lung mechanics of exposed animals primarily by constriction of smooth muscle; however, the threshold concentration for this response is quite variable, depending upon the animal species and measurement procedure used. In general, exposure to H_2SO_4 at levels <1,000 $\mu g/m^3$ does not produce physiologically significant changes in standard tests of pulmonary mechanics, except in the guinea pig. Although in this species such effects may be markers of exposure, any health significance in normal individuals is not clear. On the other hand, all subgroups of an exposed population may not be equally sensitive.

Airway Responsiveness

Some lung diseases (e.g., asthma) involve a change in airway "responsiveness", which is an alteration in the degree of reactivity to exogenous (or endogenous) bronchoactive agents, resulting in increased airway resistance at levels of these agents which would not affect airways of normal individuals. Such altered airways are called hyperresponsive. The use of pharmacologic agents capable of inducing smooth muscle contraction, a technique known as bronchoprovocation challenge testing, can assess the state of airway responsiveness after exposure to a nonspecific stimulus such as an inhaled irritant. Human asthmatics and, to some extent, chronic bronchitis, typically have hyperresponsive airways, but the exact role of this in the pathogenesis of airway disease is uncertain. Hyperresponsiveness may be a predisposing factor in clinical disease, or it may be a reflection of other changes in the airways which precede it. In any case, current evidence supports the hypothesis that an increase in airway responsiveness is a factor in the pathogenesis of obstructive airway disease (O'Connor et al., 1989).

The ability of H_2SO_4 aerosols to alter airway responsiveness has been assessed in a number of studies. Silbaugh et al. (1981a) exposed guinea pigs for 1 h to 4,000 to 40,000 $\mu g/m^3$ H_2SO_4 (1.01 μm, MMAD) and examined the subsequent response to inhaled histamine. Some of the animals showed an increase in pulmonary resistance and a decrease in compliance at H_2SO_4 concentrations $\geq$19,000 $\mu g/m^3$ without provocation challenge; only the animals showing this constrictive response during acid exposure also had major increases in histamine sensitivity. This suggested that airway constriction may have been a prerequisite for the development of hyperresponsiveness. On the other hand, Chen et al. (1992b) found bronchial hyperresponsiveness, but no change in baseline resistance, in guinea pigs exposed for 1 h to 200 $\mu g/m^3$ H_2SO_4 (0.06 μm, MMD). Perhaps the smaller size of this aerosol was responsible for producing effects at a lower concentration.

Kobayashi and Shinozaki (1993) exposed guinea pigs to fairly high H_2SO_4 levels, namely 1,000 and 3,200 $\mu g/m^3$ (0.54 μm), 24 h/day for 3, 7, 14 or 30 days, and examined airway response to inhaled histamine. Unlike the study of Silbaugh et al. (1981a) and similar to that of Chen et al. (1992b), acid exposure did not change the baseline resistance measured prior to bronchoprovocation challenge. Exposure to 3,200 $\mu g/m^3$ of acid resulted in airway hyporesponsiveness at 3 days, hyperresponsiveness at 14 days and a return to normal levels of responsiveness by 30 days of exposure. Thus, acid exposure resulted in a transient alteration in airway function. The authors speculated that the hyporesponsiveness, and eventual return to

normal, was due to changes in mucous secretion in the airways, which would affect the ability of the inhaled histamine challenge aerosol to contact airway receptors.

Airway responsiveness following chronic exposure to H_2SO_4 was examined by Gearhart and Schlesinger (1986), who exposed rabbits to 250 $\mu g/m^3$ H_2SO_4 (0.3 μm, MMD) for 1 h/day, 5 days/week, and assessed responsiveness after 4, 8 and 12 mo of exposure, using acetylcholine administered intravenously rather than inhaled. Hyperresponsiveness was evident at 4 mo, and a further increase was found by 8 mo; the response at 12 mo was similar to that at 8 mo, indicating a stabilization of effect. There was no change in baseline resistance. Thus, repeated exposures to H_2SO_4 produced hyperresponsive airways in previously normal animals.

The mechanism which underlies H_2SO_4-induced airway hyperresponsiveness is not clear. However, some recent studies have suggested possibilities. One may involve an increased sensitivity to mediators involved in airway smooth muscle control. For example, guinea pigs exposed to H_2SO_4 showed a small degree of enhanced response to histamine, but a much more pronounced sensitivity to substance P, a neuropeptide having effects on bronchial muscle tone (Stengel et al., 1993). El-Fawal and Schlesinger (1994) exposed rabbits for 3 h to 50 to 500 $\mu g/m^3$ H_2SO_4 (0.3 μm), following which bronchial airways were examined in vitro for responsiveness to acetylcholine and histamine. Exposures at ≥ 75 $\mu g/m^3$ produced increased responsiveness to both constrictor agents. Detailed examination of the response in tracheal segments suggested that the acid effect may result from interference with airway contractile/dilatory homeostatic processes, in that there was a potentiation of the response of airway constrictor receptors and a diminution of the response of dilatory receptors.

11.2.2.4 Pulmonary Morphology and Biochemistry

Morphologic alterations associated with exposure to acid aerosols are summarized in Table 11-6.

TABLE 11-6. EFFECTS OF ACIDIC SULFATE PARTICLES ON RESPIRATORY TRACT MORPHOLOGY

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique (RH)	Particle Characteristics: Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
H_2SO_4	Guinea pig	Whole body (70-90%)	32,600	1 (MMAD); 1.49	4 h	Focal atelectasis; epithelial desquamation in terminal bronchioles	Brownstein (1980)
H_2SO_4	Guinea pig, M/F Hartley, 2-3 mo	Whole body (80%)	1,200, 9,000, 27,000	0.8-1 (MMAD); 1.5-1.6	6 h	At 27,000 $\mu g/m$: interstitial edema only in "responders"; no change in "nonresponders" or at 1,000 and 10,000 $\mu g/m^3$. Concentration-dependent increase in height of tracheal mucus layer at all concentrations.	Wolff et al. (1986)
H_2SO_4	Rabbit, M mixed, 2.5-2.7 kg	Oral tube or nose- only (80%)	250-500	0.3 (MMAD); 1.6	1 h/day, 5 days/week, 4 weeks	Increased epithelial thickness in small airways; increase in secretory cells in mid to small airways	Schlesinger et al. (1983)
H_2SO_4	Rabbit, M mixed, 2.5-2.7 kg	Nose-only (80%)	250	0.3 (MMAD); 1.6	1 h/day, 5 days/week up to 52 weeks	Increase in secretory cell no. density throughout bronchial tree increase in number of small airways	Gearhart and Schlesinger (1988)
H_2SO_4	Rabbit, M NZ White, 3-3.5 kg	Nose-only (60%)	125	0.3 (MMD); 1.6	2 h/day, 5 days/week up to 12 mo	No bronchial inflammation; increase in secretory cell number density in small airways at 12 mo	Schlesinger et al. (1992b)
H_2SO_4	Rat	Whole body (40-60%)	2,000	0.3 (MMD); ≈2	8 h/day, 82 days	Some hypertrophy of epithelial cells, mainly at alveolar duct level; no effect on turnover rate of terminal bronchiolar epithelial or Type II cells	Juhos et al. (1978)
H_2SO_4	Rat	Whole body (50%)	700-1,200	0.03-0.04 (CMD); 1.8-2.1	Continuous, up to 180 days	No effect	Moore and Schwartz (1981)
H_2SO_4	Rat	Whole body (≤60%)	45,000 68,000 172,000	0.52 (CMD) 0.4 (MMAD) 0.45 (CMD)	11 days 6 days 7 days	No effect in nasal passages, trachea, bronchi, alveolar region	Schwartz et al. (1977)
H_2SO_4	Rhesus monkey	Whole body (≤60%)	150,000 361,000 502,000	0.3-0.5 (CMD) 0.43 (MMAD); 1.6 0.48 (MMAD); 1.5	3 days 7 days 7 days	No effect	Schwartz et al. (1977)
H_2SO_4	Guinea Pig	Whole body (≤60%)	30,000 38,000 71,000	0.31 (MMAD); 1.6 0.31 (MMAD); 1.6 0.52 (CMD)	7 days 7 days 4 days	At 71,000 $\mu g/m^3$: focal edema, necrosis of alveolar septa, inflammatory cell infiltration; necrosis of bronchiolar epithelium; focal epithelial necrosis in larger bronchi; ciliary denudation. At 38,000 $\mu g/m^3$: minimal effects; some change in density and length of cilia	Schwartz et al. (1977)

TABLE 11-6 (cont'd). EFFECTS OF ACIDIC SULFATE PARTICLES ON RESPIRATORY TRACT MORPHOLOGY

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique (RH)	Particle Characteristics: Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
H_2SO_4	Mouse	Whole body (≤60%)	140,000 170,000	0.32 (MMAD); 1.4 0.62 (MMAD); 1.7	14 days 10 days	Lesions in larynx and upper trachea; epithelial ulceration, edema, inflammatory infiltration	Schwartz et al. (1977)
H_2SO_4	Rat	Whole body	1,000-100,000	0.6-1.1 (MMAD); 1.7-1.8	6 h	At 100,000 μg/m : some cilia loss; ulceration of larynx. <100,000 $\mu g/m^3$: no effect	Henderson et al. (1980a)
H_2SO_4	Rat, M/F, F344/Crl 12-16 weeks	Whole body (80%)	1,100, 11,000, 96,000	0.8-1 (MMAD); 1.6-1.8	6 h	Laceration of larynx and cilia loss in bronchi at 96,000 $\mu g/m^3$; no deep lung lesions; some thickening of mucus lining in trachea at 11,000 and 96,000 $\mu g/m^3$	Wolff et al. (1986)
H_2SO_4	Rat, M Fischer, 250-300 g	Whole body (55%)	10,000 30,000 100,000	0.89 (MMD) 0.83 (MMD) 0.72 (MMD)	5 days 5 days 5 days	No effect	Cavender et al. (1977b)
H_2SO_4	Guinea pig	Whole body (55%)	10,000 30,000 100,000	0.89 (MMD) 0.83 (MMD) 0.72 (MMD)	5 days 5 days 5 days	No effect } Mortality }	Cavender et al. (1977b)
$(NH_4)_2SO_4$	Guinea pig, M, Hartley adult	Whole body	1,030	0.42 (MMD); 2.25	6 h/day, 5 days/week, 20 days	Interstitial thickening; hypertrophy and hyperplasia of Type II cells and secretory cells in bronchioli	Busch et al. (1984)
$(NH_4)_2SO_4$	Rat, M, SD/Crl, 70-75 g	Whole body	5000	0.8-1 (MMD); 1.8-2.0	7 days	No effect (proximal acinar region)	Last et al. (1983)
$(NH_4)_2SO_4$	Hamster, M, Syrian, 10 weeks	Whole body	187	0.3 (MMD); 2.02	6 h/day, 5 days/week, 15 weeks	Emphysematic lesions; no hyperplasia of bronchial glands or metaplasia of goblet cells	Godleski et al. (1984)
$(NH_4)_2SO_4$	Rat, M, adult	Whole body	300,000	1-2 (MMAD)	8 h/day, 1-14 days	No effect	Pepelko et al. (1980a)
$(NH_4)_2SO_4$	Rat, M, SD adult	Whole body	1,030	0.42 (MMAD); 2.25	6 h/day, 5 days/week, 20 days	Interstitial thickening	Busch et al. (1984)
$(NH_4)_2SO_4$	Rat	Nose-only	70	0.2 (MMAD)	4 h/day, 4 days/week, 8 weeks	Increased alveolar septal thickness; decreased average alveolar diameter	Kleinman et al. (1995)

Single or multiple exposures to H_2SO_4 at fairly high levels (>1,000 $\mu g/m^3$) produce a number of characteristic morphologic responses (e.g., alveolitis, bronchial and/or bronchiolar epithelial desquamation, and edema). As with other endpoints, the sensitivity to H_2SO_4 is dependent upon the animal species. Comparative sensitivities of the rat, mouse, rhesus monkey and guinea pig were examined by Schwartz et al. (1977), using concentrations of $H_2SO_4 \geq 30{,}000$ $\mu g/m^3$ at comparable particle sizes (0.3 to 0.6 μm) and assessing airways from the larynx to the deep lung. Both the rat and monkey were quite resistant, while the guinea pig and mouse were the more sensitive species. The nature of the lesions in the latter pair were similar, but differed in location; this was, perhaps, a reflection of differences in the deposition pattern of the acid droplets. Mice would tend to have greater deposition in the upper respiratory airways than would the guinea pig (Schlesinger, 1985), which could account for the laryngeal and upper tracheal location of the lesions seen in the mice. The relative sensitivity of the guinea pig and relative resistance of the rat to acid sulfates is supported by results from other morphological studies (Busch et al., 1984; Cavender et al., 1977b; Wolff et al., 1986).

Repeated or chronic exposures to H_2SO_4 at concentrations $\leq 1{,}000$ $\mu g/m^3$ produce a response characterized by hypertrophy and hyperplasia of epithelial secretory cells. In morphometric studies of rabbits exposed to 125 to 500 $\mu g/m^3$ H_2SO_4 (0.3 μm) for 1 to 2 h/day, 5 d/week (Schlesinger et al., 1983; Gearhart and Schlesinger, 1988; Schlesinger et al., 1992b), increases in the relative number density of secretory cells (as determined by histochemical staining) have been found to extend to the bronchiolar level, where these cells are normally rare or absent. Depending upon the study, the changes began within 4 weeks of exposure and persisted for up to 3 mo following the end of exposure. The mechanism underlying increases in secretory cell numbers at low H_2SO_4 exposure levels is also unknown; it may involve an increase in secretory activity of existing cells, or a transition from another cell type.

An increase in the relative number of smaller airways (<0.25 mm) in rabbits was found by 4 mo of exposure to 250 $\mu g/m^3$ (0.3 μm) for 1 h/day, 5 days/week (Gearhart and Schlesinger, 1988). Changes in airway size distribution due to irritant exposure, specifically cigarette smoke, has been reported in humans (Petty et al., 1983; Cosio et al., 1977), and this seems to be an early change relevant to clinical small airways disease.

The specific pathogenesis of acid-induced lesions is not known. As with pulmonary mechanics, both a direct effect of deposited acid droplets on the epithelium and/or indirect effects, perhaps mediated by humoral factors, may be involved. For example, similar lesions have been produced in guinea pig lungs by exposure to either histamine or H_2SO_4 (Cavender et al., 1977a). In addition, some lesions may be secondary to reflex bronchoconstriction, to which guinea pigs are very vulnerable, rather than primary effects separable from constriction. Thus, damage at the small bronchi and bronchiolar level may be due not only to direct acid droplet-induced injury, but to indirect, reflex-mediated injury as well (Brownstein, 1980).

Morphologic and cellular damage to the respiratory tract following exposure to acid aerosols may be determined by methods other than direct microscopic observation. Analysis of bronchoalveolar lavage fluid can also provide valuable information, and this procedure has seen increasing use since publication of the previous CD. Levels of cytoplasmic enzymes, such as lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6PD), are markers of cytotoxicity; increases in lavageable protein suggest increased permeability of the alveolar epithelial barrier; levels of membrane enzymes, such as alkaline phosphatase, are markers of disrupted membranes; the presence of fibrin degradation products (FDP) provides evidence of general damage; and sialic acid, a component of mucoglycoprotein, indicates mucus-secretory activity. (It should, however, be noted that lavage analysis may not be able to provide identification of the site of injury nor indicate effects in the interstitial tissue.)

Henderson et al. (1980b) exposed rats for 6 h to H_2SO_4 (0.6 μm, MMAD) at 1,500, 9,500, and 98,200 $\mu g/m^3$, and found FDP in blood serum after exposure at all concentrations. No FDP was found in lavage fluid, but since the washing procedure did not include the upper respiratory tract (i.e., anterior to and including the larynx), FDP in the serum was concluded to be an indicator of upper airway injury. A concentration-dependent increase in sialic acid content of the lavage fluid was also observed, indicating increased secretory activity within the tracheobronchial tree.

Chen et al. (1992a) exposed guinea pigs to fine (0.3 μm) and ultrafine (0.04 μm) aerosols of H_2SO_4 at 300 $\mu g/m^3$ for 3 h/day for 1 or 4 days. Animals were sacrificed 24 h after each of these exposures. Following the single exposure to either size, lavage fluid showed increases in LDH and total protein, and the change in LDH was evident at 24 h with

the fine, but not the ultrafine, particles. These responses did not occur following the 4 day exposure.

Wolff et al. (1986) exposed both rats and guinea pigs for 6 h to H_2SO_4 (0.8 to 1 μm, MMAD), at concentrations of 1,100 to 96,000 $\mu g/m^3$ for rats and 1,200 to 27,000 $\mu g/m^3$ for guinea pigs. No changes in lavageable LDH, protein, or sialic acid were found in the rat. However, some of the guinea pigs exhibited bronchoconstriction after exposure to 27,000 $\mu g/m^3$, and only these animals showed increased levels of lavageable protein, sialic acid and LDH. In other studies, no changes in lavageable protein were found in the lungs of rats exposed for 3 days to 1,000 $\mu g/m^3$ (0.4 to 0.5 μm, MMAD) H_2SO_4 (Warren and Last, 1987), nor for 2 days to 5,000 $\mu g/m^3$ (0.5 μm, MMAD) $(NH_4)_2SO_4$ (Warren et al., 1986).

An important group of biological mediators of the inflammatory response, as well as of smooth muscle tone, are the eicosanoids, (e.g., prostaglandins and leukotrienes). Modulation of these mediators could be involved in damage to the respiratory tract due to inhaled particles. Preziosi and Ciabattoni (1987) exposed isolated, perfused guinea pig lungs for 10 min to aerosols of H_2SO_4 (no concentration or particle sizes were given). An increase in thromboxane B_2 but no change in leukotriene B_4 in the perfusate was found. Schlesinger et al. (1990b) exposed rabbits to 250 to 1,000 $\mu g/m^3$ H_2SO_4 (0.3 μm) for 1 h/day for 5 days. Lungs were lavaged and the fluid assayed for eicosanoids. A concentration-dependent decrease in levels of prostaglandins E_2 and $F_{2\alpha}$ and thromboxane B_2 were noted, while there was no change in leukotriene B_4. The effects, which were determined to be due to the hydrogen ion rather than the sulfate ion, indicate that acid sulfates can upset the normally delicate balance of eicosanoid synthesis/metabolism which is necessary to maintain pulmonary homeostasis. Since some of the prostaglandins are involved in regulation of muscle tone, this imbalance may be involved in the development of airway hyperresponsiveness found with exposure to acid sulfates.

Other biochemical markers of pulmonary damage have been used to assess the toxicity of acid sulfate particles. The proline content of the lungs may provide an index of collagen metabolism. No change in soluble proline content was found in rat lungs after exposure for 7 days to 4,840 $\mu g/m^3$ (0.5 μm, MMAD) $(NH_4)_2SO_4$, nor due to a 7 day exposure to 1,000 $\mu g/m^3$ (0.5 μm) H_2SO_4 (Last et al., 1986). A series of studies assessed collagen synthesis in rat lung minces after in vivo exposure; this is a possible indicator of the potential

for pollutants to produce fibrosis. Exposure for 7 days to H_2SO_4 at 40, 100, 500, and 1,000 $\mu g/m^3$ (0.4 to 0.5 μm, MMAD) resulted in an increase in collagen synthesis rate only at 100 $\mu g/m^3$; higher levels had no effect (Warren and Last, 1987). No effect on collagen synthesis by rat lung minces was found due to 7-day exposures to $(NH_4)_2SO_4$ at 5,000 $\mu g/m^3$ (0.8 to 1 μm, MMAD) (Last et al., 1983).

Other parameters of pulmonary damage are changes in lung DNA, RNA, or total protein content. No significant changes in any of these parameters were found in rats after exposure to 1,000 $\mu g/m^3$ H_2SO_4 (<1 μm) for 3 days (Last and Cross, 1978), nor in protein content in rats exposed for up to 9 days to a similar concentration of H_2SO_4 (Warren and Last, 1987).

11.2.2.5 Pulmonary Defenses

Responses to air pollutants often depend upon their interaction with an array of non-specific and specific respiratory tract defenses. The former consists of nonselective mechanisms protecting against a wide variety of inhaled materials; the latter requires antigenic stimulation of the immune system for activation. Although these systems may function independently, they are linked, and response to an immunologic insult may enhance the subsequent response to nonspecific materials. The overall efficiency of lung defenses determines the local residence times for inhaled deposited material, which has a major influence upon the degree of pulmonary response; furthermore, either depression or over-activity of these systems may be involved in the pathogenesis of lung diseases.

Studies of altered lung defenses resulting from inhaled acid aerosols have concentrated on conducting and respiratory region clearance function and nonspecific activity of macrophages; there are only a few studies of effects upon immunologic competence.

Clearance Function: Clearance, a major nonspecific defense mechanism, is the physical removal of material that deposits on airway surfaces. As discussed in Chapter 10, the mechanisms involved are regionally distinct. In the tracheobronchial region, clearance occurs via the mucociliary system, whereby a mucus "blanket" overlying the ciliated epithelium is moved by the coordinated beating of the cilia towards the oropharynx. In the alveolar region of the lungs, clearance occurs via a number of mechanisms and pathways, but the major one for both microbes and nonviable particles is the alveolar macrophage (AM).

These cells exist freely within the fluid lining of the alveolar epithelium, where they move by ameboid motion. The phagocytic ingestion of deposited particles helps prevent particle penetration through the alveolar epithelium and subsequent translocation to other sites. These cells contain proteolytic enzymes, which digest a wide variety of organic materials, and they also kill bacteria through oxidative mechanisms. In addition, AMs are involved in the induction and expression of immune reactions. Thus, the AM provides a link between the lung's non-specific and specific defense systems. These cells also are in the effector chain for lung damage (e.g., by release of proinflammatory cytokines).

Mucociliary Transport: The assessment of acid effects upon mucociliary clearance often involved examination only of mucus transport rates in the trachea, since this is a readily accessible airway and tracheal mucociliary clearance measurements are more straightforward to perform than are those aimed at assessing clearance from the entire tracheobronchial tree. Table 11-7 outlines studies of acid sulfate effects upon tracheal mucociliary clearance.

Although many of the studies involved fairly high concentrations of acid aerosols, most demonstrated a lack of effect. The most likely explanation for this is that the sizes of the aerosols were such that significant tracheal deposition did not occur. This is supported by the results of Wolff et al. (1981), who found tracheal transport rates in dogs to be depressed only when using 0.9 μm H_2SO_4; no effect was seen with a 0.3 μm aerosol at an equivalent mass concentration. In addition, the use of tracheal clearance rate as a sole toxicologic endpoint may be misleading, inasmuch as a number of studies have demonstrated alterations in bronchial clearance patterns in the absence of changes in tracheal mucous transport.

Studies assessing the effects of acid aerosols upon bronchial mucociliary clearance are also outlined in Table 11-7. Responses following acute exposure to H_2SO_4 indicate that the nature of clearance change (i.e., a slowing or speeding) is concentration (C) and exposure-duration (t) dependent; stimulation of clearance generally occurs after low Ct exposures, and retardation generally occurs at higher Ct levels. However, the actual Ct needed to alter clearance rate may depend upon the anatomic location within the bronchial tree from which clearance is being measured, in relation to the region which is most affected by the deposited acid. Studies in humans indicated that low H_2SO_4 concentrations (i.e., $\approx$100 to 500 $\mu g/m^3$) may accelerate clearance, compared to unexposed subjects, from the large proximal airways

TABLE 11-7. EFFECTS OF ACIDIC SULFATE PARTICLES ON RESPIRATORY TRACT CLEARANCE

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique (RH)	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
	Tracheal						
H_2SO_4	Dog, M/F Beagle, 3 years	Nose-only (80%)	1,000 5,000 1,000 500	0.3 (MMAD); 1.2 0.3 (MMAD); 1.2 0.9 (MMAD); 1.3 0.9 (MMAD); 1.3	1 h 1 h 1 h 1 h	NC NC ↓ ↓	Wolff et al. (1981)
H_2SO_4	Donkey, M/F adult	Nasopharyngeal catheter (45%)	200-1,400	0.4 (MMAD); 1.5	1 h	NC	Schlesinger et al. (1978)
H_2SO_4	Rat	Whole body (82%)	1,000-100,000	0.6-0.8 (MMAD); 1.5-2.6	6 h	↑	Wolff et al. (1980)
H_2SO_4	Rat	Nose-only (80%)	10,000-100,000	0.4-0.6 (MMAD); 1.3-1.4	0.5 h	↑	
H_2SO_4	Rat, M/F F344/Crl 12-16 weeks	Whole body (80%)	1,100, 11,000, 96,000	0.9-1 (MMAD); 1.6-1.8	6 h	↑ at 96,000 $\mu g/m$	Wolff et al. (1986)
H_2SO_4	Guinea pig, M/F Hartley 2-3 mo	Whole body (80%)	1,400, 9,000, 27,000	0.8-0.9 (MMAD); 1.5-1.6	6 h	↓ at 1,400 $\mu g/m$	
NH_4HSO_4	Sheep	Head-only (20-30%)	1,000	0.1 (CMD); 2.1	4 h	NC	Sackner et al. (1981)
$(NH_4)_2SO_4$	Donkey	Nasopharyngeal catheter (45%)	300-3,000	0.4 (MMAD); 1.5	1 h	NC	Schlesinger et al. (1978)
$(NH_4)_2SO_4$	Sheep	Head-only (20-30%)	1,100	0.1 (CMD); 2.1	4 h	NC	Sackner et al. (1981)
	Bronchial						
H_2SO_4	Rabbit, M NZW/mixed, 2.5-3 kg	Oral tube (75%)	100-2,200	0.3 (MMAD); 1.6	1 h	↑,↓ (depending on concentration and duration)	Chen and Schlesinger (1983); Schlesinger et al. (1984)
H_2SO_4	Rabbit, M mixed 2.5-2.7 kg	Oral tube or nose-only (80%)	250-500	0.3 (MMAD); 1.6	1 h/days, 5 days/week, 4 weeks	↑; persistent	Schlesinger et al. (1983)
H_2SO_4	Rabbit, M NZW 2.5-3 kg	Nose-only (80%)	250	0.3 (MMAD); 1.6	1 h/day, 5 days/week, 12 mo	↓ by 1 week; progressive slowing after 19 weeks; persistent	Gearhart and Schlesinger (1988)
H_2SO_4	Rabbit, M NZW 2.5-3 kg	Nose-only (60%)	125	0.3 (MMD); 1.6	2 h/day, 5 days/week up to 12 mo	↑ followed by ↓ PE; persistent	Schlesinger et al. (1992b)

TABLE 11-7 (cont'd). EFFECTS OF ACIDIC SULFATE PARTICLES ON RESPIRATORY TRACT CLEARANCE

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique (RH)	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ	Exposure Duration	Observed Effect	Reference
Bronchial							
H_2SO_4	Rabbit, M mixed 6 mo	oral tube nose-only	250; 250; 500	0.3 (MMAD); 1.6	1 h/day, 5 days/week, 4 weeks	↑ only some days at 250/oral and 500/nasal; persistent ↑ up to 14 days PE for all.	Schlesinger et al. (1983)
H_2SO_4	Donkey	Nasopharyngeal catheter (45%)	200-1,400	0.4 (MMAD); 1.5	1 h	↓ in some animals at all concentrations; progressive slowing in some animals with continued exposures.	Schlesinger et al. (1978)
H_2SO_4	Rat, M SD 200 g	Nose-only (39%; 85%)	3,600	1.0 (MMAD); 1.9-2.3	4 h	NC	Phalen et al. (1980)
NH_4HSO_4	Rabbit, M mixed 2.5-2.7 kg	Oral tube (78%)	600-1,700	0.4 (MMAD); 1.6	1 h	↓ at 1,700 $\mu g/m$	Schlesinger (1984)
$(NH_4)_2SO_4$	Rabbit, M mixed 2.5-2.7 kg	Oral tube (78%)	2,000	0.4 (MMAD); 1.6	1 h	NC	Schlesinger (1984)
$(NH_4)_2SO_4$	Rat, M SD 200 g	Nose-only (39%; 85%)	3,600	0.4 (MMAD); 1.9-2.3	4 h	NC	Phalen et al. (1980)
Alveolar							
H_2SO_4	Rat, M SD 200 g	Whole body (30-80%)	3,600	1.0	4 h	NC	Phalen et al. (1980)
H_2SO_4	Rabbit, M NZW 2.5-3 kg	Oral tube	1,000	0.3 (MMAD); 1.5	1 h	↑	Naumann and Schlesinger (1986)
H_2SO_4	Rabbit, M NZW 2.5-3 kg	Nose-only (80%)	250	0.3 (MMAD); 1.6	1 h/day, 5 days/week, 1, 57, 240 day	↑	Schlesinger and Gearhart (1986)

TABLE 11-7 (cont'd). EFFECTS OF ACIDIC SULFATE PARTICLES ON RESPIRATORY TRACT CLEARANCE

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique (RH)	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ	Exposure Duration	Observed Effect	Reference
Alveolar (cont'd)							
H_2SO_4	Rabbit, M NZW 3-3.5 kg	Nose-only (80%)	500	0.3 (MMAD); 1.6	2 h/day, 14 days	↓	Schlesinger and Gearhart (1987)

Key to abbreviations:
NC: No significant change
↑: Significant increase
↓: Significant decrease
PE: Post exposure

where little acid deposits, while slowing clearance from the distal ciliated airways where there is greater acid deposition. At higher concentrations, mucociliary clearance from both the proximal and distal bronchial tree becomes depressed (Leikauf et al., 1984).

Comparison of responses to H_2SO_4 show interspecies differences in the sensitivity of mucociliary clearance to acid aerosols. As an example, the acceleration of tracheal transport found by Wolff et al. (1986) in the rat with $\approx$100,000 $\mu g/m^3$ H_2SO_4 seems anomalous since, in other species, levels $\geq$1,000 $\mu g/m^3$ depress mucociliary function. The reasons for this apparent discrepancy are not known. The rat is less susceptible to the lethal effects of H_2SO_4, and it does not have strong bronchoconstrictive reflex responses following H_2SO_4 exposures. These characteristics suggest that the mucociliary system of the rat may also differ in sensitivity from the other species studied, a view supported by the lack of effect of H_2SO_4 on bronchial clearance found by Phalen et al. (1980) following exposure at 3,600 $\mu g/m^3$ for 4 h and by the similarity in bronchial clearance response in donkeys and rabbits to single 1-h exposures of H_2SO_4 (Table 11-7). Although the lack of response of tracheal transport in the guinea pig at H_2SO_4 levels >1,000 $\mu g/m^3$ is also surprising, its response at 1,000 $\mu g/m^3$ is also different from that of the rat and more in line with other species (Wolff, 1986).

The relative potency of acid sulfate aerosols, in terms of altering mucociliary clearance, is related to their acidity (H^+ content). Schlesinger (1984) exposed rabbits for 1 h to submicrometer aerosols of NH_4HSO_4, $(NH_4)_2SO_4$, and Na_2SO_4. Exposure to NH_4HSO_4 at concentrations of $\approx$600 to 1,700 $\mu g/m^3$ significantly depressed clearance rate only at the highest exposure level. No significant effects were observed with the other sulfur oxides at levels up to $\approx$2,000 $\mu g/m^3$. When these results are compared to those from a study using H_2SO_4 (Schlesinger et al., 1984), the ranking of potency was $H_2SO_4 > NH_4HSO_4 > (NH_4)_2SO_4$, Na_2SO_4; this strongly suggests a relation between the hydrogen ion concentration and the extent of alteration in bronchial mucociliary clearance.

The mechanism by which deposited acid aerosol alters clearance is not certain. The effective functioning of mucociliary transport depends upon optimal beating of cilia and the presence of mucus having appropriate physicochemical properties, and both ciliary beating as well as mucus viscosity may be affected by acid deposition. At alkaline pH, mucus is more fluid than at acid pH, so a small increase in viscosity due to deposited acid could "stiffen"

the mucus blanket, perhaps promoting the clearance mechanism and, thus, increasing its efficiency (Holma et al., 1977). Such a scenario may occur at low H_2SO_4 exposure concentrations, where ciliary activity would not be directly affected by the acid, and is consistent with clearance acceleration observed at these concentrations with acute exposure. However, the exact relation between mucus viscosity and transport rate is not certain.

High concentrations of H_2SO_4 may affect ciliary beating, as discussed in the previous CD (U.S. Environmental Protection Agency, 1982; Schiff et al., 1979; Grose et al., 1980). An additional mechanism by which deposited acid may affect mucociliary clearance is via altering the rate and/or amount of mucus secreted. A small increase in mucus production could facilitate clearance, while more excessive production could result in a thickened mucus layer which would be ineffectively coupled to ciliary beat. Finally, the airways actively transport ions, and the interaction between transepithelial ion transport and consequent fluid movement is important in maintaining the mucus lining. A change in ion transport due to deposited acid particles may alter the depth and/or composition of the sol layer (Nathanson and Nadel, 1984), perhaps affecting clearance rate. In any case, the pathological significance of transient alterations in bronchial clearance rates in healthy individuals is not certain, but such changes are an indication of a lung defense response. On the other hand, persistent impairment of clearance may lead to the inception or progression of acute or chronic respiratory disease and, as such, may be a plausible link between inhaled acid aerosols and respiratory disease.

Short-term exposures to acid aerosols may lead to persistent clearance changes, as indicated previously (Schlesinger et al., 1978). The effects of long-term exposures were investigated by Schlesinger et al. (1983), who exposed rabbits to 250 or 500 $\mu g/m^3$ H_2SO_4 (0.3 μm, MMAD) for 1 h/day, 5 days/week for 4 weeks, during which time bronchial mucociliary clearance was monitored. Clearance was accelerated on individual days during the course of the acid exposures, especially at 500 $\mu g/m^3$. In addition, clearance was significantly faster, compared to preexposure levels, during a 2 week follow- up period after acid exposures had ceased.

Another long-term exposure at relatively low H_2SO_4 levels was conducted by Gearhart and Schlesinger (1988). Rabbits were exposed to 250 $\mu g/m^3$ H_2SO_4 for 1 h/day, 5 days/week for up to 52 weeks, and some animals were also provided a 3 mo follow-up

period in clean air. Clearance was slower during the first month of exposure and this slowing was maintained throughout the rest of the exposure period. After cessation of exposure, clearance became extremely slow and did not return to normal by the end of the follow-up period. Differences in the nature of clearance change between this study and that of Schlesinger et al. (1983) may be due to differences in exposure protocol daily (duration) and/or concentration. In both studies, however, and as discussed earlier, histologic analyses indicated the development of increased numbers of epithelial secretory cells, especially in small airways, the likely consequence of which would be an increase in mucus production. In addition, the slowing of clearance seen by Gearhart and Schlesinger (1988) was also associated with a shift in the histochemistry of mucus towards a greater content of acidic glycoproteins; this would tend to make mucus more viscous.

The longest duration study at the lowest concentration of H_2SO_4 yet reported is that of Schlesinger et al. (1992b), in which rabbits were exposed to 125 $\mu g/m^3$ H_2SO_4 for 2 h/day, 5 days/week for up to 52 weeks. The variability of measured bronchial clearance time was increased with acid exposure, and acceleration of clearance was noted at various times during the one-year exposure period. However, following a 6-mo observation period after exposures had ceased, a trend towards slowing of clearance was noted (compared to both control and rates during acid exposure). In addition, and consistent with previous studies, an increase in the number density of epithelial secretory cells was observed in small airways (<0.5 mm) following 12 mo of acid exposure. This histological change had resolved by the end of the 6-mo post-exposure period.

Alveolar Region Clearance and Alveolar Macrophage Function: Only a few studies have examined the ability of acid aerosols to alter clearance of particles from the alveolar region of the lungs (Table 11-7). Rats exposed to 3,600 $\mu g/m^3$ H_2SO_4 (1μm) for 4 h showed no change in clearance (Phalen et al., 1980). On the other hand, acceleration of clearance was seen in rabbits exposed for 1 h to 1,000 $\mu g/m^3$ H_2SO_4 (0.3 μm, MMAD) (Naumann and Schlesinger, 1986).

Two studies involving repeated exposures to acid aerosols have been reported. In one, rabbits were exposed to 250 $\mu g/m^3$ (0.3 μm, MMAD) H_2SO_4 for 1 h/day, 5 days/week, and inert tracer particles were administered on days 1, 57 and 240 following the start of the acid exposures (Schlesinger and Gearhart, 1986). Clearance (measured for 14 days after each

tracer exposure) was accelerated during the first test, and this acceleration was maintained throughout the acid exposure period. In the other study (Schlesinger and Gearhart, 1987), rabbits were exposed 2 h/day for 14 days to 500 $\mu g/m^3$ H_2SO_4 (0.3 μm, MMAD); retardation of early alveolar region clearance of tracer particles administered on the first day of exposure was noted. The results of these two studies suggest a graded response, whereby a low exposure concentration accelerates early alveolar region clearance and a high level retards it, such as was seen with mucociliary transport following acute H_2SO_4 exposure.

The mechanisms responsible for the altered alveolar region clearance patterns seen in the above studies are not known. Observed clearance is the net consequence of a number of differential underlying responses, which can include change in mucociliary transport rates and altered functioning of AMs.

A number of studies have examined the functional response of AMs following acidic sulfate aerosol exposures. To adequately perform their role in clearance, AMs must be competent in a number of functions, including phagocytosis, mobility and attachment to a surface. Alterations in any one, or combination, of these individual functions may affect clearance function. Naumann and Schlesinger (1986) noted a reduction in surface adherence and an enhancement of phagocytosis in AMs obtained by lavage from rabbits following a 1-h exposure to 1,000 $\mu g/m^3$ H_2SO_4 (0.3 μm). The acid exposure produced no change in the viability or numbers of recoverable AMs.

In a study with repeated H_2SO_4 exposures, AMs were lavaged from rabbits exposed to 500 $\mu g/m^3$ H_2SO_4 (0.3 μm) for 2 h/day for up to 13 consecutive days (Schlesinger, 1987). Macrophage counts increased after 2 of the daily exposures, but returned to control levels thereafter. Neutrophil counts remained at control levels throughout the study, suggesting no acute inflammatory response. Random mobility of AMs decreased after 6 and 13 of the daily exposures. The number of phagocytically active AMs and the level of such activity increased after 2 exposures, but phagocytosis became depressed by the end of the exposure series. Although such studies demonstrate that H_2SO_4 can alter AM function, they have not as yet been able to provide a complete understanding of the cellular mechanisms which may underly the changes in pulmonary region clearance observed with exposure to acid aerosols.

The relative potency of acidic sulfate aerosols in terms of altering AM numbers or function has been examined. Aranyi et al. (1983) found no change in total or differential

counts of free cells lavaged from mice exposed to 1,000 $\mu g/m^3$ $(NH_4)_2SO_4$ for 3 h/day for 20 days; this suggests a lack of inflammatory response to this sulfate aerosol. Additional studies seem to suggest that the response to acid sulfates of AM is a function of the H^+. Schlesinger et al. (1990a) examined phagocytic activity of AMs recovered from rabbits exposed for 1 h/day for 5 days to either 250 to 2,000 $\mu g/m^3$ H_2SO_4 (0.3μm) or 500 to 4,000 $\mu g/m^3$ NH_4HSO_4 (0.3 μm); the levels were chosen such that the H^+ concentration in the exposure atmospheres were equivalent for both sulfate species. Phagocytic activity of AMs was reduced following exposure to $\geq$1,000 $\mu g/m^3$ H_2SO_4 or to 4,000 $\mu g/m^3$ NH_4HSO_4; exposure to 2,000 $\mu g/m^3$ NH_4HSO_4 resulted in increased phagocytic activity. While these exposure concentrations were quite high, the interesting observation was that for a given level of sulfate, the response to H_2SO_4 was greater than that to NH_4HSO_4. However, even when the data were assessed in terms of H^+ concentration in the exposure atmosphere, it was noted that exposure to the same concentrations of H^+ did not result in identical responses for the two different acid sulfate species; H^+ appeared to be more effective as the H_2SO_4 species. On the other hand, when AMs were incubated in acidic environments in vitro, the phagocytic activity response was identical, regardless of the sulfate species used, as long as the pH was the same. These results suggested an enhanced potency of H_2SO_4 during inhalation exposures. Experimental evidence provided by Schlesinger and Chen (1994) indicated that this difference noted in vivo was likely a reflection of different degrees of neutralization by respiratory tract ammonia of the two species of inhaled acid aerosols. It was shown that, for a given concentration of ammonia and within a given residence time within the respiratory tract, more total H^+ remained available from inhaled sulfuric acid than from inhaled ammonium bisulfate when the exposure atmospheres had the same total H^+ concentration. Thus, the greater observed potency of inhaled sulfuric acid compared to ammonium bisulfate for exposure atmospheres containing the same total H^+ concentration is likely due to a greater degree of neutralization of the latter, and a resultant greater loss of H^+ prior to particle deposition onto airway surfaces. Thus, the respiratory "fate" of inhaled acid sulfate particles should be considered in assessing the relationship between exposure atmosphere and biological response, since a lower H^+ concentration will likely deposit onto lung tissue than is inhaled at the mouth or nose.

Interspecies differences in the effects of acid sulfates on AM function were examined by Schlesinger et al. (1992a). Based upon in vitro exposures of AM to acidic media, a ranking of response in order of decreasing sensitivity to acidic challenge and subsequent effect on phagocytic activity was found to be: guinea pig>rat>rabbit>human.

As noted with other endpoints, the effect of H_2SO_4 upon AM function may be dependent upon particle size. Chen et al. (1992a) observed that 300 $\mu g/m^3$ H_2SO_4 enhanced the phagocytic activity of AMs recovered from guinea pigs after 4 days (3 h/day) of exposure to fine particles (0.3 μm), while an identical exposure to ultrafine particles (0.04 μm) depressed phagocytic function.

The effects of acid sulfates upon the intracellular pH of AMs has been examined, because this may be one of the determinants of the rate of many cellular functions (Nucitelli and Deamer, 1982). Internal pH of AMs recovered from guinea pigs exposed to 300 $\mu g/m^3$ H_2SO_4 was depressed after a single 3-h exposure to both 0.3 and 0.04 μm particles, but the depression persisted for 24 h following exposure to the smaller size (Chen et al., 1992a). A depression in pH was also noted 24 h following 4 days of exposure to the ultrafine, but not the fine, aerosol. Thus, acid exposure produced a change in intracellular pH of the AMs and the effect was particle size dependent.

It is possible that this and other differences in response between fine and ultrafine particles reflect, to some extent, differences in the number of particles in aerosols of these two size modes, in that at a given mass concentration of acid sulfate, there are a greater number of ultrafine than fine particles. To examine this possibility, Chen et al. (1995) noted that changes in intracellular pH of macrophages obtained following inhalation exposure to H_2SO_4 aerosols were dependent both upon the number of particles as well as upon the total mass concentration of H^+ in the exposure atmosphere, with a threshold existing for both exposure parameters. The role of size in modulating toxicity due to PM is discussed further in Section 11.4. It should, however, be noted that aside from number, differences in deposition and neutralization may also affect differential responses to fine and ultrafine particles.

A possible mechanism underlying the acid-induced alterations in intracellular pH was examined by Qu et al. (1993), who exposed guinea pigs to 969 $\mu g/m^3$ H_2SO_4 (0.3 μm MMD, σg 1.73) for 3 h or to 974 $\mu g/m^3$ for 3 h/day for 5 days. Macrophages were

obtained following the end of each exposure protocol and examined for the ability of internal pH to recover from an added intracellular acid load. Both H_2SO_4 exposures resulted in a depression of internal pH recovery compared to air control. Subsequent analysis indicated that this alteration in internal pH regulation was attributable to effects on the Na^+/H^+ exchanger located in the cell membrane.

Macrophages are the source of numerous biologically active chemicals, and the effects of acid sulfate upon some of these have been investigated. Zelikoff and Schlesinger (1992) exposed rabbits to 50 - 500 $\mu g/m^3$ H_2SO_4 (0.3 μm) for 2 h. AM recovered by lavage following exposure were assessed for effects on tumor necrosis factor (TNF) release/activity and production of superoxide radical, both of which are biological mediators involved in host defense. Exposure to H_2SO_4 at $\geq$ 75 $\mu g/m^3$ produced a reduction in TNF cytotoxic activity, as well as a reduction in stimulated production of superoxide radical. Subsequently, Zelikoff et al. (1994) exposed rabbits for 2 h/day for 4 days to sulfuric acid at 500, 750 or 1,000 $\mu g/m^3$. AM recovered from animals exposed at the highest acid level showed a reduction in TNF and interleukin (IL)-1α production/activity, both immediately and 24 h following the last exposure. On the other hand, increased release of TNF from macrophages obtained from guinea pigs was observed immediately following a single 3 h exposure, and 24 h following a 3 h/day 4 day exposure, to 300 $\mu g/m^3$ H_2SO_4 (0.3 μm or 0.04 μm) (Chen et al., 1992a); in addition, production of hydrogen peroxide by these cells was enhanced immediately after the 4 day exposure. These differences in TNF may reflect interspecies differences in response to acid exposure and/or differences in experimental conditions.

Resistance to Infectious Disease

The development of an infectious disease requires both the presence of the appropriate pathogen, as well as host vulnerability. There are numerous anti-microbial host defenses with different specific functions for different microbes (e.g., there are some differences in defenses against viruses and bacteria). The AM represents the main defense against gram positive bacteria depositing in the alveolar region of the lungs. The ability of acid aerosols to modify resistance to bacterial infection could result from a decreased ability to clear microbes, and a resultant increase in their residence time, due to alterations in AM function. To test this possibility, a rodent infectivity model has been frequently used. In this

technique, mice are challenged with a bacterial aerosol after exposure to the pollutant of interest; mortality rate and/or survival time are then examined within a particular postexposure time period. Any decrease in the latter or increase in the former indicates impaired defense against respiratory infection. A number of studies which have used the infectivity model (primarily with *Streptococcus sp.*) to assess effects of acid aerosols were discussed in the previous CD (U.S. Environmental Protection Agency, 1982). It was evident that acute exposures to H_2SO_4 aerosols at concentrations up to 5,000 $\mu g/m^3$ were not very effective in enhancing susceptibility to this bacterially-mediated respiratory disease in the murine model. More recent studies with mice, shown in Table 11-8, continue to support this conclusion.

However, a study using another animal suggests that H_2SO_4 may indeed alter antimicrobial defense. Zelikoff et al. (1994) exposed rabbits for 2 h/day for 4 days to 500, 750, or 1,000 $\mu g/m^3$ H_2SO_4. Intracellular killing of a bacterium, *Staphylococcus aureus,* by AMs recovered by lavage 24 h following the last exposure at the two highest acid concentrations was reduced; bacterial uptake was also reduced at the same time point, but only at the highest acid level. Thus, repeated H_2SO_4 exposures may reduce host resistance to bacteria in the rabbit, in contrast to no effect on this endpoint in the mouse.

Specific Immune Response

Most of the database involving effects of acid aerosols on lung defense is concerned with non-specific mechanisms. Little is known about the effects of these pollutants on humoral (antibody) or cell-mediated immunity. Since numerous potential antigens are present in inhaled air, the possibility exists that acid sulfates may enhance immunologic reaction and, thus, produce a more severe response, and one with greater pulmonary pathogenic potential. Pinto et al. (1979) found that mice which inhaled H_2SO_4 for 0.5 h daily and were then exposed weekly to a particulate antigen (sheep red blood cells) exhibited higher serum and bronchial lavage antibody titers than did animals exposed to the antigen alone; unfortunately, neither the exposure mass concentration nor particle size of the H_2SO_4 was described. The combination of acid with antigen also produced morphologic damage, characterized by mononuclear cell infiltration around the bronchi and blood vessels, while

TABLE 11-8. EFFECTS OF ACID SULFATES ON BACTERIAL INFECTIVITY IN VIVO

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique (RH)	Mass Concentration ($\mu g/m^3$)	Particle Characteristics	Exposure Duration	Observed Effect	References
				Size (μm); σ_g			
H_2SO_4	Mouse, F CD-1 30 days	Head-only (31%)	543	0.08 (VMD); 2.3	2 h	NC	Grose et al. (1982)
H_2SO_4	Mouse, F CD-1 30 days	Head-only (31%)	365	0.06 (VMD); 2.3	2 h/day, 5 days	NC	Grose et al. (1982)
$(NH_4)_2SO_4$	Mouse, F CD-1 30 days	Whole body	1,000	Submicrometer	3 h/day, 20 days	NC	Aranyi et al. (1983)

NC: No change

exposure to acid or antigen alone did not. Thus, the apparent adjuvant effect of H_2SO_4 may be a factor promoting lung inflammation.

Osebold et al. (1980) exposed mice to 1,000 $\mu g/m^3$ H_2SO_4 (0.04 μm, CMD) to determine whether this enhanced the sensitization to an inhaled antigen (ovalbumin). The exposure regimen involved intermittent 4 day exposures, up to 16 total days of exposure; no increase in sensitization compared to controls was found. Kitabatake et al. (1979) exposed guinea pigs to 1,910 $\mu g/m^3$ H_2SO_4 (<1 μm, MMAD) for 0.5 h twice per week for 4 weeks, followed by up to 10 additional paired treatments with the H_2SO_4 for 0.5 h each; the animals were then exposed to aerosolized albumin for another 0.5 h. The breathing pattern of the animals was monitored for evidence of dypsnea. Enhanced sensitization was found after $\approx$4 of the albumin exposures. A subsequent challenge with acetylcholine suggested hyperresponsive airways.

Fujimaki et al. (1992) exposed guinea pigs to 300, 1,000, and 3,200 $\mu g/m^3$ H_2SO_4 for 2 or 4 weeks, following which lung mast cell suspensions were examined for antigen-induced histamine release. Exposure for 2 weeks at the two highest concentrations resulted in enhanced histamine release, but this response dissipated by 4 weeks of exposure. Thus, H_2SO_4, at high concentrations, may affect the functional properties of mast cells; these cells are involved in allergic responses, including bronchoconstriction.

11.2.3 Mixtures Containing Acidic Sulfate Particles

Most of the toxicological data concerning effects of PM are derived from exposures using single compounds. Although such information is essential, it is also important to study responses which result from inhalation of typical combinations of materials, because population exposures are generally to mixtures. Toxicological interaction provides a basis whereby ambient pollutants may show synergism (effect greater than the sum of the parts) or antagonism (effect less than the sum of the parts). Thus, the lack of any toxic effect following exposure to an individual pollutant should always be interpreted with caution, because mixtures may act differently than expected from the same pollutants acting separately. Most toxicologic studies of pollutant mixtures involved exposures to mixtures containing only two materials. These are summarized first below for mixtures containing

acidic aerosols (see Table 11-9); complex acid aerosol mixture studies (i.e., those using more than 2 compounds) are then discussed.

The extent of any toxicological interaction involving acidic sulfate aerosols has been shown to depend on the endpoint being examined, as well as on the co-inhalant. Most studies of interactions using acidic sulfates employed ozone (O_3) as the co-pollutant. Depending upon the exposure regimen, endpoint, and animal species, either additivity, synergism, or antagonism has been observed. These studies are summarized in the O_3 criteria document (U.S. Environmental Protection Agency, 1995). Interaction studies of H_2SO_4 and nitrogen dioxide (NO_2) are discussed in the nitrogen oxides criteria document pollutant (U.S. Environmental Protection Agency, 1993). The nature of interactions was dependent on the protocol; no unifying principles emerged. It is important to recognize that the nature of particle-pollutant interactions are specific for a given endpoint and set of exposure conditions and no attempt should be made to generalize from those specific observations discussed in the O_3 and NO_x criteria documents.

Kitabatake et al. (1979) exposed guinea pigs to H_2SO_4 aerosol (average 1910 $\mu g/m^3$) or SO_2 + H_2SO_4 aerosol (average 145 ppm and 1890 $\mu g/m^3$) for 30 min, twice a week for 4 weeks prior to albumin exposure. After the preexposures, the guinea pigs were treated 10 times with paired exposures to the sulfur oxides for 30 min followed by treatment with the antigen (albumin) aerosol for another 30 min. The results indicate that exposures to high concentrations of sulfur oxides (SO_2 + H_2SO_4 aerosol or H_2SO_4 aerosol alone) may increase hyperreactivity to albumin in guinea pigs.

In a study designed to determine if effects of exposure to H_2SO_4 aerosol were exacerbated in the presence of other particulate matter, Henderson et al. (1980a) exposed rats to H_2SO_4 aerosol (MMAD = .8 μm, σ_g = 1.7) in the presence or absence of 70,000 $\mu g/m^3$ fly ash (MMAD = 6.0 μm, σ_g = 2.0). Lung damage in the rats was determined by BAL one day after exposure to the fly ash and 1,000, 10,000, or 100,000 $\mu g/m^3$ H_2SO_4 for 6 h. BAL from animals exposed to high levels of sulfuric acid alone, to the ash alone, or to both showed an increase in sialic acid and in acid phosphatase activity. Lactate dehydrogenase and glutathione reductase activities were elevated in the combined exposures. The presence of a separate particulate aerosol did not greatly modify the response of the rat lung to H_2SO_4.

TABLE 11-9. TOXICOLOGIC EFFECTS OF MIXTURES CONTAINING ACIDIC AEROSOLS

Co-Pollutant			Acid Particle								
Chemical	μg/m	ppm[3]	Chemical	μg/m (μm)	Exposure Regime[3]	Exposure Conditions	Species, Gender Strain, Age and Body Weight	Endpoints	Response to Mixture	Interaction	Reference
ZnO	(0.05 μm, MMAD, σ_g = 1.86)		H_2SO_4	25 or 84	3 h	Nose-only	GP, M Hartley 250-300 g	BAL eicosanoids PE		Concentration dependent ↑ in PGF2α compared to ZnO alone	Chen et al. (1989)
ZnO	(0.05 CMD, σ_g = 2)		H_2SO_4 (coated on particles)	24 or 84		Nose-only	Guinea pig, M, Hartley 260-325 g	Pulmonary function	Animals exposed to acid had greater decrease in lung volume and DL_{co}	Acid layered on particle enhanced response to subsequent O_3 or acid exposure	Chen et al. (1991)
O_3	0.15		H_2SO_4 pure	300 (0.08)							
ZnO	up to 2,760 μg/m[3] (0.05 μm MMAD, σ_g = 2.0)		H_2SO_4 (coated on particle)	20-30 μg/m[3] (0.05 μm MMAD, σ = 2.0)	1 h	Head-only	Guinea pig	Airway responsiveness to acetylcholine		Acid-coated particles caused hyperresponsiveness	Chen et al. (1992b)
			H_2SO_4	202 μg/m[3] (0.06 μm MMAD, σ = 1.36)						Similar changes at 10 × concentration of coated particles	
SO_2		145	H_2SO_4	1,890 (<1 μm, MMAD)	0.5 h, twice weekly for 4 weeks; then 0.5 h twice weekly with antigen or constrictor challenge	Head-only	Guinea pig	Sensitization to inhaled antigen (albumin); responsitivity to acetylcholine		Enhanced response compared to H_2SO_4 alone	Kitabatake et al. (1979)
Fly ash	70,000 (6 μm, MMAD)		H_2SO_4	1,000, 10,000, 100,000 (0.8 μm, MMAD, σ_g = 1.7-1.8)	6 h	Chamber	Rat	Lavage indices (LDH, acid phosphatase, glutathione reductase)		Minimal interaction: response largely due to H_2SO_4; increase in LDH and glutathione reductase only in combined exposure	Henderson et al. (1980a)

TABLE 11-9 (cont'd). TOXICOLOGIC EFFECTS OF MIXTURES CONTAINING ACIDIC AEROSOLS

Co-Pollutant			Acid Particle							
Chemical	μg/m^3	ppm	Chemical	μg/m^3 (μm)	Exposure Regime	Exposure Conditions	Species, Gender Strain, Age and Body Weight	Endpoints	Response to Mixture	Interaction	Reference
HNO_3 (vapor) Diesel exhaust	380 460 (0.15)		H_2SO_4	180 (no size stated)	5 h/day, 5 days		Rat, M, Sprague-Dawley	Macrophage phagocytosis; receptor activity	Macrophage phagocytosis, F_c receptor activity decreased	Not determined	Prasad et al. (1988)
HNO_3 (vapor) Diesel exhaust	380 550 (0.15 μm MMAD)		H_2SO_4	180 (no size stated)	5 h/day, 5 days	Nose-only	Rat, M, Sprague-Dawley, 6 wk	Macrophage phagocytosis; morphology; tracheobronchial and mucociliary clearance	No change in cell turnover in nose, trachea, alveolar epithelium; no deep lung lesions; ↓ phagocytosis; no clearance effects	Not determinable	Prasad et al. (1990)
ZnO	up to 2500 μg/m^3 (0.05 μm emd, σ_g = 2)		H_2SO_4 (coated on ZnO particles)	20-30 μg	3 h/day for 5 days		Guinea pig	Pulmonary function	Reductions in total lung vol. vital capacity, DL_{co} severity inc. with increasing exposure duration, inc. protein, PMNs in BAL		Amdur and Chen (1989)

A few studies have examined the effects of exposure to multicomponent (complex) atmospheres containing acidic sulfate particles. Studies of mixtures containing O_3 or NO_2 are summarized elsewhere (U.S. Environmental Protection Agency, 1993, 1995).

A series of studies discussed in the previous PM/SO_x CD (U.S. Environmental Protection Agency, 1982) involved exposure of dogs to simulated auto exhaust atmospheres (e.g., Hyde et al., 1978) for 16 h/day for 68 mo followed by a 32- to 36-mo period in clean air. The mixture consisted of 90 $\mu g/m^3$ H_2SO_4+ 1,100 $\mu g/m^3$ SO_2, with and without irradiated auto exhaust (which results in production of oxidants) and nonirradiated auto exhaust. The results were dependent on the time of examination, exposure, and the endpoint. The primary finding was that groups exposed to SO_2 and H_2SO_4 showed emphysema like changes, observed 32- to 36-mo postexposure. The authors considered the specific changes to be analogous to an incipient stage of human centrilobular emphysema. SO_2 alone would be unlikely to produce such a deep lung response. Also, from the pulmonary function results, it did not appear that auto exhaust exacerbated the effects of the SO_2-H_2SO_4 mixture.

Prasad et al. (1988) exposed rats for 5 h/day for 5 days to an atmosphere consisting of 460 $\mu g/m^3$ diluted diesel exhaust (0.15 μm), 380 $\mu g/m^3$ HNO_3 vapor, and 180 $\mu g/m^2$ H_2SO_4 (present as a surface coat on the diesel particles). Reduced activity of macrophage surface (Fc) receptors and phagocytosis were noted, but interaction could not be determined since the individual components were not tested separately. In another related study, Prasad et al. (1990) examined particle clearance, lung histology and macrophage phagocytic activity following nose-only exposures of rats (Sprague-Dawley, M, 6 weeks) for 5 h/day for 5 days to atmospheres consisting of 380 $\mu g/m^3$ HNO_3 vapor, 550 $\mu g/m^3$ diluted diesel exhaust, and 180 $\mu g/m^3$ H_2SO_4 coated on the diesel particles (0.15 μm). There was no change in tracheobronchial or pulmonary clearance of tracer particles with this mixture, compared to air controls. While no deep lung lesions nor any change in turnover rate of epithelial cells from the nose, trachea or alveolar region were noted, there was a decrease in the percentage of total macrophages assessed which had internalized diesel particles following exposure to the mixture, compared to cells recovered from animals exposed to the diesel particles alone. Furthermore, phagocytosis was depressed up to 3 days following exposure to the mixture.

The enhanced effect of the particles with the surface acid coat is consistent with studies, described below, with other acid-coated particles.

Amdur and Chen (1989) exposed guinea pigs to simulated primary emissions from coal combustion processes, produced by mixing ZnO, SO_2, and water in a high temperature combustion furnace. The animals were exposed 3 h/day for 5 days to ultrafine (0.05 μm CMD, σg=2) aerosols of zinc oxide (ZnO) at up to 2,500 $\mu g/m^3$ having a surface coating of H_2SO_4 resulting from this process (ZnO had no effect in this study). Levels of SO_2 in the effluent ranged from 0.2 to 1 ppm. Acid sulfate concentrations as low as 20 to 30 $\mu g/m^3$ as equivalent H_2SO_4 delivered in this manner resulted in significant reductions in total lung volume, vital capacity, and DLco. The effects appeared to be cumulative, in that the severity was increased with increasing exposure duration. These exposures also resulted in an increase in the protein content of pulmonary lavage fluid and an increase in PMNs. The investigators noted that much higher exposure levels of pure H_2SO_4 aerosol were needed to produce comparable results, suggesting that the physical state of the associated acid in the pollutant mixture was an important determinant of response. But one confounder in these studies was that the number concentration was greater for the coated particles than for the pure acid particles and, as mentioned earlier, both number and mass concentrations of the exposure atmosphere likely play roles in the biological responses.

Other studies have examined responses to acid-coated particles. Chen et al. (1989) exposed (nose-only) guinea pigs (male, Hartley, 250 to 300g) for 3 h to ultrafine ZnO (0.05 μm, σg=1.86) onto which was coated 25 or 84 $\mu g/m^3$ H_2SO_4. Selected eicosanoids were examined in lavage fluid obtained at 0, 72, and 96 h post-exposure. Immediately following exposure, animals exposed to the higher acid concentration showed increased levels of prostaglandin F2α compared to those found in animals exposed to ZnO alone. Levels of prostaglandins E1 and 6-keto-PGF1α, thromboxane B2 and leukotriene B4 were similar to those found in animals exposed to the metal alone. During the post-exposure period, changes in prostaglandin E1, leukotriene B4 and thromboxane B2 were noted. But the authors suggested that there was no causal relationship between these changes and alterations in pulmonary function noted earlier (Amdur et al., 1986).

Chen et al. (1992b) exposed guinea pigs to acid-coated ZnO for 1 h, and examined airway responsiveness to acetylcholine administered 1.5 h after exposure. In this study, the

equivalent concentrations of H_2SO_4 were 20 and 30 $\mu g/m^3$ coated on the 0.05 μm ZnO particles. Animals were also exposed to pure H_2SO_4 droplets at 202 $\mu g/m^3$ and having a similar size as the coated particles (0.06 μm, σg=1.36). Hyperresponsiveness was found in animals exposed to the acid-coated particles, but not in those exposed to furnace gases (particle-free control) or to the ZnO alone. A similar quantitative change was noted in those animals exposed to the pure droplet at about 10 times the concentration of the coated particles (Amdur and Chen, 1989).

Amdur and Chen (1989) exposed guinea pigs for 3 h or for 3 h/day for 5 days to a similar atmosphere as above and examined pulmonary function. Levels of 30 $\mu g/m^3$ H_2SO_4 produced a significant depression in diffusing capacity (DLco). Repeated exposures at the equivalent of 21 $\mu g/m^3$ H_2SO_4 resulted in reduced DLco after the 4th exposure day; at the higher (30 $\mu g/m^3$) level of coated acid, DLco decreased gradually from the first exposure day.

The interaction of acid coated particles with ozone was examined by Chen et al. (1991). Guinea pigs (male, Hartley, 260 to 325 g) were exposed (nose-only) to sulfuric acid coated ZnO particles (0.050 μm CMD, σg=2) at 24 or 84 $\mu g/m^3$ H_2SO_4 or pure acid (0.08 μm) at 300 $\mu g/m^3$ for 2 h, followed by 2 h rest period and 1 h additional exposure (whole body) to air or 0.15 ppm O_3. Other animals were exposed to acid coated ZnO having an equivalent acid concentration (24 $\mu g/m^3$) for 3 h/day for 5 days. This was followed by exposure for 1 h to 0.15 ppm O_3 on day 9, or to two additional 3 h exposures to 24 $\mu g/m^3$ H_2SO_4 layered-ZnO on days 8 and 9. In the single exposure series, animals exposed only to the higher coated acid concentration followed by ozone showed greater than additive changes in vital capacity and DLco, while those exposed first to the pure acid droplet did not show any change greater than that due to ozone alone. Animals exposed repeatedly and then to the two added acid exposures showed greater reductions in lung volumes and DLco than did those that did not receive the additional acid exposures. Finally, animals exposed to ozone after acid showed reduced lung volumes and DLco not observed in animals exposed to either ozone alone or acid alone. In terms of acid alone, neither single exposure to the coated acid affected the endpoints, while exposure to the pure acid decreased DLco. The investigators concluded that single or multiple exposures to the acid-coated ZnO resulted in an enhanced response to subsequent exposures to acid or ozone and that the manner in which the acid was

delivered (i.e., as a pure droplet or as a surface coating) affected whether or not any interaction occurred. However, it is likely that the number concentration of particles was greater in the zinc oxide aerosol than in the pure acid aerosol, and the interaction may reflect this greater particle number. It should also be noted that ZnO itself may have produced some biological response, or contributed to any interaction with the acid, in some of the studies reported for some endpoints.

Wong et al. (1994) exposed rats (M; F-344, nose-only) for 4 h/day, 4 days/week for 8 weeks to a complex mixture consisting of 350 $\mu g/m^3$ California road dust (5 μm MMAD) + 65 $\mu g/m^3$ $(NH_4)_2SO_4$ (0.3 μm) + 365 $\mu g/m^3$ NH_4NO_3 (0.6μm) + O_3 (0.2 ppm), as well as to O_3 alone. Animals were sacrificed at 4 or 17 days after the last exposure to assess stress inducible heat shock protein as an indicator of early pulmonary injury. An increase in heat shock protein was observed with the mixture at both time points, but the effect of O_3 was greater than that due to the mixture.

Mannix et al. (1982) examined the effects of a 4 h exposure of rats to a SO_2-sulfate mix, consisting of SO_2 (13,000 $\mu g/m^3$) plus 1,500 $\mu g/m^3$ (0.5 μm, MMAD) of an aerosol containing $(NH_4)_2SO_4$ and $Fe_2(SO_4)_3$. No change in particle clearance from the tracheobronchial tree or pulmonary region was found.

11.3 METALS

11.3.1 Introduction

The metals discussed in this section are generally present in the ambient atmosphere of U.S. urban areas in concentrations greater than 0.5 $\mu g/m^3$ (see Chapter 3, Table 3-10) and include arsenic, cadmium, copper, iron, lead, vanadium, and zinc. While other metals are present in the ambient air, they are found at concentrations less than 0.5 $\mu g/m^3$ and are not reviewed here. There are no reported toxicological studies of acute effects of inhaled metals at or below this concentration.

The information presented has primarily been obtained from occupational and laboratory animal studies. Both of these data sources have limitations that affect their usefulness to ambient particulate matter discussion. In the occupational studies, the exposures are not well-characterized and may be confounded by exposure to other materials

such as PAH, toxic gases, and other respirable particulate. Moreover, the concentrations of metals experienced in occupational settings as well as the exposure concentrations and the doses administered in the laboratory animal studies are generally hundreds to several thousand times greater than the concentrations found in the ambient air (about 1-14 $\mu g/m^3$).

These sections are intended as general summaries of each metal since the majority, with the exception of lead, do not have current documentation or health risk standards. However, review articles and criteria documents from other agencies are cited as sources of additional information. While there are many studies available using higher concentrations and other routes of administration than inhalation, a select summary only of the effects of inhaling metals on humans and animals is presented in Table 11-10 where an attempt was made, where possible, to focus on those studies that reported effects at the lowest exposures. Each section briefly discusses data on acute and chronic effects from inhaling metals in humans and laboratory animals. Endpoints (developmental effects and other non-respiratory endpoints) not immediately related to the epidemiological findings presented in Chapter 12 are not included in this discussion but are presented in the references cited. End points seen with routes of exposure other than inhalation are not discussed.

11.3.2 Arsenic

Human Data: The toxicity data on inhalation exposures to arsenic are limited in number and quality. Long-term occupational exposure to arsenic leads to a range of health effects such as lung cancer, skin changes and peripheral nerve damage in workers. Most of the available human inhalation data on arsenic are based on occupational exposures to arsenic trioxide.

In humans, acute symptoms are seen after airborne exposure to high levels of arsenic trioxide in an occupational setting. Symptoms include severe irritation of the nasal mucosa, larynx, and bronchi (Holmqvist, 1951; Pinto and McGill, 1953). It is not clear if these effects were chemically related to arsenic or a result of irritation due to the dusts inhaled. Irritation of mucous membranes of the nose and throat leading to hoarseness, laryngitis, bronchitis, or rhinitis and sometimes perforation of the nasal septa have been reported in workers exposed to arsenic dusts (Pinto and McGill, 1953), but effect levels cannot be set due to insufficient exposure data. Increased peripheral vasospastic disorders and Raynaud's

TABLE 11-10. RESPIRATORY SYSTEM EFFECTS OF INHALED METALS ON HUMANS AND LABORATORY ANIMALS

Metal (Ambient Concentrations)[a]	Subjects	Effects [b]	References
Arsenic (0.002-2.32 $\mu g/m^3$)	In humans	Resp. tract irritation, laryngitis, bronchitis, rhinitis. Effects absent at 100-1,000 $\mu g/m^3$.	Agency for Toxic Substances and Disease Registry (1993)
	In animals	Decreased bactericidal activity, inc mortality in streptococcal assay at 500-940 $\mu g/m^3$.	Aranyi et al. (1985)
Cadmium (0.0002-7.0 $\mu g/m^3$)	In humans	Acute exposure: resp. tract irritation, bronchiolitis, alveolitis, impaired lung function, and emphysema; mild and reversible symptoms with exposure to 200-500 $\mu g/m^3$. Chronic exposure: kidneys and resp. tract affected; effects include proteinuria and emphysema, with exposure to 20 $\mu g/m^3$ for 27 years.	Agency for Toxic Substances and Disease Registry (1989)
	In animals	Mild inflammation; AM and epithelial hyperplasia in rat at 500 $\mu g/m^3$ for 3 h; lesions repaired at 7-15 days postexposure. Effects similar to humans. Dose-dependent fibrotic lesions in lungs of rats exposed to 300-1,000 $\mu g/m^3$ for 12 weeks.	Buckley and Bassett (1987)
		Hyperplasia of terminal bronchioles, cell flattening, inflammation and proliferation of fibroblasts in rat at $\geq$300 $\mu g/m^3$, 6 h/day, 5 days/week, 62 days.	Kutzman et al. (1986)
		BAL fluid changes at 1,600 $\mu g/m^3$, 3 h/day, 5 days/week, 1-6 weeks indicative of lung damage. Aggregates of PMNs in interstitium, thickening of alveolar septa. Effects peaked at 2 weeks, then dec.	Hart (1986)
		Inc number and size of AM in rat at 100 $\mu g/m^3$, 22 h/day, 7 days/week, 30 days, returning to normal 2 mo postexposure.	Glaser et al. (1986a)
		In rabbit at 400 $\mu g/m^3$, 6 h/day, 5 days/week, 4-6 weeks, inc lung weight, interstitial infiltration of PMNs and lymphocytes, intraalveolar accumulation of large, vacuolated macrophages, inc phospholipid content.	Johansson et al. (1984)
		In mouse at 30-90 $\mu g/m^3$, 8 or, 19 h/day, 5 days/week, 42-69 weeks inc incidence of alveolar lipoproteinosis, interstitial fibrosis, bronchoalveolar hyperplasia.	Heinrich et al. (1989a)

TABLE 11-10 (cont'd). RESPIRATORY SYSTEM EFFECTS OF INHALED METALS ON HUMANS AND LABORATORY ANIMALS

Metal (Ambient Concentrations)[a]	Subjects	Effects [b]	References
Copper (0.003-5.14 $\mu g/m^3$)	In humans	Subjective symptoms and clinical tests (CBC, LDH determination, urinalysis) after outbreak of metal fume fever: fever, dyspnea, chills, headache, nausea, myalgia, cough, shortness of breath, sweet metallic taste, vomiting, 1-10 h occup exposure. Complaints of discomfort similar to onset of common cold; chills or warmth; stuffiness of the head, 75-120 $\mu g/m^3$, few weeks occup exposure.	Agency for Toxic Substances and Disease Registry (1990)
	In animals	Mild respiratory tract effects in hamster: Decreased cilia beating frequency and abnormal epithelium at 3,300 $\mu g/m^3$, 3 h/day.	Agency for Toxic Substances and Disease Registry (1990)
		In mouse exposed for 3 h/day, 5 days/week, 1-2 weeks slight alveolar thickening and irregularities after 5 exposures at 120 $\mu g/m^3$, extensive thickening with many walls fused into irregular masses and dec mean survival time after 10 exposures at 130 $\mu g/m^3$. Dec bactericidal activity in both exposure groups.	Agency for Toxic Substances and Disease Registry (1990)
Iron (0.13-13.80 $\mu g/m^3$)	In humans	Subjective symptoms, chest X ray: siderosis in 3 males. Note: concurrent exposure to several other chemicals; $\geq$10,000 $\mu g/m^3$, 2 mo-12 years (occup).	Sentz and Rakow (1969)
		34% prevalence of siderosis; complaints of chronic coughing and breathlessness, 3,500-269,000 $\mu g/m^3$, 10 year (avg).	Teculescu and Albu (1973)
	In animals	Respiratory tract cell injury (not specified) in hamsters, alveolar fibrosis, 14,000 $\mu g/m^3$, 1 mo.	Creasia and Nettesheim (1974)
		Impaired respiration in rats, blood nasal discharge at 6,800 and 22,000 $\mu g/m^3$, 6 h/day 5 days/week, 4 weeks.	BASF Corporation (1991)

TABLE 11-10 (cont'd). RESPIRATORY SYSTEM EFFECTS OF INHALED METALS ON HUMANS AND LABORATORY ANIMALS

Metal (Ambient Concentrations)[a]	Subjects	Effects [b]	References
Vanadium (0.0004-1.46 $\mu g/m^3$)	In humans	Bronchial irritation (cough, mucous formation) postexposure at 60 $\mu g/m^3$. Cough at 100, 600 $\mu g/m^3$ 8 h lasted about 1 week.	Zenz and Berg (1967)
		Productive cough, runny nose, sore throat, wheezing, 100-300 $\mu g/m^3$, 2 years (occup).	Lewis (1959)
	In humans	Rhinitis, nasal discharge, irritated throat, bronchopneumonia, "asthmatic" bronchitis, est $\leq$6,500, 1-2 years (occup)	Sjöberg (1950)
	In animals	Alveolar proteinosis in rat at 17,000 $\mu g/m^3$, 6 h/day, 5 days/week, 2 weeks; dose-related inc lung weight, inc accumulation of macrophages, collagen deposition, lung lipid content, and Type II pneumocytes.	Lee and Gillies (1986)
		Reduced lung function in monkey at 2,500 $\mu g/m^3$, 6 h, inc pulmonary resistance; inc leukocytes in bronchoalveolar lavage.	Knecht et al. (1985)
		In rat, nasal discharge (sometimes containing blood), difficulty breathing, dec BW; hemorrhages in lung, heart, liver, kidney, brain. bronchitis, focal interstitial pneumonia in lungs. Effects mainly in lungs at low concentration. Mild signs of toxicity at 2,800 $\mu g/m^3$.	Roshchin (1967a)
	In rats	Capillary congestion, perivascular edema, hemorrhages in lungs. Also focal edema and bronchitis in some cases, lymphocyte infiltration of interstitial spaces, constriction of small bronchi, 1,700-2,800 $\mu g/m^3$, 2 h/every other day, 3 mo.	Roshchin (1967a)

TABLE 11-10 (cont'd). RESPIRATORY SYSTEM EFFECTS OF INHALED METALS ON HUMANS AND LABORATORY ANIMALS

Metal (Ambient Concentrations)[a]	Subjects	Effects [b]	References
Zinc (0.015-8.328 $\mu g/m^3$)	In humans	Symptoms metal fume fever: Nausea, chills, shortness of breath and chest pains at 320,000-580,000 $\mu g/m^3$, 1-3 h.	Agency for Toxic Substances and Disease Registry (1994)
		Fever, chills, chest tightness, muscle/joint pain, sore throat, headache at 4-8 h postexposure; inc airway resistance of 16%, 4,900 $\mu g/m^3$, 2 h/day, 1 day (face mask).	Gordon et al. (1992)
		Significant correlation between change in peak expiratory flow rate and dust concentration, 6-8 h workshift.	Marquart et al. (1989)
		BAL fluid changes; inc number of leukocytes, T cells, T suppressor cells, and NK cells; inc PMN leukocytes, with 77,000-153,000 $\mu g/m^3$, 15-30 min (occup).	Blanc et al. (1991)
		Miminal substernal irritation and throat irritation during exposure, 3.6 $\mu g/m^3$, 2 h.	Linn et al. (1981)
	In animals	BAL fluid: Inc protein, LDH, and β-glucuronidase, inflammation at 2,200 $\mu g/m^3$, 3 h/day 1 day in rat.	Gordon et al. (1992)
		BAL fluid: Inc protein, LDH, and β-glucuronidase (suggesting altered macrophage function), inflammation at 2,200 $\mu g/m^3$, 3 h/day 1 day in guinea pig.	
		Impaired lung function (dec compliance and lung volume, inc pulmonary resistance, dec CO diffusing capacity at 3,700 $\mu g/m^3$, 3 h/day 6 day in guinea pig.	Lam et al. (1985)
		Inc lung weight; inflammation, and increased interstitial thickening, fibroblasts, and interstitial infiltrates at 4,300 $\mu g/m^3$.	
		Dec pulmonary compliance, followed by inc during 2-h postexposure, at 730 $\mu g/m^3$, 1 h in guinea pig.	Amdur et al. (1982)

[a]Ambient air concentration range associated with metal particulate matter in the United States atmosphere (see Chapter 1, Table 1-4).
[b]Abbreviations: dec = decreased; inc = increased; occup = occupational; PAH = polycyclic aromatic hydrocarbons; ALK = alkaline phosphatase; BAL = bronchoalveolar lavage; AM = pulmonary alveolar macrophage; PMN = polymorphonuclear leukocyte; res = respiratory.

phenomenon were found in Swedish arsenic workers exposed to airborne arsenic dusts (Lagerkvist et al., 1986).

Laboratory Animal Data: Limited acute data were available on the inhalation toxicity of arsenic in animals. Aranyi et al. (1985) exposed mice to an aerosol of arsenic trioxide for 3 h at levels of 0, 270, 500, or 940 μg arsenic/m^3. Additional groups were exposed for 3 h/day for 5 or 20 days. At the end of exposure, mice were challenged with an aerosol exposure of viable streptococci, and death of exposed and controls was recorded over 14 days. Separate groups were challenged with aerosols of ^{35}S-labeled *Klebsiella pneumoniae* to evaluate macrophage function (bacterial killing) in a 3-h period. In the streptococcal assay, a concentration-related increase in mortality occurred. Bactericidal activity was markedly decreased after a single exposure to 940 μg arsenic/m^3, but no consistent or significant effects were seen at lower exposure levels after one or several exposures.

In a chronic inhalation study, male Wistar rats (20 to 40/group) were continuously exposed to 0, 60, or 200 μg arsenic/m^3 as arsenic trioxide for 18 mo (Glaser et al., 1986b). No effects on body weight, hematology, clinical chemistry, or macroscopic and microscopic examination outcomes were observed.

11.3.3 Cadmium

Recent reviews and health criteria documents have detailed the toxicological and carcinogenic effects of cadmium by different routes of administration including inhalation (Oberdörster, 1989a,b; Waalkes and Oberdörster, 1990; International Agency for Research on Cancer, 1993). Acute and chronic health effects observed after cadmium exposure were mostly related to occupational settings and occurred after exposures to concentrations far exceeding those occurring environmentally. Average airborne cadmium concentrations in rural areas range from 0.0002 to 0.006 μg/m^3, and in urban areas concentrations from 0.002 to 0.025 μg/m^3 have been found which can increase in industrial areas by a factor of 3 to 5. Health effects at these low airborne concentrations of cadmium have not been reported; the following summary indicates that health effects observed in humans and animals are correlated with higher occupational exposure concentrations ranging up to the mg/m^3 levels. Thus, exposure to much lower ambient environmental airborne concentrations of cadmium

are unlikely to contribute to acute health effects. It should also be considered that exposure to cadmium occurring in cigarette smoke by far exceeds background ambient air concentrations. When evaluating health effects of inhaled cadmium compounds it should be considered that *in vivo* solubility of the different cadmium compounds is different from their water solubility. For example, CdO and CdS are both insoluble in water, yet CdO is rapidly soluble in the lung, possibly in the acidic milieu of alveolar macrophages after phagocytosis, whereas CdS is highly insoluble in the lung, behaving more like a low toxicity particle (Oberdörster, 1989b).

11.3.3.1 Health Effects

Human Data: Table 11-10 summarizes data from studies of occupationally-exposed workers which show that the main target organs for cadmium toxicity are the kidney and the respiratory tract. This table is restricted to those studies where exposures to airborne cadmium concentrations were less than 100 $\mu g/m^3$ since it is felt that effects observed from exposures to higher airborne cadmium concentrations are irrelevant for low concentrations of environmental cadmium and particulate matter. With respect to renal damage, these low environmental concentrations will not lead to significant accumulation of cadmium in the kidney to reach the critical concentration of 200 $\mu g/g$ which will result in symptoms of kidney damage, e.g., proteinuria. Earlier studies found evidence of proteinuria after occupational exposures to 50 $\mu g/m^3$ for up to 12 years (Kjellstrom et al., 1977). More recent analyses found the threshold of cadmium exposure for proteinuria at close to 1,000 $\mu g/m^3 \times$ year (Elinder et al., 1985a,b; Mason et al., 1988). Obviously, these exposure concentrations are far above those encountered environmentally and will not be considered further in the context of this document.

Acute respiratory effects of inhaled cadmium have been reported as pneumonitis and edema if exposure concentrations exceed 1,000 $\mu g/m^3$ for periods of 1 h or more. Chronic cadmium exposures resulting in emphysema and dyspnea have also been reported when exposure concentrations are very high, exceeding for extended periods of time several hundred $\mu g/m^3$. Chronic exposure concentrations below 100 $\mu g/m^3$ at occupational settings have been associated with induction of lung tumors (International Agency for Research on Cancer, 1993). Recent analyses of English and Swedish cohorts as well as an American

cohort found a statistically significant excess risk of lung cancer in the highest exposure groups (Elinder et al., 1985c; Sorahan, 1987; Thun et al., 1985). Based on these studies, IARC determined that cadmium is a human carcinogen. However, environmentally encountered airborne cadmium concentrations are too low to induce lung cancer, unless it is postulated that a combination of cadmium plus other air contaminants results in a synergistic carcinogenic effect. Excess of prostate cancer due to occupational inhalation of cadmium observed in earlier epidemiological studies have not been confirmed in later studies (IARC, 1993).

Laboratory Animal Data: Health effects of inhaled cadmium compounds are summarized in Table 11-10. Like with the human studies, only those studies are listed where exposure concentrations below 100 $\mu g/m^3$ were used. These studies in laboratory animals confirm that inhalation exposure to cadmium compounds can result in respiratory tract injury. Very high exposure concentrations (mg/m^3) are needed to cause acute effects such as lung edema and alveolar epithelial cell necrosis, whereas lower exposure concentrations at ~50 - 100 $\mu g/m^3$ can induce chronic inflammatory responses including bronchoalveolar hyperplasia, proliferation of connective tissue leading to interstitial fibrosis (Takenaka et al., 1983). The most striking effect at low exposure concentrations in rats is that different cadmium compounds were shown to cause lung cancer (Takenaka et al., 1983; Glaser et al., 1990). These studies reported primary lung tumors (bronchoalveolar adenoma, adenocarcinoma, squamous cell tumors) following exposure to $CdCl_2$, $CdSO_4$, CdS and CdO inhaled as dust or fume. Exposure concentrations were as low as 10 $\mu g/m^3$, adding to the evidence from human occupational exposure studies that inhaled Cd-compounds can induce lung tumors. In contrast to rats, mice and hamsters exposed to the different cadmium compounds at similar concentrations did not induce lung tumors (Heinrich et al., 1989a). The reason for the significant species differences may be the different inducibility of metallothionein (MT) as well as different baseline levels of MT in the lungs of mice and rats which was demonstrated by Oberdörster et al. (1994a). These authors found that a four-week inhalation exposure to $CdCl_2$ aerosols at an exposure concentration of 100 $\mu g/m^3$ caused greater and more persistent inflammation and cell proliferation in the lungs of mice than in rats. At the same time MT was induced to a greater degree in mice, possibly protecting the lungs of this species from the cytotoxic effects of inhaled cadmium.

In summary, these studies demonstrate that measured low environmental cadmium concentrations alone are not likely to be causally associated with acute effects on mortality and morbidity observed in epidemiological studies; nor are they likely to cause long-term chronic effects. Cadmium exposure at relatively high exposure concentrations has been shown to lead to a decreased immune response in mice (Graham et al., 1978; Krzystyniak et al., 1987) which could suggest that people with a compromised immune system may also be affected more than healthy people by exposure to cadmium. However, environmental low level cadmium concentrations have not been shown to induce these effects.

11.3.4 Copper

Human Data: The data on human exposure to copper by inhalation are limited. The major target organ appears to be the respiratory system, but the data are limited to occupational studies. Data are primarily based on subjective symptoms without indications of pulmonary function changes as a result of occupational exposure to copper. The observed symptoms may also be due to exposure to copper by both oral and inhalation routes since exposures were confounded. The lack of control workers is also a limitation in evaluating the human data available for copper exposure by inhalation. A combination of respiratory symptoms has been reported following acute inhalation exposure to copper in humans. Armstrong et al. (1983) reported the following symptoms (in order of number of workers affected): fever, dyspnea, chills, headache, nausea, myalgia, cough, shortness of breath, a sweet metallic taste and vomiting in factory workers accidentally exposed to copper dust or fumes for 1 to 10 h as a result of cutting pipes known to contain copper. These symptoms are consistent with metal fume fever, an acute disease induced by inhalation of metal oxides that temporarily impairs pulmonary function but does not progress to chronic lung disease (Stokinger, 1981a). Airborne copper concentration during the exposure period was not reported. It was reported that 5 of 12 workers hospitalized following the acute exposure had urine copper levels greater than 50 μg/L. Since the major route of excretion of copper is biliary, the elevated urine copper levels reported suggest that the exposure concentration was relatively high. Copper levels were not determined for control workers in this study which limits the interpretation of the urinary copper values as an indicator of copper inhalation exposure. Armstrong et al. (1983) also reported evidence of minimal elevation of serum

lactate dehydrogenase (in 3 of 14 workers evaluated) and leukocytosis (in 21 of 24 workers evaluated). Nonspecific complaints of discomfort and chills were reported among several workers within a few weeks of beginning operation of a copper plate polishing operation. Exposure levels of 75 to 120 $\mu g/m^3$ were measured (Gleason, 1968).

In a epidemiological study by Suciu et al. (1981), factory workers exposed to copper dust received annual physical and clinical examinations during a 4 year exposure period. The reported air copper levels were not reported for the first year, were 464,000 μg Cu/m^3 in the second year; 132,000 μg Cu/m^3 in the third year; and 111,000 μg Cu/m^3 in the fourth year. Although inhalation was considered to be the major route of exposure for these workers, it was likely that a portion of the airborne copper was trapped in the upper respiratory tract and swallowed. This assumption was made based on the gastrointestinal effects that were observed in these workers in addition to the respiratory effects. Respiratory effects reported included symptoms of coughing, sneezing, yellowish-green expectoration, dyspnea, and thoracic pain. Radiography revealed linear pulmonary fibrosis and in some cases nodulation. Limitations of this study include the absence of a control group, poor description of study design and the lack of statistical analysis of data.

Respiratory effects were also noted in a report by Askergren and Mellgren (1975). Nose and throat examinations were performed in sheet-metal workers exposed to copper dust. Six of 11 workers had nasal mucosa characterized by increased vascularity and superficial epistatic vessels. This was accompanied by symptoms of runny nose and mucosal irritation in the mouth and eyes. However, the levels of airborne copper were not measured.

Laboratory Animal Data: As with human exposure, the respiratory system appears to be the primary site of injury following inhalation exposure to copper. Drummond et al. (1986) reported a decrease in tracheal cilia beating frequency following a single exposure to 3,300 μg Cu/m^3 (as a copper sulfate aerosol) in hamsters, but not in mice exposed to the same level. Alveolar thickening was observed in mice exposed repeatedly and the severity of the effect increased with the duration of exposure. Histological examination of the trachea revealed abnormal epithelium in mice at 5 exposures at 120 μg Cu/m^3, extensive thickening and decreased mean survival time after 10 exposures at 130 μg Cu/m^3.

Immunological effects were observed in mice (Drummond et al., 1986) and in rabbits (Johansson et al., 1983) exposed to copper sulfate aerosols. Mice exposed to either a single

concentration of 560 μg Cu/m^3 or 10 exposures to 130 μg Cu/m^3, and simultaneously challenged with an aerosol of *Streptococcus zooepidemicus* had decreased survival time (Drummond et al., 1986). Decreased bactericidal activity was also observed in mice after exposure to an aerosol of *Klebsiella pneumonia* after single or repeated exposures to copper sulfate aerosols (Drummond et al., 1986), suggesting that copper can inhibit the function of alveolar macrophages. After inhalation exposure, Johansson et al. (1983) also observed a slight increase in the amount of lamellated cytoplasmic inclusions in alveolar macrophages. Exposures of rabbits to copper chloride aerosols for 4 to 6 weeks resulted in a minor increase in volume density of alveolar Type 2 cells and minor levels of lymphocytic or eosinophilic inflammatory infiltrates (Johansson et al., 1984).

11.3.5 Iron

Human Data: Most of the available human inhalation data on iron are based on occupational exposures to iron oxide, with effects limited to respiratory symptoms and dysfunction. There are no acute human inhalation data on the effects of iron exposure. Health effects information via inhalation route is limited to iron pentacarbonyl. No information was located on the soluble iron salts including ferric chloride, ferric nitrate, and ferric sulfate.

Occupational exposure occurs from mining of iron ores, consisting mainly of oxide forms. During the mining and during smelting and welding process, workers are often exposed to dust containing iron oxides and silica, as well as other metals and substances. It is known that exposure to iron oxides results in roentgenological changes in the lung due to deposition of inhaled iron particles (Doig and McLaughlin, 1936; Musk et al., 1988; Plamenac et al., 1974), designated variously as siderosis, iron pneumoconiosis, hematite pneumoconiosis, iron pigmentation of the lung, and arc welder lung (Elinder, 1986). Siderosis is prevalent in 5 to 15% of iron workers exposed for more than 5 years (Buckell et al., 1946; Schuler et al., 1962; Sentz and Rakow, 1969). Exposure levels were reported to exceed 10,000 μg iron/m^3 by Sentz and Rakow (1969); but no exposure data were presented for the other studies. A Romanian study (Teculescu and Albu, 1973) reported a 34% prevalence of siderosis in workers exposed to ferric oxide dust (3,500 to 269,000 μg/m^3); but radiological evidence of lung fibrosis was not observed. Complaints of

chronic coughing were reported by 80% of the workers. Morgan (1978) found a male subject exposed chronically to ferric oxide (magnetite; Fe_3O_4) had symptoms of coughing and sputum for 8-9 years and exhibited an abnormal chest x-ray, but pulmonary function tests revealed no abnormalities. Stokinger (1984) reviewed the literature on occupational exposure to iron oxide fumes, and concluded that most investigators considered the roentgenological pulmonary changes, secondary to inhalation of iron dust (i.e., siderosis), as benign and did not suspect them to progress to fibrosis. Although several case reports have described iron oxide workers, with coughing and shortness of breath, exhibiting diffuse fibrosis in their chest x-rays (Charr, 1956; Friede and Rachow, 1961; Stanescu et al., 1967), concurrent exposure to other chemicals may have contributed to this finding (Chan-Yeung et al., 1982; Sitas et al., 1989).

Several studies report high incidence of lung cancer mortality among workers exposed to iron oxide in mines and smelters; but, in all cases, there was simultaneous exposure to other potentially carcinogenic substances (Boyd et al., 1970; Faulds, 1957). Improvements in dust control and ventilation of mines after 1967 have also resulted in reduction of lung cancer mortality in iron ore mine workers (Kinlen and Willows, 1988).

Iron oxide particles have been used both as a tracer and as a carrier particle for radioactive tracers (e.g., Te) in human (Leikauf et al., 1984; Gerrard et al., 1986; Ilowite et al., 1989; Bennett et al., 1992; Bennett and Zeman, 1994; Bennett et al., 1993) and laboratory animal studies (Okuhata et al., 1994, Brain et al., 1994; Warheit and Hartsky, 1993; Dorries and Valberg, 1992; Warheit et al., 1991a,c; Bellmann et al., 1991; Lehnert and Morrow, 1985; Brain et al., 1984; Valberg, 1984; Skornik and Brain, 1983) to measure different aspects of pulmonary deposition and clearance. In general, the exposures were brief and the concentrations of iron used in these studies were extremely high compared to those found in the ambient atmosphere. There were no reported acute effects of exposure to these iron oxide particles.

Laboratory Animal Data*:* Two acute inhalation studies reported clinical signs relating to respiratory distress in rats exposed to iron pentacarbonyl for 4 h or 1 mo (BASF Corporation, 1991; Bio/Dynamics Incorporated, 1988). However, histopathology was not performed on the lungs. Acute exposure of rats to 500,000 μg iron/m^3 as iron oxide for greater than 30 min also resulted in coughing, respiratory difficulties, and nasal irritation

(Hewitt and Hicks, 1972 as cited in Elinder, 1986) and histopathology of the lungs revealed iron oxide particles in macrophage cells. Ten intratracheal installations of ferric oxide in hamsters produced loss of ciliated cells, and hyperplasia and proliferation of non-ciliated epithelial cells in the lungs (Port et al., 1973). Intratracheal instillation of iron oxides in female rats produced tumors in 70% of the animals but did not reduce the life-span (Pott et al., 1994). At a longer duration of 1 mo, hamsters inhaling 14,000 μg iron/m^3 as ferric oxide dust (MMAD of 0.11 μm) revealed respiratory tract cell injury and alveolar fibrosis (Creasia and Nettesheim, 1974).

See also the discussion below on transition metals (Section 11.3.8) regarding ferric iron (Fe^{3+}) complexed on the surface of silicates. There it is noted, for example that newly emerging studies by Ghio et al. (1992) and others suggest that Fe^{3+} complexed on the surface of silicate particles may be responsible for inflammatory responses associated with silicate inhalation.

11.3.6 Vanadium

Human Data: Acute and chronic inhalation studies in humans are generally limited to occupational case studies and epidemiology studies in workers engaged in the industrial production and use of vanadium. Based on these studies, the respiratory tract is the primary target of vanadium inhalation. Most of the reported exposures are to vanadium pentoxide dusts.

Acute and chronic respiratory effects were most commonly seen following exposure to vanadium pentoxide dusts. Mild respiratory distress (cough, wheezing, chest pain, runny nose, or sore throat) was observed in workers exposed to vanadium pentoxide dusts or vanadium in fuel oil smoke for as few as 5 h (Levy et al., 1984; Musk and Tees, 1982; Thomas and Stiebris, 1956; Zenz et al., 1962) or as long as 6 years (Lewis, 1959; Orris et al., 1983; Sjöberg, 1956; Vintinner et al., 1955; Wyers, 1946). Most clinical signs reflect the irritative effects of vanadium on the respiratory tract; only at concentrations >1,000 μg vanadium/m^3 were more serious effects on the lower respiratory tract observed (bronchitis, pneumonitis). Rhinitis, pharyngitis, bronchitis, chronic productive cough, wheezing, shortness of breath, and fatigue were reported by workers following chronic inhalation of vanadium pentoxide dusts (Sjöberg, 1956; Vintinner et al., 1955; Wyers, 1946).

Two volunteers exposed to 60 μg vanadium/m^3 as vanadium pentoxide reported a delay of 7 to 24 h in the onset of mucus formation and coughing (Zenz and Berg, 1967).

Vanadium induced asthma in vanadium pentoxide refinery workers without previous history of asthma, with symptoms continuing for 8 weeks following cessation of exposure (Musk and Tees, 1982). Increased neutrophils in the nasal mucosa were reported in chronically exposed workers (Kiviluoto, 1980; Kiviluoto et al., 1979, 1981c).

Chronic occupational exposure to vanadium dusts was also associated with some electrocardiographic changes (Sjöberg, 1950). Vanadium dusts had no effect on hematology following acute exposure (Zenz and Berg, 1967) or chronic exposure (Kiviluoto et al., 1981a; Sjöberg, 1950; Vintinner et al., 1955). Blood pressure and gross neurologic signs were not affected following chronic exposure to vanadium pentoxide dusts at levels up to 58,800 μg vanadium/m^3 (Vintinner et al., 1955), although other authors reported anemia or leukopenia (Roshchin, 1964; Watanabe et al., 1966). Based on serum biochemistry and urinalysis, there was no indication of kidney or liver toxicity in workers chronically exposed to 200 to 58,800 μg vanadium/m^3 as vanadium dusts (Kiviluoto et al., 1981a,b; Sjöberg, 1950; Vintinner et al., 1955). Vanadium green discoloration of the tongue resulting from direct deposition of vanadium is often reported (Orris et al., 1983; Lewis, 1959; Musk and Tees, 1982).

Laboratory Animal Data: Acute and chronic laboratory animal studies support the respiratory tract as the main target of inhaled vanadium compounds. The animal data indicate that vanadium toxicity increases with increasing compound valency, and that vanadium is toxic both as a cation and as an anion (Venugopal and Luckey, 1978).

The mechanism of vanadium's effect on the respiratory system is similar to that of other metals. *In vitro* tests show that vanadium damages alveolar macrophages (Castranova et al., 1984; Sheridan et al., 1978; Waters et al., 1974; Wei and Misra, 1982) by affecting the integrity of the alveolar membrane, thus impairing the cells' phagocytotic ability, viability, and resistance to bacterial infection. Cytotoxicity, tested on rabbit alveolar macrophages *in vitro*, was directly related to solubility in the order $V_2O_5 > V_2O_3 > VO_2$. Dissolved vanadium pentoxide (6 μg/ml) also reduces phagocytosis (Waters, 1977).

Respiratory effects in laboratory animals following acute inhalation of vanadium compounds include increased pulmonary resistance and significantly increased

polymorphonuclear leukocytes in bronchioalveolar lavage fluid. These effects were observed in monkeys 24 h following a 6-h inhalation exposure to 2,800 μg vanadium/m^3 as vanadium pentoxide (Knecht et al., 1985). In addition, increased lung weight and alveolar proteinosis were observed in rats after inhaling bismuth orthovanadate 6 h daily for two weeks (Lee and Gillies, 1986). Rabbits exposed to high concentrations of vanadium pentoxide dust for 1 to 3 days exhibited dyspnea and mucosal discharge from the nose and eyes (Sjöberg, 1950). In a follow-up experiment, rabbits had difficulty breathing following a daily 1-h exposure for 8 mo (Sjöberg, 1950).

The effects of acute exposure to 5,600 to 39,200 μg vanadium/m^3 as vanadium pentoxide fume or 44,800 to 392,000 μg vanadium/m^3 as vanadium pentoxide dust were investigated by Roshchin (1967a); the exposure duration was not described in the available literature. For vanadium pentoxide fume, "mild toxicity" occurred at 5,600 μg vanadium/m^3, and deaths were observed at the high level. The vanadium pentoxide dust was described as one-fifth as toxic as the fume. Effects at the lower levels were mostly observed in the lungs. These included irritation of respiratory mucosa, perivascular and focal edema, bronchitis, and interstitial pneumonia. In a subchronic experiment, rats were exposed to vanadium pentoxide fume (1,700 to 2,800 μg vanadium/m^3) or vanadium pentoxide dust (5,600 to 17,000 μg vanadium/m^3) for 2 h every other day for 3 to 4 mo (Roshchin, 1967a). Histopathological effects were limited to the lungs and were similar to those observed following acute exposure. The study author concluded that vanadium inhalation resulted in irritation of the respiratory mucosa, hemorrhagic inflammation, a spastic effect on smooth muscle of the bronchi, and vascular changes in internal organs (at higher levels). Similar effects were observed with the trivalent vanadium compounds vanadium trioxide and vanadium trichloride, although vanadium trichloride caused more severe histological changes in internal organs (Roshchin, 1967b); further details were not available.

Rats exposed to vanadium pentoxide condensation aerosol (15 μg vanadium/m^3) continuously for 70 days developed marked lung congestion, focal lung hemorrhages, and extensive bronchitis (Pazynich, 1966).

11.3.7 Zinc

Inhalation of zinc compounds, most notably zinc oxide fumes, can result in significant pulmonary irritation and inflammation referred to as metal fume fever. However, zinc is an essential element with low intrinsic toxicity, and exposure concentrations have to be in the mg/m^3 range to induce these symptoms which are accompanied by increased inflammatory cell and protein levels in pulmonary lavage both in experimental animals and humans (Gordon et al., 1992). A number of studies in experimental animals and also in humans occupationally-exposed to zinc fumes have been reported, and almost all of these were related to high exposure concentrations which are irrelevant for low environmental exposure levels. A recent review of the toxicity of inhaled metal compounds including zinc in the respiratory tract (Gordon, 1995) describes a number of studies from which it can be concluded that inhaled zinc compounds including zinc oxide are rapidly solubilized in the lung and do not appear to accumulate in the respiratory tract. Elevated levels of zinc can be found in blood and urine of exposed workers as well as in exposed animals. Occupational exposures at concentrations below 50 $\mu g/m^3$ have not resulted in the occurrence of metal fume fever (Marquart et al., 1989; Linn et al., 1981). Higher exposure concentrations inhaled repeatedly result in the development of tolerance after initial symptoms of zinc fume fever subside (Gordon et al., 1992). Effects observed after acute high level exposures include dyspnea, cough, pleuritic chest pain, bilateral diffuse infiltrations, pneumothorax and acute pneumonitis from respiratory tract irritations. However, exposure concentrations have to be extremely high for the more severe symptoms to occur which has no relevance for ambient low level particulate pollutants.

11.3.8 Transition Metals

An area of current investigation is the potential for the particle-associated transition metals to induce oxidant injury. The transition metals are characterized by being electronically stable in more than one oxidation state and, as a result, have the ability to catalyze the oxidative deterioration of biological macromolecules. Considering that the transition metals can catalyze the oxidative deterioration of biological macromolecules it is plausible that inhalation of PM containing these metals could cause oxidative injury to the

respiratory tract. However, the data available thus far is derived from studies using in vitro systems and intratracheal administration and can not be used for risk estimation.

Iron, the best studied of the transition metals, has the ability to catalyze the formation of reactive oxygen species (ROS) and initiate lipid peroxidation (Aust, 1989; Minotti and Aust, 1987; Imlay et al., 1988; Halliwell and Gutteridige, 1986). Guilianelli et al. (1993) studied the importance of iron to the toxicity of iron-containing particles in cultured tracheal epithelial cells. Nemalite, the most cytotoxic of the three minerals tested, contained the most surface Fe^{2+}. Moreover, pretreatment with the iron chealating compound desferrioxamine, reduced the toxic effects of nemalite.

Garrett et al. (1981a) exposed rabbit alveolar macrophages in vitro to fly ash with and without surface coatings of various metal oxides. Cellular viability and cellular adenosine triphosphate content were reduced only with the metal-coated ash particles. Berg and co-workers (1993) measured the release of ROS from bovine alveolar macrophages stimulated with heavy metal-containing dusts <4 μm in diameter. Dusts, derived from waste incineration, sewage sludge incineration, an electric power station, and from two factories, incubated with alveolar macrophages caused a concentration-dependent increase in ROS release. The ratio of superoxide anion ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2) secreted varied, depending on the dust, but the release of H_2O_2 correlated best, in descending order, with the content of iron, manganese, chromium, vanadium, and arsenic in the dusts. The positioning of iron first in this array is consistent with other studies examining the biological effects of iron coating the surface of particles.

Certain particles, including silica, crocidolite, kaolinite, and talc, complex considerable concentrations of ferric iron (Fe^{3+}) onto their surfaces. The potential biological importance of iron complexation was assessed by Ghio and co-workers (1992) who examined the effects of surface Fe^{3+} on several indices of oxidative injury. Three varieties of silicate dusts were studied: (1) iron-loaded, (2) unmodified, and (3) desferrioxamine-treated. The ability of silicates to catalyze oxidant generation in an ascorbate/H_2O_2 system in vitro, to trigger respiratory burst activity and leukotriene B_4 release by alveolar macrophages, and induce lung inflammation in the rat following intra-tracheal instillation all increased in proportion to the amount of Fe^{3+} complexed onto their surfaces. Ghio and Hatch (1993) noted that an extracellular accumulation of surfactant following instillation of silica into the lungs of rats

was associated with the concentration of Fe^{3+} complexed to the surface of the particles, and that surfactant-enriched material was a target for oxidants, the production of which was catalyzed by Fe^{3+}. Moreover, iron, drawn from body stores, has been shown to complex to the surface of intratracheally instilled silica particles and increase concentrations of iron in bronchoalveolar lavage fluid, lung tissue and plasma, and decrease antioxidant molecules in lung tissue, including ascorbate, urate, and glutathione (Ghio et al., 1994).

Surface complexed iron has been implicated in pulmonary injury due to a variety of environmental particles (Costa et al., 1994a,b; Tepper et al., 1994). Three particle types (Mt. St. Helen's volcanic ash, ambient particles of Dusseldorf, Germany, and residual oil fly ash), which represented a range of inflammatory potential, were intratracheally instilled into rats. Both the degree of acute inflammation (as measured by assessing PMNs, eosinophils, LDH and protein in lavage) and nonspecific bronchial responsiveness correlated with the iron (specifically Fe^{+3}) loading of the particles. An interesting observation was that surface iron was correlated with particle acidity, yet when instillation of H_2SO_4 at comparable pH was performed, the lavage analysis indicated much less inflammation with the pure acid compared to the high surface iron particles. In fact, neutralization of the fly ash instillate (which could occur if similar particles were inhaled, due to endogeneous respiratory tract ammonia) actually enhanced particle toxicity, while the pulmonary response diminished when iron was removed from the fly ash by acid washing. These preliminary results generally support the notion that oxidant generation by iron present on the surface of particles may increase lung injury; but, clearly, other factors are likely to contribute to this response.

Tepper et al. (1994) reported that the concentration of iron (Fe^{3+}) complexed on the surface of a particle was associated with the ability of the particle to support electron transfer and to generate oxidants in vitro and to increase lung inflammation and airway hyperresponsiveness in vivo. Particles with or without iron complexed on the surface were instilled into the lungs of rats and evaluated for their potential to produce inflammation and airway hyperactivity. The effects of a high-iron particle (coal fly ash) before and after surface iron was removed by acid washing and the effects of an inert particle (titanium), with or without iron added to the particle surface, were evaluated. The effects of pretreating the rats with drugs to reduce iron-associated ROS formation also were studied. Although coal ash caused considerable inflammation and hyperactivity, acid washing to remove surface iron

reduced the deleterious effects of the particle. However, compared to titanium alone, instillation of a titanium particle coated with iron did not increase lung injury. Pretreatment with allopurinol partially blocked lung inflammation, but desferrioxamine and an anti-neutrophil antibody were less effective. The authors concluded that the results generally support the hypothesis that ROS generation by iron on the surface of particles may exacerbate lung injury.

The inflammatory potential of 10 different metal-containing dusts of either natural or anthropogenic origin was evaluated following intratracheal instillation in rats (Pritchard et al., 1995). Measurements included (1) oxidized products of deoxyribose catalyzed by particulates, (2) induction of a neutrophilic alveolitis after particulate instillation, (3) increments in airway reactivity after particulate instillation, and (4) mortality after exposures to both dust and a microbial agent. Except for titanium, in vitro generation of oxidized products of deoxyribose increased with ionizable concentrations of all metals associated with the particles. After intratracheal instillation of the dusts in rats, the neutrophil influx and lavage protein both increased with ionizable concentrations of the same metals. Changes in airway reactivity following instillation of the dusts also appeared to be associated with the ionizable concentrations of these metals. Similarly, mortality after instillation of particles in mice followed by exposure to aerosolized Streptococcus zooepidemicus reflected metal concentrations. The authors concluded that in vitro measures of oxidant production and in vivo indices of lung injury increased with increasing concentrations of the metals instilled intratracheally.

Thus, it is clear that ROS produced through chemical reactions involving iron can initiate lipid peroxidation, cell injury, and ultimately cell death. It may be possible that other transition metals, by virtue of their ability to redox between valence states, also can generate ROS in the presence of precursor oxidants and reducing agents. However, it has not been established in inhalation studies that these reactions can occur in vivo.

11.3.9 Summary

Data from occupational studies and laboratory animal studies indicate that acute exposures to high levels or chronic exposures to low levels (albeit high compared to ambient levels) of metal particulate can have an effect on the respiratory tract. However, it is

doubtful that the metals at concentrations present in the ambient atmosphere (1 to 14 $\mu g/m^3$) could have a significant acute effect in healthy individuals.

Acute and chronic inhalation exposures to arsenic, cadmium, copper, iron, and vanadium are associated with respiratory effects, and, in the case of cadmium, renal effects. However, in general, the levels used in the laboratory animal studies or experienced in occupational settings are considerably higher (at least 10-fold and as much as 10^3- or 10^4-fold) than those found in the ambient environment, and the results of these studies provide little insight into the morbidity and mortality studies discussed in Chapter 12. This is not unexpected because of the patterns of exposure and the total exposures, as well as differences in the populations exposed. Some of the effects noted in the human occupational studies such as respiratory tract irritation, bronchitis, impaired pulmonary function, cough, wheezing, are also observed in the epidemiological studies discussed in Chapter 12 and may indicate a general effect of PM. However, these effects are evident at exposures much greater than experienced in the ambient atmosphere. Nevertheless, the toxicological studies of the metals do not appear to provide insight into the effects observed in the epidemiological studies discussed in Chapter 12. While studies examining the potential for the transition metals to cause lung injury have been conducted in vitro and in animals by intratracheal instillation are interesting, these results thus far are of limited value.

11.4 ULTRAFINE PARTICLES

This section on ultrafine particles is designed to provide an overview of current concepts concerning the potential pulmonary toxicity of this class of particulates. The occurrence of ultrafine particles in the ambient environment as well as their sources are reviewed in Chapters 3 and 6. Studies assessing the comparative toxicity of particles of different sizes using intratracheal instillation are reviewed in Section 11.9.1. Particles used in toxicological studies are mainly in the fine and coarse mode size range. This section addresses the hypothesis that ultrafine particles can cause acute lung injury and focuses on experimental studies in which ultrafine particles generated as fumes were used. The ultrafine (nucleation mode) particle phase has a median diameter of $\approx$20 nm (see Figure 3-13). Ultrafine particles with a diameter of 20 nm have an approximately 6 order of magnitude

higher number concentration than a 2.5 μm diameter particle when inhaled at the same mass concentration; particle surface area is also highly increased (Table 11-1).

At present, no toxicological studies with relevant ambient ultrafine particles have been performed. Although ultrafine particles have been used in animal inhalation studies, the studies did not focus on two potentially important aspects of ultrafine particles which are addressed in this chapter; their presence in the exposure atmosphere as single particles rather than aggregates and their low solubility. Single ultrafine particles occur regularly in the urban atmosphere at high number concentrations (5×10^4 - 3×10^5 particles/cm^3) but very low mass concentrations (Brand et al., 1991; 1992; Castellani, 1993). These single ultrafine particles are not very stable and eventually aggregate with larger particles but they are always freshly-generated by a number of natural anthropogenic sources (e.g., gas to particle conversion; combustion processes; incinerator emissions). Because results of studies with relevant ambient ultrafine particles at relevant low mass concentrations (10 to 50 μg/m^3) are not available in the literature, effects of single ultrafine particles generated as polymer fumes are discussed in this section. Obviously, polymer fume particles do not occur in the ambient atmosphere and they serve only as a surrogate to indicate the toxic potential that some inhaled ultrafine particles may have. The hypothesis that other ultrafine particles have this toxic potential as well needs still to be tested but cannot be refuted at this time since studies with ultrafine copper oxide particles described in this section also indicate their potential to cause acute effects. Human exposure to very fine acid aerosols ($\approx$100 nm; 1,500 μg/m^3) have also been conducted (Horvath et al., 1987). No pulmonary function or symptom responses were observed suggesting that the soluble nature of these particles or their tendency to either grow or aggregate may be responsible for the fact that they did not induce responses similar to other (less soluble) ultrafine particles.

Inhalation studies in rats with aggregated ultrafine particles have shown that these particles still required high concentrations (in the mg/m^3 range) and repeated exposures to produce effects in laboratory animals, although they were more active than larger-sized particles of the same composition. These particles included ultrafine TiO_2 aggregates (Ferin et al., 1992; Oberdörster et al., 1992; Heinrich, 1994), aggregated carbon black particles (Heinrich, et al., 1995; Mauderly et al., 1994a; Nikula et al., 1995), and diesel soot (White and Garg, 1981; Rudell et al., 1990). Effects observed after subchronic or chronic exposure

of rats included chronic pulmonary inflammation, pulmonary fibrosis, and induction of lung tumors. No acute effects were observed, even at the highest exposure concentrations. Although the studies with TiO_2 and carbon black involved particles of ultrafine size (~20 nm), they were inhaled as aggregates which are considerably larger than single 20 nm ultrafine particles. Thus, these results may not fully reflect the toxicity of single 20 nm particles.

From these studies with aggregate ultrafine particles, it appeared that particle surface area is an important parameter for expressing exposure-response and dose-response relationships of inhaled highly insoluble particles. The significantly increased pulmonary inflammatory response of aggregated ultrafine particles is presumably because of their highly increased surface area. If the dose for particles of different sizes is expressed relative to their surface area, then responses elicited by ultrafines would be comparable with those for larger-sized particles (Oberdörster et al., 1992, 1994b). The finding that ultrafine particles can penetrate into the interstitium more easily than larger-sized particles (Takenaka et al., 1986; Ferin et al., 1992) is also very important. Transport across the epithelium appears to be facilitated if ultrafine aggregates deaggregate upon deposition and are present as single particles.

As stated above, acute pulmonary effects were not observed after inhalation of aggregates of ultrafine particles. In contrast, specific types of inhaled single ultrafine particles described below can induce severe acute lung injury at low inhaled mass concentrations relative to aggregated ultrafine particles (Oberdörster, 1995). Such model ultrafine particles can be generated by heating of polytetrafluoroethylene (Teflon®; PTFE); the resulting condensation aerosol consists of single ultrafine particles. More than 25 years ago it was recognized that the toxicity of pyrolysis products of PTFE is associated with the particulate phase rather than with gas phase constituents (Waritz and Kwon, 1968). It was demonstrated more recently that these particles are of ultrafine size (Lee and Seidel, 1991a,b; Seidel et al., 1991). These particles form upon heating of Teflon® to a critical temperature of $\approx$420 to 450 °C and have diameters from <10 - 60 nm (median diameter of $\approx$26 nm) (Oberdörster et al., 1995a). The toxicity of PTFE fumes has been recognized dating back to the 1950's, when exposures of rabbits, guinea pigs, rats, mice, cats, and dogs resulted in acute mortality (Treon et al., 1955). Further studies in experimental animals by several

investigators (Scheel et al., 1968; Coleman et al., 1968; Griffith et al., 1973; Lee et al., 1976; Alarie and Anderson, 1981) confirmed that these fumes are highly toxic to birds and mammals. Extensive pulmonary epithelial and interstitial damage and alveolar flooding occurred after only short-durations of exposure. Accidental exposures of humans to fumes generated from polymers also demonstrated the high toxicity of these fumes for humans (Nuttall et al., 1964; Goldstein et al., 1987; Dahlqvist et al., 1992). Associated effects include pulmonary edema, nausea and headaches, together characterized by the term "polymer fume fever" in analogy to the well-known symptoms of metal fume fever (Rose, 1992).

The toxicity of polymer fumes was initially thought to be associated with toxic gas phase products, such as hydrogen fluoride (HF), carbonyl fluoride, and perfluoroisobutylene (PFIB). However, detailed studies by Waritz and Kwon (1968) as well as more recent studies have shown that the high toxicity is associated with the particulate phase. For example, HF studies showed that concentrations as high as 1300 ppm are needed to cause effects in the upper respiratory tract of exposed rats; effects did not occur in the lung periphery where the fume particles have been shown to be most effective (Stavert et al., 1991). Concentrations of HF in fumes generated at the critical temperature are only $\approx$10 ppm, and therefore, cannot be responsible for the observed toxicity of the fumes (Oberdörster et al., 1995a). The more toxic gas phase compounds, carbonyl fluoride and PFIB are generated only at temperatures approaching 500°C when heating PTFE (Coleman et al., 1968; Waritz and Kwon, 1968). Furthermore, rat inhalation studies with PFIB alone showed that lung pathology was detected only when a high concentration of 90,000 $\mu g/m^3$ was exceeded (Lehnert et al., 1993). Further proof that the particles of polymer fumes represent the toxic entity is provided by studies in which the particulate phase was removed by filters and subsequently the gas phase compounds did not show toxicity in exposed rats (Waritz and Kwon, 1968; Warheit et al., 1990; Lee and Seidel, 1991a).

It has also been suggested that highly toxic radicals on the surface of the polymer fume particles may cause the acute effects. However, studies by Seidel et al. (1991) with fumes from different polymers showed similar toxicities to the lung regardless as to whether significant amounts of radicals could be detected on those particles or not. Although this still does not exclude that some reactive toxic compounds may be attached to the particle surface,

all of these studies provide strong evidence that the ultrafine particles are the cause of the PTFE fume-associated, acute lung injury. It has also been shown that aging of the fumes leading to particle aggregation diminishes their toxicity, indicating that the presence of ultrafine particles as singlets is highly important for the toxicity of these particles (Lee and Seidel, 1991b; Warheit et al., 1990).

To exclude the possibility that oxygen-derived radicals from the generation process may be responsible for the observed pulmonary toxicity, PTFE particles were generated in a nitrogen atmosphere (Waritz and Kwon, 1968) or in an argon gas atmosphere (Oberdörster et al., 1995b). Results showed that the inhaled PTFE fumes generated in this way showed the same high pulmonary toxicity in rats that was observed with PTFE fumes generated in air. The toxicity consisted of severe hemorrhagic, pulmonary edema and influx of PMNs into the alveolar space within 4 h after a 15-min exposure of healthy rats to an ultrafine particle mass concentration of about 40 to 50 $\mu g/m^3$; this was accompanied by high mortality (Oberdörster et al., 1995a; Johnston et al., 1995). It was also determined by these investigators that a number concentration of 1×10^5 PTFE particles/cm^3 is equivalent to a mass concentration of ≈ 10 $\mu g/m^3$. Pulmonary lavage data showed that up to 80% of lavageable cells consisted of PMNs. Acute mortality was also observed in up to 50% of rats exposed to these concentrations of 5×10^5 particles/cm^3. Epithelial as well as endothelial cell damage occurred, resulting in both interstitial and alveolar edema. The authors concluded that freshly-generated ultrafine PTFE particles inhaled as singlets at low mass concentrations can cause severe acute lung injury and that ultrafine particles, in general, penetrate readily through epithelial-endothelial barriers.

Additional results from studies with ultrafine PTFE particles directed at evaluating mechanistic events in the lung by using in situ hybridization techniques on lung tissue showed that the highly inflammatory reaction was characterized by significant increases in message for the pro-inflammatory cytokine TNFα and the low molecular weight protein metallothionein (Johnston et al., 1995). Furthermore, increases in abundance for messages encoding IL-1α, IL-1β, IL-6, TNFα and the antioxidants MnSOD and metallothionein were found in RNA extracted from lung tissues. In addition to the increase in message of these pro-inflammatory cytokines and antioxidants, abundance for message of inducible NOS was also increased, whereas message for VEGF (vascular endothelial growth factor) was

decreased in the acute phase (Johnston et al., 1995). The authors suggested that the acute lung damage affecting epithelial and endothelial barrier functions may be due to the activities of reactive oxygen species originating from activated inflammatory cells and reactive nitrogen species produced via inducible NOS.

In another effort to evaluate acute effects and disposition of inhaled ultrafine particles Stearns et al. (1994) exposed hamsters for 60 minutes to ultrafine CuO, Cu_2O and $Cu(OH)_2$ particles (11 nm diameter, $\sigma_g = 1.8$; approximately 10^9 particles/cm^3). A marked 4-fold increase in pulmonary resistance was found which persisted for 24 hours. Immediately after exposure, using electron spectroscopic imaging, copper oxide particles were found not only on and within airway mucus and extracellular alveolar lining layers but also in airway and alveolar epithelial cells, in the pulmonary interstitium and in alveolar macrophages. These particles were even found in the alveolar capillaries and in pulmonary lymphatics. In addition, animals at 24 hours post-exposure showed evidence of a pulmonary inflammatory response, including the appearance of neutrophils and eosinophils.

Roth et al. (1994) demonstrated in human subjects that clearance of ultrafine particles is delayed. These workers exposed three male subjects to ultrafine particles (18 nm CMD; 27 nm MMD) of ^{111}In-labeled indium oxide for two or three breathing cycles and measured radioactivity present in the head, chest, and stomach immediately after inhalation and for 4 to 8 days at ensuing intervals. The clearance curves showed a fast clearance for particles deposited in the thorax with a mean value of 7% and a slow clearance fraction with a mean value of 93%. The half-life of the slow phase appeared to be on the order of 40 days, indicating greater persistence of the ultrafine particles rather than the larger particles (>2 μm) in the lung.

Hatch et al. (1994) evaluated to what extent ultrafine particles (<100 nm) are present in ambient air by determining their presence in alveolar macrophages of healthy people. Alveolar macrophages isolated from lung lavage samples of 7 workers of an oil-fired power plant, 4 welders of the power plant and 3 university employees (no known occupational or environmental exposures) were studied by electron energy loss spectroscopy and electron spectroscopic imaging. Regardless of the occupation, ultrafine particles were observed in phagolysosomes of macrophages of all volunteers, there was no correlation of ultrafine particle quantity with occupation. Spectral analysis of the ultrafine particles revealed a

variety of metals including cadmium, vanadium, titanium and iron. This study demonstrates the presence of large numbers of ultrafine particles in alveolar macrophages of healthy people even in the absence of specific occupational exposure. Whether all of these particles have been inhaled as ultrafines or whether some of them dissolved in the macrophages from larger particles to the ultrafine size is not known. However, since ultrafine particles occur in the ambient air (Chapter 6) their presence in large numbers in alveolar macrophages of people demonstrates that they are effectively deposited in the deep lung, although some of them may have been inhaled as particles adsorbed to larger particles as suggested by the authors. The high deposition efficiency of inhaled single ultrafine particles in the alveolar region (Chapter 10) contributes to the plausibility of the suggestion by Hatch et al. (1994) that many of these particles were inhaled as ultrafines.

In summary, certain freshly-generated ultrafine particles, when inhaled as singlets at very low mass concentrations (<50 $\mu g/m^3$), can be highly toxic to the lung. After inhalation and deposition in the lung, ultrafine particles of low solubility can rapidly penetrate epithelial cell barriers and penetrate to interstitial and endothelial sites (Stearns et al., 1994). Obviously, ultrafine particles studied in experimental animals so far (PTFE-fume, copper oxides) are not constituents of the ambient atmosphere and it is not clear how well these particles might serve as surrogates for ambient ultrafines.

Mechanisms responsible for a potential high toxicity could include: (1) high pulmonary deposition efficiencies of inhaled single ultrafine particles; (2) the large numbers per unit mass of these particles; (3) their increased surface area available for reaction; (4) their rapid penetration of epithelial layers and access of pulmonary interstitial sites; and (5) the presence of radicals and perhaps acids on the particle surface depending on the process of generation of the particles. Results of studies with model ultrafine particles indicate that particle number or total particle surface area could be more important than mass concentration (see Table 11-1).

11.5 DIESEL EXHAUST EMISSIONS

Diesel engines emit both gas phase pollutants (hydrocarbons, oxides of nitrogen, and carbon monoxide) and carbonaceous PM into the ambient atmosphere. The concentration of diesel particulate in the ambient atmosphere although low is ubiquitous. The concentration of

diesel particulate in the ambient atmosphere has been estimated to be about 1-6 $\mu g/m^3$ in Los Angeles (Health Effects Institute, 1995). A description of the diesel engine, its combustion system, pollutant formation mechanisms and emission factors as well as the cancer and noncancer health effects of diesel exhaust emissions have been recently reviewed elsewhere in the Health Assessment Document for Diesel Emissions (U.S. Environmental Protection Agency, 1994) and in Diesel Exhaust: A Critical Analysis of Emissions, Exposure and Health Effects (Health Effects Institute, 1995). The endpoints discussed in this section are those associated with diesel particulate and directly related to the epidemiological results discussed in Chapter 12. Other components of diesel exhaust, such as sulfur dioxide (SO_2), nitrogen dioxide (NO_2), formaldehyde, acrolein, and sulfuric acid may contribute to some of these potential health effects. Endpoints not directly related to the epidemiological findings are not included in the discussion but are presented elsewhere (International Agency for Research on Cancer, 1989; Claxton, 1983; Lewtas, 1982; Ishinishi et al., 1986; Pepelko and Peirano, 1983; Pepelko et al., 1980b,c; U.S. Environmental Protection Agency, 1994; Health Effects Institute, 1995).

Within the text, exposures are expressed in terms of the mass concentration of diesel particles. Other major measured components in the studies are presented in the tables which have additional details about the studies, including references. The Health Assessment Document for Diesel Emissions (U.S. Environmental Protection Agency, 1994) that is in preparation and the Diesel Exhaust Document (Health Effects Institute, 1995) should be consulted for a complete evaluation of the health effects associated with diesel emissions.

11.5.1 Effects of Diesel Exhaust on Humans

It is difficult to study the health effects of diesel exhaust in the general population because diesel emissions are diluted in the ambient air; hence, exposure is very low. Thus, populations occupationally exposed to diesel exhaust are studied to determine the potential health effects in humans. The occupations involving potential high exposure to diesel exhaust are miners, truck drivers, transportation works, railroad workers, and heavy-equipment operators. All the occupational studies considered in this section have a common problem—an inability to measure accurately the actual exposure to diesel exhaust.

The effects of short term exposure to diesel exhaust have been investigated primarily in occupationally-exposed workers (Table 11-11). Symptoms of acute exposure to high levels of diesel exhaust include mucous membrane, eye, and respiratory tract irritation (including chest tightness and wheezing) and neuropsychological effects of headache, lightheadedness, nausea, heartburn, vomiting, weakness, and numbness and tingling in the extremities. Diesel exhaust odor can cause nausea, headache, and loss of appetite.

There have been a few experimental exposures of humans to diesel exhaust, but all were single exposures. No significant changes in respiratory function were found in subjects exposed for 1 (Battigelli 1965) or 3.7 (Ulfvarsson et al., 1987) hours to diesel exhaust at approximately 1,000 μg soot/m^3 or less.

Rudell et al. (1990, 1994) exposed eight healthy subjects in an exposure chamber to diluted exhaust from a diesel engine for one hour, with intermittent exercise. Dilution of the diesel exhaust was controlled to provide a median NO_2 level of approximately 1.6 ppm. Median particle number was $4.3 \times 10^6/cm^3$, and median levels of NO and CO were 3.7 and 27 ppm, respectively (particle size and mass concentration were not provided). There were no effects on spirometry or on closing volume using nitrogen washout. Five of eight subjects experienced unpleasant smell, eye irritation, and nasal irritation during exposure. Bronchoalveolar lavage was performed 18 hours after exposure and was compared with a control BAL performed 3 weeks prior to exposure. There was no control air exposure. Small but statistically significant reductions were seen in BAL mast cells, AM phagocytosis of opsonized yeast particles, and lymphocyte CD4/CD8 ratios. A small increase in recovery of PMNs was also observed. These findings suggest that diesel exhaust may induce mild airway inflammation in the absence of spirometric changes.

In underground miners, bus garage workers, dock workers, and locomotive repairmen exposed to diesel exhaust, minimal and not statistically significant changes were reported in respiratory symptoms and pulmonary function over the course of a workshift. In diesel bus garage workers, there was an increased reporting of burning and watering of the eyes, cough, labored breathing, chest tightness, and wheezing, but no reductions in pulmonary function associated with exposure to diesel exhaust. In stevedores pulmonary function was adversely affected over a workshift exposure to diesel exhaust but normalized after a few days without exposure.

TABLE 11-11. HUMAN STUDIES OF DIESEL EXHAUST EXPOSURE

Study	Description	Findings
Kahn et al. (1988)	13 Cases of acute exposure, Utah and Colorado coal miners.	Acute reversible sensory irritation, headache; nervous system effects, bronchoconstriction were reported at unknown exposures.
El Batawi and Noweir (1966)	161 Workers, two diesel bus garages.	Eye irritation (42%), headache (37%), dizziness (30%), throat irritation (19%), and cough and phlegm (11%) were reported in this order of incidence by workers exposed in the service and repair of diesel powered buses.
Battigelli (1965)	Six subjects, eye exposure chamber, three dilutions.	Time to onset was inversely related and severity of eye irritation was associated with the level of exposure to diesel exhaust.
Katz et al. (1960)	14 Persons monitoring diesel exhaust in a train tunnel.	Three occasions of minor eye and throat irritation; no correlation established with concentrations of diesel exhaust components.
Hare and Springer (1971) Hare et al. (1974)	Volunteer panelists who evaluated general public's response to odor of diesel exhaust.	Slight odor intensity, 90% perceived, 60% objected; slight to moderate odor intensity, 95% perceived, 75% objected; almost 75% objected; almost 95% objected.
Linnell and Scott (1962)	Odor panel under highly controlled conditions determined odor threshold for diesel exhaust.	In six panelists, the volume of air required to dilute raw diesel exhaust to an odor threshold ranged from a factor of 140 to 475.
Battigelli (1965)	13 Volunteers exposed to three dilutions of diesel exhaust for 15 min to 1 h.	No significant effects on pulmonary resistance were observed as measured by plethysmography.
Reger (1979)	Five or more VC maneuvers by each of 60 coal miners exposed to diesel exhaust at the beginning and end of a work shift.	FEV_1, FVC, and PEFR were similar between diesel and non-diesel-exposed miners. Smokers had an increased number of decrements over shift than nonsmokers.
Ames et al. (1982)	Pulmonary function of 60 diesel-exposed compared with 90 non-diesel-exposed coal miners over work shift.	Significant work shift decrements occurred in miners in both groups who smoked; no significant differences in ventilatory function changes between miners exposed to diesel exhaust and those not exposed.
Jorgensen and Svensson (1970)	240 Iron ore miners matched for diesel exposure, smoking and age were given bronchitis questionnaires and spirometry pre- and postwork shift.	Among underground (surrogate for diesel exposure) miners, smokers and older age groups, frequency of bronchitis was higher. Pulmonary function was similar between groups and subgroups except for differences accountable to age.

TABLE 11-11 (cont'd). HUMAN STUDIES OF DIESEL EXHAUST EXPOSURE

Study	Description	Findings
Gamble et al. (1979)	200 Salt miners performed before and after workshift spirometry. Personal environmental NO_2 and inhalable particle samples were collected.	Smokers had greater but not significant reductions in spirometry than ex- or nonsmokers. NO_2, but not particulate, levels significantly decreased FEV1, FEF_{25}, FEF_{50}, and FEF_{75} over the workshift.
Gamble et al. (1987a)	232 Workers in four diesel bus garages were administered acute respiratory questionnaires and before and after workshift spirometry. Compared to lead, acid battery workers previously found to be unaffected by their exposures.	Prevalence of burning eyes, headache, difficult or labored breathing, nausea, and wheeze were higher in diesel bus workers than in comparison population.
Ulfvarson et al. (1987)	Workshift changes in pulmonary function were evaluated in crews of roll-on/ roll-off ships and car ferries and bus garage staff. Pulmonary function was evaluated in six volunteers exposed to diluted diesel exhaust, 2.1 ppm NO_2, and 600 $\mu g/m^3$ particulate matter.	Pulmonary function was affected during a workshift exposure to diesel exhaust, but it normalized after a few days with no exposure. Decrements were greater with increasing intervals between exposures. No effect on pulmonary function was observed in the experimental exposure study.
Battigelli et al. (1964)	210 Locomotive repairmen exposed to diesel exhaust for an average of 9.6 years in railroad engine houses were compared with 154 railroad yard workers of comparable job status but no exposure to diesel exhaust.	No significant differences in VC, FEV_1, peak flow, nitrogen washout, or diffusion capacity nor in the prevalence of dyspnea, cough, or sputum were found between the diesel exhaust-exposed and nonexposed groups.
Gamble et al. (1987b)	283 Male diesel bus garage workers from four garages in two cities were examined for impaired pulmonary function (FVC, FEV_1, and flow rates). Study population with a mean tenure of 9 ± 10 years S.D. was compared to a nonexposed "blue collar" population.	Analyses within the study populations population showed no association of respiratory symptoms with tenure. Reduced FEV_1 and FEF_{50} (but not FEF_{75}) were associated with increasing tenure. The study population had a higher incidence of cough, phlegm, and wheezing unrelated to tenure. Pulmonary function was not affected in the total cohort of diesel-exposed of diesel-exposed but was reduced with 10 or more years of tenure.

TABLE 11-11 (cont'd). HUMAN STUDIES OF DIESEL EXHAUST EXPOSURE

Study	Description	Findings
Purdham et al. (1987)	**Respiratory symptoms and pulmonary function were evaluated in 17 stevedores exposed to both diesel and gasoline exhausts in car ferry operations; control group was 11 on-site office workers.**	**No differences between the two groups for respiratory symptoms. Stevedores had lower baseline lung function consistent with an obstructive ventilatory defect compared with controls and those of Sydney, Nova Scotia, residents. Caution in interpretation is warranted due to small sample size. No significant changes in lung function over workshift nor difference between two groups.**
Reger et al. (1982)	**Differences in respiratory symptoms and pulmonary function were assessed in 823 coal miners from six diesel equipped mines compared to 823 matched coal miners not exposed to diesel exhaust.**	**Underground miners in diesel-use mines reported more symptoms of cough and phlegm and had lower pulmonary function. Similar trends were noted for surface workers at diesel-use mines. Pattern was consistent with small airway disease but factors other than exposure to diesel exhaust thought to be responsible.**
Ames et al. (1984)	**Changes in respiratory symptoms and function were measured during a 5-year period in 280 diesel-exposed and 838 nonexposed U.S. underground coal miners.**	**No decrements in pulmonary function or increased prevalence of respiratory symptoms were found attributable to diesel exhaust. In fact, 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust than in miners exposed to diesel exhaust.**
Attfield (1978)	**Respiratory symptoms and function were assessed in 2,659 miners from 21 underground metal mines (1,709 miners) and nonmetal mines (950 miners). Years of diesel usage in the mines were surrogate for exposure to diesel exhaust.**	**Questionnaire found an association between an increased prevalence of cough and aldehyde exposure; this finding was not substantiated by spirometry data. No adverse symptoms or pulmonary function decrements were related to exposure to NO_2, CO, CO_2, dust, or quartz.**
Attfield et al. (1982)	**Respiratory symptoms and function were assessed in 630 potash miners from six potash mines using a questionnaire, chest radiographs and spirometry. A thorough assessment of the environment of each mine was made concurrently.**	**No obvious association indicative of diesel exposure was found between health indices, dust exposure, and pollutants. A higher prevalence of cough and phlegm, but no differences in FVC and FEV_1, were found in these diesel-exposed potash workers when compared to predicted values from a logistic model based on blue- collar staff working in nondusty jobs.**

TABLE 11-11 (cont'd). HUMAN STUDIES OF DIESEL EXHAUST EXPOSURE

Study	Description	Findings
Gamble et al. (1983)	Respiratory morbidity was assessed in 259 miners in 5 salt mines by respiratory symptoms, radiographic findings and spirometry. Two mines used diesels extensively, 2 had limited use, one used no diesels in 1956, 1957, 1963, or 1963 through 1967. Several working populations were compared to the salt mine cohort.	After adjustment for age and smoking, salt miners showed no symptoms, increased prevalence of cough, phlegm, dyspnea or air obstruction (FEV_1/FVC) compared to aboveground coal miners, potash workers or blue collar workers. FEV_1, FVC, FEF_{50}, and FEF_{75} were uniformly lower for salt miners in comparison to all the comparison populations. No changes in pulmonary function were associated with years of exposure or cumulative exposure to inhalable particles or NO_2.
Gamble and Jones (1983)	Same as above. Salt miners were grouped into low, intermediate and high exposure categories based on tenure in jobs with diesel exposure.	A statistically significant dose-related association of phlegm and diesel exposure was noted. Changes in pulmonary function showed no association with diesel tenure. Age- and smoking-adjusted rates of cough, phlegm, and dyspnea were 145, 169, and 93% of an external comparison population. Predicted pulmonary function indices showed small but significant reductions; there was no dose-response relationship.
Edling and Axelson (1984)	Pilot study of 129 bus company employees classified into three diesel exhaust exposure categories clerks (0), bus drivers (1), and bus garage workers.	The most heavily exposed group (bus garage workers) had a fourfold increase in risk of dying from cardiovascular disease, even after correction for smoking and allowing for 10 years of exposure and 15 years or more of induction latency time.
Edling et al. (1987)	Cohort of 694 male bus garage employees followed from 1951 through 1983 were evaluated for mortality from cardiovascular disease. Subcohorts categorized by levels of exposure were clerks (0), bus drivers (1), and bus garage employees (2).	No increased mortality from cardiovascular disease was found among the members of these five bus companies when compared with the general population or grouped as sub-cohorts with different levels of exposure.
Rudell et al. (1989, 1990, 1994)	Eight healthy non-smoking subjects exposed for 60 min in chamber to diesel exhaust (3.7 ppm NO, 1.5 ppm NO_2, 27 ppm CO, 0.5 mg/m^3 formaldehyde, particles 4.3×10^6/cm^3). Exercise, 10 of each 20 min (75 W).	Odor, eye and nasal irritation in 5/8 subjects. BAL findings small decrease in mast cells, lymphocyte subsets and macrophage phagocytosis, small increase in PMNs.

The chronic effects of exposure to diesel exhaust have been evaluated in humans in epidemiologic studies of occupationally exposed workers. Most of the epidemiologic data indicate the absence of an excess of chronic respiratory disease associated with exposure to diesel exhaust. In a few of these studies, a higher prevalence of respiratory symptoms, primarily cough, phlegm, or dyspnea was observed in the exposed workers. Reductions in several pulmonary function parameters including FVC and FEV_1, and to a lesser extent forced expiratory flow at 50 and 75% of vital capacity (FEF_{50} and FEF_{75}), have also been reported. Two studies (Reger et al., 1982; Purdham et al., 1987), each with methodological problems, detected statistically significant decrements in pulmonary function when compared with matched controls. These two studies coupled with other reported nonsignificant trends in respiratory flow-volume measurements suggest that diesel exhaust exposure may impair pulmonary function among occupational populations. A preliminary study of the association of cardiovascular mortality and exposure to diesel exhaust found a risk ratio of 4.0. A more comprehensive study by the same investigators, however, found no significant difference between the observed and expected number of deaths due to cardiovascular disease.

The results of the epidemiologic studies addressing noncarcinogenic health effects resulting from exposure to diesel exhaust must be interpreted cautiously because of a myriad of methodological problems, including incomplete information on the extent of exposure to diesel exhaust, the presence of confounding variables (smoking, occupational exposures to other toxic substances), and the short duration and low intensity of exposure. These limitations restrict definitive conclusions about diesel exhaust being the cause of any noncarcinogenic health effects, observed or reported.

11.5.2 Effects of Diesel Exhaust on Laboratory Animals

In short-term and chronic exposure studies, toxic effects have been related to high concentrations of diesel particulate matter. Data from short-term exposures indicate minimal effects on pulmonary function, even though histological and cytological changes were observed in the lungs (Table 11-12). Exposures for several months or longer to levels markedly above environmental ambient concentrations resulted in accumulation of particles in the lungs, increases in lung weight, increases in AMs and leukocytes, macrophage aggregation, hyperplasia of alveolar epithelium, and thickening of the alveolar septa. Similar

TABLE 11-12. SHORT-TERM EFFECTS OF DIESEL EXHAUST ON LABORATORY ANIMALS

Species/Sex	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rat, F-344, M; Mouse, A/J; Hamster, Syrian	20 h/day 7 days/week 10-13 weeks	1,500 0.19 μm, MMD	2,100,000 to 2,730,000	6.9	0.49	—	Increase in lung wt; increase in thickness of alveolar walls; no species difference	Kaplan et al. (1982)
Rat, F-344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/week 19 weeks	210 1,000 4,400	140,000 665,000 2,926,000	— — —	— — —	— — —	No effects on lung function; increase in PMNs and proteases and AM aggregation in both species	Mauderly et al. (1981)
Cat, Inbred, M	20 h/day 7 days/week 4 weeks	6,400	3,584,000	14.6	2.1	2.1	Few effects on lung function; focal pneumonitis or alveolitis	Pepelko et al. (1980d)
Rat, Sprague-Dawley, M	20 h/day 7 days/week 4 weeks	6,400 6,800[a]	3,584,000 3,808,000	16.9 16.1[a]	2.49 2.76[a]	2.10 1.86[a]	Decreased body wt; arterial blood pH reduced; both vital and total lung capacities increased	Pepelko (1982a)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 4 weeks	6,800[a]	3,808,000	16.7	2.9	(<0.01 ppm O_3)[a]	Exposure started when animals were 4 days old; increase in pulmonary flow; bradycardia	Wiester et al. (1980)
Rat, F-344, M	20 h/day 5.5 days/week 4 weeks	6,000 6.8 μm, MMD	2,640,000	—	—	—	Macrophage aggregation; increase in PMNs; Type 2 cell proliferation; thickened alveolar walls	White and Garg (1981)
Guinea Pig, Hartley M, F	20 h/day 7 days/week 8 weeks	6,300	7,056,000	17.4	2.3	(<0.01 ppm O_3)[a]	Increase in relative lung wt; AM aggregation; hypertrophy of goblet cells; focal hyperplasia of alveolar epithelium	Weister et al. (1980)

[a]Irradiated exhaust.
PMN = Polymorphonuclear leukocyte.
AM = Alveolar macrophage.

Source: quoted from U.S. Environmental Protection Agency (1994).

histological changes, as well as reductions in growth rates and alterations in indices of pulmonary function, have been observed in chronic exposure studies. Chronic studies have been carried out using rats, mice, guinea pigs, hamsters, cats, and monkeys. Reduced resistance to respiratory tract infections has been reported in mice exposed to diesel exhaust.

Reduced growth rates have been observed most often in studies with exposures of at least 2,000 $\mu g/m^3$ diesel particulate matter which lasted for 16 h or more per day (Table 11-13). No effects on growth or survival were noted at levels of 6,000 to 8,000 $\mu g/m^3$ of PM when the daily exposures were only 6 to 8 h/day.

Changes in pulmonary function have been noted in a number of different species chronically exposed to diesel exhaust (Table 11-14). The lowest exposure levels that resulted in impaired pulmonary function varied among the species tested but were in excess of 1,000 $\mu g/m^3$.

Histological changes occurring in the respiratory tract tissue of animal exposed chronically to high concentrations of diesel exhaust include alveolar histiocytosis, macrophage aggregation, tissue inflammation, increases in polymorphonuclear leukocytes, hyperplasia of bronchiolar and alveolar Type 2 cells, thickened alveolar septa, edema, fibrosis, and emphysema (Table 11-15). Biochemical changes in the lung associated with these histopathological findings included increases in lung DNA, total protein, and activities of alkaline and acid phosphatase, and glucose-6-phosphate dehydrogenase; increased synthesis of collagen; and release of inflammatory mediators such as leukotriene LTB and prostaglandin $PGF_{2\alpha}$. Some studies have also suggested that there may be a threshold of exposure to diesel exhaust below which pathologic changes do not occur. These no-effect levels were reported to be 2,000 $\mu g/m^3$ for cynomolgus monkeys, 110 to 350 $\mu g/m^3$ for rats, and 250 $\mu g/m^3$ PM for guinea pigs exposed for 7 to 20 h/day, 5 to 5.5 days/week for 104 to 130 weeks.

The pathological effects of diesel exhaust particulate matter appear to be strongly dependent on the relative rates of pulmonary deposition and clearance (Table 11-16). At particle concentrations of about 1,000 $\mu g/m^3$ or above, pulmonary clearance becomes reduced, with concomitant focal aggregations of particle-laden AMs. The principal mechanism of reduced particle clearance appears to be the result of impaired AM function. This impairment seems to be nonspecific and applies to insoluble particles deposited in the

TABLE 11-13. EFFECTS OF CHRONIC EXPOSURES TO DIESEL EXHAUST ON SURVIVAL AND GROWTH OF LABORATORY ANIMALS

Species/Sex	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rat, F-344, M, F; Monkey, cynomolgus, M	7 h/day 5 days/week 104 weeks	2,000 0.23–0.36 µm, MMD	7,280,000	11.5	1.5	0.8	No effects on growth or survival	Lewis et al. (1989)
Rat, F344, M; Guinea Pig, Hartley, M	20 h/day 5 days/week 106 weeks	250 750 1,500 0.19 µm, MMD	2,650,000 7,950,000 15,900,000	2.7[a] 4.4[a] 7.1[a]	0.1[b] 0.27[b] 0.5[b]	— — —	Reduced body weight in rats at 1,500 $\mu g/m^3$	Schreck et al. (1981)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6,000 12,000	8,736,000 17,472,000	— —	— —	— —	No effect on growth	Vinegar et al. (1981a,b)
Rat, Wistar, M	6 h/day 5 days/week 87 weeks	8,300 0.71 µm, MMD	21,663,000	50.0	4.0–6.0	—	No effect on growth or mortality rates	Karagianes et al. (1981)
Rat, F-344, M, F; Mouse CD-1	7 h/day 5 days/week 130 weeks	350 3,500 7,000 0.25 µm, MMD	1,592,000 15,925,000 31,850,000	2.9 16.5 29.7	0.05 0.34 0.68	— — —	No effect on growth or mortality rates	Mauderly et al. (1984, 1987b)
Rat, Wistar, F; Mouse, MMRI, F	19 h/day 5 days/week 104 weeks	4,240 0.35 µm, MMD	41,891,0000	12.5	1.5	1.1	Reduced body wts; increased mortality in mice	Heinrich et al. (1986a)
Rat, F-344 M, F	16h/day 5 days/week 104 weeks	700 2,200 6,600	5,824,000 18,304,000 54,912,000	— — 32.0	— — —	— — —	Growth reduced at 2,200 and 6,600 $\mu g/m^3$	Brightwell et al. (1986)
Rat[c] F-344/Jcl.	16 h/day 6 days/week 130 weeks	110[d] 410[d] 1,080[d] 2,310[d] 3,720[e] 0.2–0.3 µm, MMD	1,373,000 5,117,000 13,478,000 28,829,000 46,426,000	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	Concentration-dependent decrease in body weight; earlier deaths in females exposed to 3,720 $\mu g/m^3$, stabilized by 15 mo	Research Committee for HERP Studies (1988)

[a]Estimated from graphically depicted mass concentration data.
[b]Estimated from graphically presented mass concentration data for NO_2 (assuming 90% NO and 10% NO_2).
[c]Data for tests with light-duty engine; similar results with heavy-duty engine.
[d]Light-duty engine.
[e]Heavy-duty engine.

Source: Quoted from U.S. Environmental Protection Agency (1994).

TABLE 11-14. EFFECTS OF DIESEL EXHAUST ON PULMONARY FUNCTION OF LABORATORY ANIMALS

Species/Sex	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rat, F-344 M, F	7 h/day 5 days/week 104 weeks	2,000 0.23–0.36 µm MMD	7,280,000	11.5	1.5	0.8	No effect on pulmonary function	Lewis et al. (1989)
Monkey, M Cynomolgus	7 h/day 5 days/week 104 weeks	2,000 0.23-0.36 µm, MMD	7,280,000	11.5	1.5	0.8	Decreased expiratory flow; no effect on vital or diffusing capabilities	Lewis et al. (1989)
Rat, F-344, M	20 h/day 5.5 days/week 87 weeks	1,500 0.19 µm, MMD	14,355,000	7.0	0.5	—	Increased functional residual capacity, expiratory volume and flow	Gross (1981)
Rat, Wistar, F	7-8 h/day 5 days/week 104 weeks	3,900 0.1 µm, MMD	14,196,000- 16,224,000	18.5	1.2	3.1	No effect on minute volume, compliance or resistance	Heinrich et al. (1982)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6,000 12,000	8,736,000 17,472,000	— —	— —	— —	Decrease in vital capacity, residual volume, and diffusing capacity; increase in static deflation lung volume	Vinegar et al. (1980, 1981a,b)
Rat, F-344, M, F	7 h/day 5 days/week 130 weeks	350 3,500 7,000 0.23–0.26 µm, MMD	1,593,000 15,925,000 31,850,000	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Diffusing capacity, lung compliance reduced at 3,500 and 7,000 $\mu g/m^3$	Mauderly et al. (1988) McClellan et al. (1986)
Hamster, Syrian M, F	19 h/day 5 days/week 120 weeks	4,240 0.35 µm, MMD	48,336,000	12.5	1.5	1.1	Significant increase in airway resistance	Heinrich et al. (1986a)
Rat, F-344; Hamster Syrian	16 h/day 5 days/week 104 weeks	700 2,200 6,600	5,824,000 18,304,000 54,912,000	— — —	— — —	— — —	Large number of pulmonary function changes consistent with obstructive and restrictive airway diseases at 6,600 $\mu g/m^3$ (no specific data provided)	Brightwell et al. (1986)
Rat, Wistar, F	19 h/day 5 days/week 140 weeks	4,240 0.35 µm, MMD	56,392,000	12.5	1.5	1.1	Decrease in dynamic lung compliance; increase in airway resistance	Heinrich et al. (1986a)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6,000[a] 12,000[b]	41,664,000 83,328,000	20.2 33.3	2.7 4.4	2.1 5.0	Decrease in vital capacity, total lung capacity, and diffusing capacity after 2 years; no effect on expiratory flow	Pepelko et al. (1980e, 1981) Moorman et al. (1985)

[a]1 to 61 weeks exposure.
[b]62 to 124 weeks of exposure.

Source: Quoted from U.S. Environmental Protection Agency (1994).

TABLE 11-15. HISTOPATHOLOGICAL EFFECTS OF DIESEL EXHAUST IN THE LUNGS OF LABORATORY ANIMALS

Species/Sex	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rat, F-344, M Mouse A/J, M; Hamster, Syrian, M	20 h/day 7 days/week 12-13 weeks	1,500 0.19 µm, MMD	2,520,000- 2,730,000	—	—	—	Inflammatory changes; increase in lung weight; increase in thickness of alveolar walls	Kaplan et al. (1982)
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2,000 0.23–0.36 µm, MMD	7,280,000	11.5	1.5	0.8	AM aggregation; no fibrosis, inflammation or emphysema	Lewis et al. (1989)
Rat, F-344, M, F	7 h/day 5 days/week 104 weeks	2,000 0.23–0.36 µm, MMD	3,640,000	11.5	1.5	0.8	Multifocal histiocytosis; inflammatory changes; Type II cell proliferation; fibrosis	Bhatnagar et al. (1980) Pepelko (1982a)
Rat, Sprague-Dawley, M; Mouse, A/HEJ, M	8 h/day 7 days/week 39 weeks	6,000	13,104,000	—	—	—	Increase in lung protein content and collagen synthesis but a decrease in overall lung protein synthesis in both species; prolyl-hydroxylase activity increased in rats in utero	Bhatnagar et al. (1980) Pepelko (1982a)
Hamster, chinese, M	8 h/day 5 days/week 26 weeks	6,000 12,000	6,240,000 12,480,000	— —	— —	— —	Inflammatory changes; AM accumulation; thickened alveolar lining; Type II cell hyperplasia; edema; increase in collagen	Pepelko (1982b)
Hamster, Syrian, M, F	7-8 h/day 5 days/week 120 weeks	3,900 0.1 µm, MMD	16,380,000- 18,720,000	18.5	1.2	3.1	Inflammatory changes, 60% adenomatous cell proliferation	Heinrich et al. (1982)
Rat, Wistar, M	6 h/day 5 days/week 87 weeks	8,300 0.71 µm, MMD	21,663,000	50.0	4.0-6.0	—	Inflammatory changes; AM aggregation; aleovar cell hypertrophy; interstitial fibrosis, emphysema (diagnostic metho-dology not described)	Karagianes et al. (1981)
Rat, F-344, F	8 h/day 7 days/week 104 weeks	4,900	28,538,000	7.0	1.8	13.1	Type II cell proliferation; Inflammatory changes; bronchial hyperplasia; fibrosis	Iwai et al. (1986)
Rat, F-344, M, F; Mouse CD-1, M, F	7 h/day 5 days/week 130 weeks	350 3,500 7,000 0.23 µm, MMD	1,592,000 15,925,000 31,850,000	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Alveolar and bronchiolar epithelial metaplasia in rats at 3,500 and 7,000 $\mu g/m^3$; fibrosis at 7,000 $\mu g/m^3$ in rats and mice; inflammatory changes	Mauderly et al. (1987a,b) Henderson et al. (1988)

TABLE 11-15 (cont'd). HISTOPATHOLOGICAL EFFECTS OF DIESEL EXHAUST IN THE LUNGS OF LABORATORY ANIMALS

Species/Sex	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rat, M, F, F-344/Jcl.	16 h/day 6 days/week 130 weeks	110[a] 410[a] 1,080[a] 2,310[a] 3,720[b]	1,373,000 5,117,000 13,478,000 28,829,000 46,336,000	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	Inflammatory changes; Type II cell hyperplasia and lung tumors seen at >400 $\mu g/m^3$; shortening and loss of cilia in trachea and bronchi	Research Committee for HERP Studies (1988)
Hamster, Syrian, M, F	19 h/day 5 days/week 120 weeks	4,240	48,336,000	12.5	1.5	1.1	Inflammatory changes; thickened alveolar septa; bronchioalveolar hyperplasia; emphysema (diagnostic methodology not described)	Heinrich et al. (1986a)
Mouse, NMRI, F	19 h/day 5 days/week 120 weeks	4,240	48,336,000	12.5	1.5	1.1	Inflammatory changes; bronchio-alevolar hyperplasia; alveolar lipo-proteinosis; fibrosis	Heinrich et al. (1986a)
Rat, Wistar, F	19 h/day 5 days/week 140 weeks	4,240	56,392,000	12.5	1.5	1.1	Thickened alveolar septa; AM aggregation; inflammatory changes; hyperplasia; lung tumors	Heinrich et al. (1986a)
Guinea Pig, Hartley, M	20 h/day 5.5 days/week 104 weeks	250 750 1,500 6,000	2,860,000 8,580,000 17,160,000 68,640,000	— — — —	— — — —	— — — —	Minimal response at 250 and ultrastructural changes at 750 $\mu g/m^3$; thickened alveolar membranes; cell proliferation; fibrosis at 6,000 $\mu g/m^3$; increase in PMN at 750 $\mu g/m^3$ and 1,500 $\mu g/m^3$	Barnhart et al. (1981, 1982) Vostal et al. (1981)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6,000[c] 12,000[d]	41,664,000 83,328,000	20.2 33.2	2.7 4.4	2.1 5.0	Inflammatory changes; AM aggregation; bronchiolar epithelial metaplasia; Type II cell hyperplasia; peri-bronchiolar fibrosis	Plopper et al. (1983) Hyde et al. (1985)
Rat, Wistar, F	18 h/day 5 days/week up to 24 mo	840 2,500 7,000	7,400,000 21,800,000 61,700,000	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	No effect on mortality. Reduced body wt., bronchioalveolar hyperplasia, and Inc. lung wt. at 2,500 and 7,000 ug/m^3 Alveolar clearance rates reduced in all groups at 3 mo. BAL showed clear exposure-related effects in all except lowest diesel exposure group	Heinrich et al. (1995)

TABLE 11-15 (cont'd). HISTOPATHOLOGICAL EFFECTS OF DIESEL EXHAUST IN THE LUNGS OF LABORATORY ANIMALS

Species/Sex	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rats, M, F F-344/N	16 h/day 5 day/week up to 24 mo	2,500 6,500	— —	10.3 26.9	0.73 3.78	— —	Higher mortality in males. Reduced body weight in males and females at 6,500 $\mu g/m^3$. Inc lung weight in males and females at 2,500 and 6,500 $\mu g/m^3$. Dose related increases in AM hyperplasia, alveolar epithelial hyperplasia, chronic active inflammation, septal fibrosis, alveolar proteinosis bronchioalveolar metaplasia, focal fibrosis with alveolar epithelial hyperplasia, squamous metaplasia, and squamous cysts	Nikula et al. (1995)
Mice NMRI/C5L F	18 h/day 5 days/week up to 24 mo	4,500	39,000,000	14.2	2.3	2.8	Reduced body weight, inc. lung weight.	Heinrich et al. (1995)

[a]Light-duty engine.
[b]Heavy-duty engine.
[c]1 to 61 weeks exposure.
[d]62 to 124 weeks of exposure.

AM = Alveolar macrophage.
PMN = Polymorphonuclear leukocyte.

Source: U.S. Environmental Protection Agency (1994).

TABLE 11-16. EFFECTS OF EXPOSURE TO DIESEL EXHAUST ON THE PULMONARY DEFENSE MECHANISMS OF LABORATORY ANIMALS

Species	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Reference
ALVEOLAR MACROPHAGE STATUS								
Guinea Pig, Hartley	20 h/day 5.5 days/week 8 weeks	250 1,500 0.19 µm, MMD	220,000 1,320,000	2.9 7.5	— —	— —	No significant changes in absolute numbers of AMs	Chen et. al. (1980)
Rat, F-344, M	7 h/day 5 days/week 104 weeks	2,000 0.23–0.36 µm MMD	7,280,000	11.5	1.5	0.81	Little effect on viability, cell number, oxygen consumption, membrane integrity, lyzomal enzyme activity, or protein content of AMs; decreased cell volume and ruffling of cell membrane and depressed luminescence of AM	Castranova et al. (1985)
Rat, F-344, M	20 h/day 5.5 days/week 26, 48, or 52 weeks	250[a] 750[a] 1,500[b] 0.19 µm, MMD	715,000- 8,580,000	2.9 4.8 7.5	— — —	— — —	AM cell counts proportional to concentration of DP at 750 and 1,500 $\mu g/m^3$; AM increased in lungs in response to rate of DP mass entering lung rather than toral DP burden in lung; increased PMNs were proportional to inhaled concentrations and/or duration of exposure; PMNs affiliated with clusters of aggregated AM rather than DP	Strom (1984) Vostal et al. (1982)
Rat F-344/Crl, M, F Mouse, CD, M,F	7 h/day 5 days/week 104 weeks (rat), 78 weeks (mouse)	350 3,500 7,000 0.25 µm, MMD	1,274,000[c] 12,740,000[c] 25,480,000[c]	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Significant increases of AM in rats and mice exposed to 7,000 $\mu g/m^3$ DP for 24 and 18 mo, respectively, but not at concentrations of 3,500 or 350 $\mu g/m^3$ DP for the same exposure durations; PMNs increased in a dose-dependent fashion in both rats and mice exposed to 3,500 or 7,000 $\mu g/m^3$ DP and were greater in mice than rats	Henderson et al. (1988)
CLEARANCE								
Rat	7 h/day 5 day/week 12 weeks	200 1,000 4,500 0.25µm, MMD	84,000 420,000 1,890,000	— — —	— — —	— — —	Evidence of apparent speeding of tracheal clearance at the 4,500 $\mu g/m^3$ level after 1 week of ^{99m}Tc macroaggregated-albumin and reduced clearance of tracer aerosol in each of the three exposure levels at 12 weeks; indication of a lower percentage of ciliated cells at the 1,000 and 4,500 $\mu g/m^3$ levels	Wolff and Gray (1980)
Rat, F-344 M, F	7 h/day 5 days/week 18 weeks	150 940 4,100 <0.5 µm, MMD	94,500 592,000 2,583,000	— — —	— — —	— — —	Lung burdens of DP were concentration-related; clearance half-time of DP almost double in 4,100 $\mu g/m^3$ group compared to 150 $\mu g/m^3$ group	Griffis et al. (1983)

TABLE 11-16 (cont'd). EFFECTS OF EXPOSURE TO DIESEL EXHAUST ON THE PULMONARY DEFENSE MECHANISMS OF LABORATORY ANIMALS

Species	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Reference
Rat, F-344, M	7 h/day 5 days/week 26-104 weeks	2,000 0.23-0.36 µm MMD	1,820,000- 7,280,000	11.5	1.5	0.8	No difference in clearance of $^{59}Fe_3O_4$ particles 1 day after tracer aerosol administration; 120 days after exposure tracer aerosol clearance was enhanced; Lung burden of DP increased significantly between 12 to 24 months of exposure	Lewis et al. (1989)
Rat, Sprague-Dawley	4-6 h/day 7 days/week 0.1 to 14.3 weeks	900 8,000 17,000	2,500- 10,210,000	— — —	5.0 2.7 8.0	0.2 0.6 1.0	Impairment of tracheal mucociliary clearance in a concentration-response manner	Battigelli et al. (1966)
Rat, F-344, M, F	7 h/day 5 days/week 130 weeks	350 3,500 7,000 0.25 µm, MMD	1,593,000 15,925,000 31,850,000	2.9 16.5 29.7	0.1 0.3 0.7	— — —	No changes in tracheal mucociliary clearance after 6, 12, 18, 24, or 30 mo of exposure; increases in lung clearance half-times as early as 6 mo at 7,000 $\mu g/m^3$ level and 18 mo at 3,500 $\mu g/m^3$ level; no changes seen at 350 $\mu g/m^3$ level; after 24 mo of diesel exposure, long-term clearance half-times were increased in the 3,500 and 7,000 $\mu g/m^3$ groups	Wolff et al. (1987)
MICROBIAL-INDUCED MORTALITY								
Mice, CD-1, F	—	—	—	—	—	—	No change in mortality in mice exposed intratracheally to 100 µg of DP prior to exposure to aerosolized *Streptococcus* sp.	Hatch et al. (1985)
Mice CD-1, F	7 h/day 5 days/week 4, 12, or 26 weeks	2,000 0.23–0.36 µm MMD	280,000- 1,820,000	11.5	1.5	0.8	Mortality similar at each exposure duration when challenged with Ao/PR/8/34 influenza virus; in mice exposed for 3 and 6 mo, but not 1 mo, there were increases in the percentages of mice having lung consolidation, higher virus growth, depressed interferon levels and a four-fold reduction in hemaglutin antibody levels	Hahon et al. (1985)

TABLE 11-16 (cont'd). EFFECTS OF EXPOSURE TO DIESEL EXHAUST ON THE PULMONARY DEFENSE MECHANISMS OF LABORATORY ANIMALS

Species	Exposure Period	Particles ($\mu g/m^3$)	C × T ($\mu g \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Reference
Mice, CR/CD-1, F	8 h/day 7 days/week 2 h up to 46 weeks	5,300 to 7,900	11,000- 20,350,000	19 to 22	1.8 to 3.6	0.9 to 2.8	Enhanced susceptibility to lethal effects of *S. pyogenes* infections at all exposure durations (2 and 6 h; 8, 15, 16, 307, and 321 days); inconclusive results with *S. typhimurium* because of high mortality rates in controls; no enhanced mortality when challenged with A/PR8-3 influenza virus	Campbell et al. (1980, 1981)

[a]Chronic exposure lasted 52 weeks.
[b]Chronic exposure lasted 48 weeks.
[c]Calculated for 104-week exposure.
DP = Diesel exhaust particles.
AM = Alveolar macrophage.
PMN = Polymorphonuclear leukocyte.

Source: Quoted from U.S. Environmental Protection Agency (1994).

alveolar region. Other data suggest that the inability of particle-laden AMs to translocate to the mucociliary escalator is correlated to the average composite particle volume per AM in the lung. Data from rats indicate that when this particle volume exceeds a critical level, impairment appears to be initiated. Such data for other laboratory species and humans, unfortunately, are very limited.

There is a considerable body of evidence that the major noncancerous health hazards posed by exposure to diesel exhaust are to the lung. These data also show that the exposures that cause pulmonary injury are lower than those inducing detectable increases in lung tumors. These same data further indicate that the inflammatory and proliferative changes in the lung play a key role in the etiology of pulmonary tumors in exposed rats. A range of no adverse effect levels has been estimated as 200-400 $\mu g/m^3$ (Health Effects Institute, 1995**).**

11.5.3 Species Differences

The responses to inhaled diesel exhaust as well as other particulate differs markedly among rodents. Data on the response to diesel exhaust for a number of species has been reviewed by Mauderly (1994a). The data indicate that as with cancer, the non-cancer pulmonary effects of diesel exhaust differ greatly in rats, mice and Syrian hamsters. Thus far, all animals show epithelial proliferation with chronic high level exposure to diesel exhaust but the changes in the respiratory bronchioles of cats differ from the changes in the alveolar ducts of rodents. Rats appear to have a greater epithelial proliferative response to dusts than do mice. Guinea pigs differ from other species in that the inflammatory response to dust is eosinophil-based rather than neutrophil-based. Thus, it is unclear which of the animals used in inhalation studies is the best model for predicting the responses of humans to dust exposure. Pepelko and Perrano (1983) exposed 8 male cats to diluted DE (6000 $\mu g/m^3$) for 5 days/week for 61 weeks, then to 12,000 $\mu g/m^3$ for another 27 mo. At the end of the exposure, a restrictive respiratory function impairment with nonuniform gas distribution was observed (Moorman et al., 1985). The accompanying histopathology included peribronchiolar fibrosis and epithelial metaplasia in terminal and respiratory bronchioles (Plopper et al., 1983). The epithelial changes lessened but the fibrosis worsened during 6 mo after the exposure ended.

The rat is the species for which most information about the noncancer effects of diesel exhaust (Table 11-15) as well as other inhaled dusts has been obtained. The responses of rats chronically exposed to carbon black or diesel particulate without the organic fraction, are essentially identical to their responses to diesel exhaust (Mauderly, 1994b; Heinrich et al., 1995). Heinrich et al. (1995) also demonstrated that the noncancer responses of rats to titanium dioxide were also similar qualitatively and quantitatively. Muhle et al. (1991) reported that the responses to chronically inhaled copying toner, a plastic dust pigmented with carbon black, titanium dioxide and silica were also similar qualitatively to titanium dioxide and diesel exhaust. Similar responses resulting from chronic exposure of rats to a range of other dusts including oil shale dusts (Mauderly et al., 1994b), talc (National Toxicology Program, 1993), and coal dust (Martin et al., 1977) have been described.

Few studies have examined the effects of exposure to diesel exhaust mixed with other dusts. The response of rats chronically exposed to diesel exhaust soot and mineral dust was studied by Mauderly et al. (1994b). Male and female F344 rats were exposed 7 hours/day 5 days/week for 30 mo to diesel exhaust, raw or retorted oil shale dust, or additive combinations of diesel exhaust and shale dust. The diesel exhaust soot accumulated more rapidly in the lungs than did the shale dust, due to differences in particle size, but the lung burdens of the two types of dust were additive. The long-term effects on lung weight and density, and BALF constituents, were greater than additive, the effects on respiratory function were approximately additive, and the effects on particle clearance were less than additive. The noncancer health effects of the combined exposures were more closely correlated with the total lung dust burden than with the combined dust exposure concentrations.

Lewis et al. (1989) studied the effects of diesel exhaust and mineral dust in rats and cynomolgus monkeys exposed to either diesel exhaust or coal dust at 2,000 μg respirable particles/m^3, or to a combination of 1,000 μg/m^3 of each material. Lung burdens of the dusts were approximately additive in rats but were not measured in the monkeys. Local histopathological responses were similar and approximately additive for the two dusts in both species.

11.5.4 Effects of Mixtures Containing Diesel Exhaust

Mauderly (1993) reviewed the results of studies in which laboratory animals were exposed to complex mixtures. In a study of diesel and coal dust, rats were exposed for 24 mo to atmospheres containing diesel exhaust at 2000 $\mu g/m^3$ coal dust at the same concentration, and a combination of diesel exhaust and coal dust at 1000 $\mu g/m^3$ each. Among the health end points evaluated, the effects of diesel exhaust and coal dust were similar with coal dust being slightly less toxic. No synergistic interactions between the exposure materials were noted. In another study of diesel and shale oil dust, Mauderly et al. (1994b) exposed rats by inhalation for 7 h/day 5 days/week for up to 30 mo to raw or retorted oil shale dusts at 5,000 $\mu g/m^3$, to diesel exhaust at 3,500 $\mu g/m^3$ or to additive combinations at total particulate concentrations of 8,500 $\mu g/m^3$. The three agents all accumulated progressively in the lungs and caused similar pneumoconiotic responses. The magnitude of effects was more closely correlated to particle lung burdens than to exposure concentrations. The effects of diesel exhaust and shale dusts generally were less than additive for delay of particle clearance; additive for respiratory function impairment; and greater than additive for lung collagen, airway fluid indicators of inflammation, and lung tumors.

Mauderly (1989) discussed the susceptibility of the aging lung to inhaled pollutants**.** Although the data is extremely limited in that only two particulate pollutants are discussed, it appears that the aging lung might be more sensitive to particulate pollution. Rats were exposed repeatedly for 6 mo to diluted, whole diesel exhaust at a concentration of 3,500 $\mu g/m^3$. The results indicated that rats exposed between 6 and 12 mo were more sensitive then rats born in the chambers and exposed up to 6 mo of age. The results indicated that mice exposed as adults were more susceptible than mice exposed at the onset of breeding age but while lung maturation was still underway.

11.5.5 Particle Effect in Diesel Exhaust Studies

Diesel PM is composed of an insoluble carbon core with a surface coating of relatively soluble organic constituents. Studies of diesel particle composition have shown that the insoluble carbon core makes up about 80% of the particle mass and that the organic phase

can be resolved into a more slowly dissolving component and a more quickly dissolving component.

The relative contribution of the carbon core of the diesel particles versus organics adsorbed to the surface of the particles to cancer induction and the uncertainty involved has been reviewed (Health Effects Institute, 1995). The primary evidence for the importance of the adsorbed organics is the presence of known carcinogens among these chemicals. These include polycyclic aromatics as well as nitroaromatics. Organic extracts of particles have also been shown to induce tumors in a variety of injection, intratracheal instillation and skin painting studies, and Grimmer et al. (1987) has, in fact, shown that the great majority of the carcinogenic potential following intratracheal instillation resided in the fraction containing four- to seven-ring PAHs.

Evidence for the importance of the carbon core is provided by studies of Kawabata et al. (1986), that showed induction of lung tumors following intratracheal instillation of CB that contained no more than traces of organics and studies of Heinrich et al. (1995) that indicated that exposure via inhalation to CB (Printex 90) particles induced lung tumors at concentrations similar to those effective in diesel studies. Other particles of low solubility such as titanium dioxide (Lee et al., 1986) have also been shown to induce lung tumors, although at much higher concentrations than necessary for carbon particles or diesel exhaust. Pyrolyzed pitch, on the other hand, essentially lacking a carbon core but having PAH concentrations at least three orders of magnitude greater than diesel exhaust, was no more effective in tumor induction than was diesel exhaust (Heinrich et al., 1986b). These studies suggest that the insoluble carbon core of the particle is at least as important as the organic components and possibly more so for lung tumor induction at high particle concentrations (>2,000 $\mu g/m^3$).

Diesel soot and carbon black appear to elicit similar responses in animal inhalation studies (Mauderly et al., 1994a; Heinrich et al., 1995; Nikula et al., 1995). Macrophage accumulation, epithelial histopathology, and reduced clearance have been observed in rodents exposed to high concentrations of chemically inert particles (Morrow, 1992), furthering the possibility that the toxicity of diesel particles results from the carbon core rather than the associated organics. However, the organic component of diesel particles consists of a large number of polycyclic aromatic hydrocarbons and heterocyclic compounds and their

derivatives. A large number of specific compounds have been identified. These components of diesel particles may also be responsible for the pulmonary toxicity of diesel particles. It is not possible to separate the carbon core from the adsorbed organics in order to compare the toxicity. As an approach to this question, a study has been performed in which rats were exposed to either diesel exhaust or to carbon black, an inert analog of the carbon core of diesel particles. Rats were exposed for 16 h/day, 5 days/week, for up to 24 mo to either 2,500 or 6,500 $\mu g/m^3$ of either particle (Nikula et al., 1995). Although the study is primarily concerned with the role of particle associated organics in the carcinogenicity of diesel exhaust, non-neoplastic effects are also mentioned. Both diesel exhaust and carbon black exposure resulted in macrophage hyperplasia, epithelial hyperplasia, bronchiolar-alveolar metaplasia, and focal fibrosis. In general, the number and intensity of the lesions seems to correspond to the exposure time and concentration and that the morphological characteristics of the lesions were similar in the animals exposed to diesel and to carbon black. The results suggest that the chronic noncancer effects of diesel exhaust exposure are caused by the persistence of the insoluble carbon core of the particles, rather than by the extractable organic layer. These studies have been reviewed (Health Effects Institute, 1995) and the consensus is that particulate matter is primarily responsible for the rat lung response to diesel exhaust.

11.5.6 Gasoline Engine Emissions

Mauderly (1994c) reviewed the toxicological and epidemiological evidence for health risks from inhaled gasoline engine emissions. Although the data bank is more extensive for diesel exhaust, animal studies have shown that heavy, chronic exposure to gasoline engine exhaust can cause lung pathology and associated physiological effects.

In female beagle dogs exposed to gasoline engine exhaust for over 5 years (16 h/day, 7 days/week) there was little effect on respiratory function during the exposure. However, subsequent tests revealed increases in lung volumes, dead space ventilation, and dynamic lung compliance, and a decrease in alveolar-capillary gas exchange efficiency (Hyde et al., 1978). There were also slight but distinct histopathological changes in the tracheobronchial and alveolar regions.

The effects of gasoline engine exhaust on the lungs of rodents were evaluated in a series of studies in rats and Syrian golden hamsters exposed for up to 24 mo to two dilutions of gasoline engine exhaust with particle concentrations of approximately 50 or 100 $\mu g/m^3$ (Bellman et al., 1983; Muhle et al., 1984; Heinrich et al., 1986a). While gasoline engine exhaust did not cause any substantial histopathology or alterations of lavage fluid in either species, gasoline engine exhaust in the higher concentration increased lung weight, retarded particle clearance, reduced lung compliance, and increased acetylcholine sensitivity in rats. No significant changes in function were found at either concentration in the hamster, or at the lower concentration in the rat. In rats and hamsters exposed to gasoline engine exhaust and diesel engine exhaust (16 h/day, 5 days/week, for 24 mo), there were no significant changes in respiratory function (Brightwell et al., 1989).

While the laboratory animal toxicological data base is limited there is some indication that long term exposure to gasoline engine exhaust can produce effects on the respiratory tract. It is unclear to what extent the other constituents of gasoline engine exhaust may have contributed to the effects.

11.5.7 Summary

In summary, diesel particulate is a widespread pollutant that is present in low concentrations in the ambient atmosphere (1 to 6 $\mu g/m^3$ in Los Angeles). Data from occupational studies and laboratory animal studies indicate that acute exposures to high levels or chronic exposures to low levels (albeit high compared to ambient levels) of diesel particulate can have an effect on the respiratory tract. However, it is doubtful that the diesel particulate at concentrations present in the ambient atmosphere could have a significant effect.

Acute and chronic inhalation exposures to diesel particulate are associated with respiratory effects. However, in general, the levels used in the laboratory animal studies or experienced in occupational settings are considerably higher than those experienced in the ambient environment and the results of these studies provide little insight into the morbidity and mortality studies discussed in Chapter 12. This is not unexpected because of the patterns of exposure and the total exposures, as well as differences in the populations exposed. Some of the effects noted in the occupational studies such as respiratory tract irritation, bronchitis,

impaired pulmonary function, cough, wheezing, are also observed in the epidemiological studies discussed in Chapter 12. Although these responses were specific to diesel exhaust, the effects appear to be due to the particles, per se. However, these effects are evident at exposures much higher than those experienced in the ambient atmosphere. Accordingly, the toxicological studies of specific diesel particulate do not appear to provide insight into the effects observed in the epidemiological studies discussed in Chapter 12 which relate to PM in general.

11.6 SILICA

This section on silica particle toxicity is designed to give an overview of current concepts regarding the pulmonary toxicity of these environmental pollutants as they relate to different species, different polymorphs (crystalline vs. amorphous), and biological mechanisms of action. No attempt has been made to review all of the relevant animal toxicity data, which is voluminous. Silica is well established as a fibrogenic pollutant which causes lung tumors following chronic exposures in rats. A review of the literature on the effects of silica can be found elsewhere (U.S. Environmental Protection Agency, 1996).

The pulmonary response to inhaled silica has long been considered to be a major occupational hazard, causing disability and deaths among workers in a variety of industries. Some of the processes and work environments which are frequently associated with silica exposure include mining, sandblasting of abrasive materials, quarrying and tunneling, stonecutting, glass and pottery manufacturing, metal casting, boiler scaling, and vitreous enameling (Ziskind et al., 1976).

11.6.1 Physical and Chemical Properties of Silica

Silica is one of the most common substances to which workers are exposed. Silica particle emissions in the environment can arise from natural, industrial, and farming activities. There are only limited data on ambient air concentrations of either crystalline or amorphous silica particles, due in part, to the limits in accurately quantifying crystalline silica and to the inability, under existing measurement methods, of separating the identity of crystalline silica from other particulate matter. Davis et al., (1984) used radiographic

diffraction to determine the inhalable composition and concentration of quartz in ambient aerosols collected on dichotomous filters at 25 U.S. metropolitan areas. They reported the average weight percentage of quartz in the coarse and fine particle mass to be 4.9 (+ 2.3) and 0.4 (+ 0.7), respectively. Combining the weight percentage data for the coarse fraction and 7 year average annual arithmetic mean PM10 information available for 17 of the 25 areas, annual average and high U.S. ambient quartz levels of 3 and 8 $\mu g/m^3$, respectively, have been estimated (U.S. Environmental Protection Agency, 1996). The actual fraction of quartz in PM_{10} samples may be slightly lower than that which was estimated by Davis et al. (1984) in the coarse fraction of dichotomous filters. However, these estimated U.S. levels are considered to be reasonable upper bound estimates (U.S. Environmental Protection Agency, 1996). There are at least four polymorphs or forms of crystalline silica dust. These include quartz, cristobalite, tridymite and tripoli. Although identical chemically, they differ in their crystal parameters. The basic structural units of the silica minerals are silicon tetrahedra, arranged in such a manner so that each oxygen atom is common to two tetrahedra. However, there are considerable differences in the arrangement of the silicon tetrahedra among the various crystalline forms of silica (Coyle, 1982). Naturally occurring rocks that contain amorphous forms of silica include diatomite or diatomaceous earth, a hydrate form such as opal, and an unhydrated form, flint (Stokinger, 1981b). Silica is also a component of many naturally occurring silicate minerals in which various cations and anions are substituted into a crystalline silica matrix. Examples of such silicates are kaolin, talc, vermiculite, micas, bentonite, feldspar, asbestos, and Fuller's earth (Silicosis and Silicate Disease Committee, 1988). Commonly encountered synthetic amorphous silica, according to their method of preparation, are SiO_2 gel (silica G), precipitated SiO_2 (silica P), and fumed SiO_2 (silica F). The most outstanding characteristics of synthetic amorphous silica compounds are their particle size and high specific surface area, which determine their numerous applications (Stokinger, 1981b).

11.6.2 Health Effects of Silica

The causal relationship between inhalation of dust containing crystalline silica and pulmonary inflammation and the consequent development of silica-induced pulmonary fibrosis (i.e., silicosis) is well established (Spencer, 1977; Morgan et al., 1980; Bowden,

1987). During the acute phase of exposure, a pulmonary inflammatory response develops and may progress to alveolar proteinosis and a granulomatous-type pattern of disease in rats and other rodent species. A pattern of nodular fibrosis occurs in chronically exposed animals and humans (Ziskind, 1976; Spencer, 1977; Morgan et al., 1980; Bowden, 1987). Although there is experimental evidence that quartz can also cause lung cancer, a clear correlation between pulmonary fibrosis and neoplasia has been suggested but has not been definitively established. Acute high occupational exposures can elicit a rapid onset of lung inflammation, leading to serious, if not fatal, lung dysfunction.

The pulmonary pathological effects of inhaled crystalline silica are well established, however, there is a paucity of information on the effects of inhaled amorphous forms of silica on the respiratory tract. The limited toxicological information available suggests that the respiratory tract effects following exposures to amorphous silicates may be reversible in the absence of continuing exposures (Groth et al., 1981; Schepers, 1981; Goscicki et al., 1978; Pratt, 1983). Thus, current evidence suggests that synthetic amorphous silica is not as severe a hazard as the various polymorphs of crystalline silica.

Parameters which have been commonly used to assess the respiratory effects of silica exposure in experimental animals include lung weight, development of pulmonary fibrosis, or biomarkers for fibrosis, such as collagen content, cytotoxicity, pulmonary inflammation, biochemical indices of homogenized lung samples or bronchoalveolar lavage samples, and immunologic responses. Few studies have provided exposure dose-response data from which definitive effect levels could be derived, thus necessitating comparisons among studies in which experimental conditions may vary considerably. A review of the published laboratory animal toxicology studies is available (U.S. Environmental Protection Agency, 1996).

11.6.3 Differences Between Chemical Forms of Silica

A few studies have been carried out to compare the effects of inhaled crystalline and amorphous silica particulates (see Table 11-17). Pratt (1983) exposed guinea pigs for 21 to 24 mo to atmospheric suspensions of either cristobalite crystalline silica, amorphous diatomaceous earth, or to amorphous volcanic glass. The index of lung pathogenicity was substantially higher for the cristobalite-exposed animals compared to the other two polymorphs of amorphous silica particles (Pratt, 1983). Hemenway et al. (1986) exposed

TABLE 11-17. COMPARATIVE INHALATION TOXICITY STUDIES WITH DIFFERENT SILICA POLYMORPHS

Particle	Species, Gender	Mass Concentration	Exposure Duration	Observed Effect	References
Cristobalite	Guinea pig (GP)	151,000 $\mu g/m^3$	7-8 h/d 5.5 d/wk	Total amount of silica accum. varied inversely with the pulmonary tissue damage. Cristobalite produced the greatest pulmonary effects.	Pratt et al. (1983)
Diatomaceous earth (amorphous)	Same	100,000 $\mu g/m^3$	21-24 mo		
Volcanic glass (amorphous)	Same	>151,000 $\mu g/m^3$			
Cristobalite	Male Fischer 344 rats	58,000 & 73,000 $\mu g/m^3$	6 h/d 8 days	Cristobalite produced the most dramatic inflammation and fibrotic response. Amorph. silica-transient inflamm. AQ initial mild response but progressive.	Hemenway et al. (1986)
Alpha-quartz	Same	36,000 & 81,000 $\mu g/m^3$			
Amorphous silica (Zeofree 80)	Same	30,000 $\mu g/m^3$			
Fumed silica	Male SD rats Male Hartley GP Male cynomolgus monkeys	15,000 $\mu g/m^3$	5.5-6 h/d 5 d/wk up to 18 mo	Monkeys developed greater response to fumed silica than rats or guinea pig. Fumed silica produced greater fibrotic and pulmonary function effects compared to gel or ppt. silica	Groth et al. (1981)
Precip. silica	Same	Same	Same		
Gel silica	Same	Same	Same		
Cristobalite	Male SD rats	10,000 or 100,000 $\mu g/m^3$	6 h/d for 3 days	Exposures to cristobalite or AQ produced persistent and progressive pulmonary inflammation and ↑ biomarkers of cytotoxicity. Ludox and amorphous silica elicited transient pulmonary inflammatory responses.	Warheit et al. (1995)
Alpha-quartz (Min-U-Sil)	Same	10,000, 50,000 or 100,000 $\mu g/m^3$	6 h/d for 3 days		
Amorphous silica (Zeofree 80)	Same	10,000 or 100,000 $\mu g/m^3$	6 h/d for 3 days		
Ludox (Colloidal silica)	Same	10,000, 50,000 or 150,000 $\mu g/m^3$	6 h/d for 2 or 4 wk		

rats for 8 days to aerosols of one of three silicon dioxide species, α-cristobalite, α-quartz, and amorphous silica particulates. The greatest measure of lung injury was produced with cristobalite, which caused substantial inflammation and fibrosis. Exposures to α-quartz produced mild but progressive effects, while amorphous silica produced transient inflammation. Warheit and coworkers carried out a number of short-term inhalation studies using cristobalite, (α-quartz Min-U-Sil), Ludox colloidal silica, a form of precipitated amorphous silica, and amorphous silica. Rats were exposed to silica aerosols for periods ranging from 3 days to 4 weeks and evaluated by bronchoalveolar lavage and cellular proliferation indices at several postexposure time periods. Brief exposures to 2 different forms of crystalline silica particles at 100 $\mu g/m^3$ produced persistent pulmonary inflammation characterized by neutrophil recruitment and elevated biomarkers of cytotoxicity in BAL fluids. Progressive histopathologic lesions previously were observed within 1 mo after a 3-day exposure (Warheit et al., 1991a). In contrast, a 3-day exposure to amorphous silica, produced transient lung inflammation, and 2 or 4-week exposures to Ludox elicited pulmonary inflammation at 50,000 or 150,000 $\mu g/m^3$ but not at 10,000 $\mu g/m^3$; most elevated biochemical effects were reversible. These results demonstrated that the crystalline forms of silica dust were substantially more potent in producing pulmonary toxicity compared to the amorphous or colloidal forms of silica (Warheit et al., 1991a, 1991b, 1995). In addition, the pulmonary effects of inhaled (α-quartz particles in rats were much more potent than in the study reported by Hemenway and coworkers (1986).

11.6.4 Species Differences

The fibrogenic effects of crystalline silica exposure may vary depending on the species used in experimental studies. Rats appear to be more sensitive to the development of silica-induced lung injury and lung tumors in comparison to other rodent species such as mice and hamsters (Saffioti, 1992; Saffioti et al., 1993; Uber and McReynolds, 1982). Warheit et al., (1994) reported that inhalation exposure to silica in complement-normal and complement-deficient mice produced an acute pulmonary inflammatory response which was mild and transient, compared to the pulmonary effects observed in rats wherein silica produced a sustained and progressive pulmonary inflammatory response. In support of these results, mice intratracheally injected with silica particles had a milder fibrogenic response

when compared with rats (Hatch et al., 1984). It seems clear, however, that the silica-induced response in mice depends upon the strain, as there appear to be low and high responding strains of mice to silica (Callis et al., 1985; Hubbard, 1989).

Differences are not only apparent across and within rodent species, but also between rodents and humans. Unlike the nodules observed in human radiographs, silicosis is manifested in rat radiographs as a diffuse "haziness", described as a ground-glass appearance with some peripheral striation (Kutzman, 1984). In a chronic study by Muhle et al. (1989), the principal non-neoplastic finding in the silica-exposed rats, extensive subpleural and peribronchiolar fibrosis, was described as being unlike the nodular fibrosis observed in human silicosis. Such interspecies differences and the fact that most of the available laboratory studies only examined one dose level may limit the utility of laboratory animal data for extrapolation of the silicosis risk observed in higher exposure conditions of human occupational studies.

For additional information on the pathogenic development of silica-related lung disease in humans and experimental animals, the reader is referred to a variety of informative reviews (Ziskind et al., 1976; Spencer, 1977; Reiser and Last, 1986; Bowden, 1987; Crouch, 1990; Goldstein and Fine, 1986; Warheit and Gavett, 1993).

11.7 BIOAEROSOLS

11.7.1 Types of Health Effects Associated with Bioaerosols

Exposure to biological aerosols can produce three general classes of health effects: infections, hypersensitivity disease, and toxicoses. It is possible that these afflictions may make people more susceptible to air pollutant effects.

11.7.1.1 Infections

Infections result when a living (micro)organism invades another organism, multiplies using some component of the host as a nutrient source, and either directly (via digestion) or indirectly (via release of toxins) causes disease. The number of individual living particles required to cause disease depends on the virulence (ability to invade the host) of the organism, and on the status of the host’s immune system (Pennington, 1989). The organisms

that most commonly cause infectious disease are viruses (e.g., influenza, measles, common colds) and bacteria (e.g., Legionnaires' disease, tuberculosis). A few fungi can also cause infections in healthy people (e.g., *Histoplasma capsulatum*) or those with damaged immunity (e.g., *Aspergillus fumigatus*) (Rippon, 1988).

Particle size is an important consideration for disease. Some agents can only cause infection in the upper respiratory tract, and are best transmitted via large droplets (many common colds). Others must reach the lower airway to cause infection, and large droplets that impact in the upper airway are not usually part of the disease process (e.g., *Mycobacterium tuberculosis*) (Burge, 1989). Infectious aerosols must remain alive and be able to invade and replicate in the host in order to cause disease. Over time, infectious aerosols decay physically (becoming less concentrated) and biologically (each remaining cell becoming less able to cause disease). Airborne infectious diseases are generally caused by relatively resistant organisms that are highly virulent (Cox, 1987).

11.7.1.2 Hypersensitivity Diseases

Hypersensitivity diseases are caused by exposure to allergens (a specific type of antigen) and result from specific responses of the immune system (Pope et al., 1993). They are always caused by two step processes. Initial exposures induce sensitization (i.e., cause the production of circulating or fixed immune cells that recognize the agent), and subsequent exposures precipitate symptoms (the agent reacts with the specific immune cell and releases mediators such as histamine that result in overt symptoms). Thus the first exposure to a sensitizing agent does not cause symptoms. The kinds of hypersensitivity diseases that are caused by bioaerosols include asthma, allergic rhinitis and (rarely) allergic dermatitis (the "immediate" or IgE-mediated diseases), and hypersensitivity pneumonitis (also called allergic alveolitis) which is mediated primarily by the cellular immune system. Approximately 30% of the US population is affected by IgE-mediated allergies. The incidence of hypersensitivity pneumonitis remains unknown. Farmer's lung disease (a form of the disease) probably occurs in less than 3% of the farm population.

Very little good data have been accumulated on the actual doses of an allergen (the agent that stimulates the response) required for either sensitization or symptom development. For the IgE-mediated diseases, relatively low level long-term exposure is considered to be

important for sensitization and higher levels are needed to precipitate symptoms. For hypersensitivity pneumonitis, intense short term exposures may result in sensitization, while very low levels may induce symptoms.

Any allergen could probably cause either type of disease depending on the conditions of exposure. Pollen and fungal allergens are well-known agents that precipitate hay fever and asthma symptoms, while proteins released from dust mite fecal particles are apparently highly effective sensitizers. Historically, the agents most commonly associated with hypersensitivity pneumonitis are the thermophilic actinomycetes. In addition, fungal spores, bird droppings, bacterial enzymes, and other agents have been reported to cause the disease.

Allergen-bearing particles that induce IgE-mediated disease range in size from <0.1 μm (cat secretions) to 60 μm (some grass pollen). Apparently allergen-bearing particles must be <5 μm in order to cause hypersensitivity pneumonitis. In both diseases, there may be synergistic effects between allergens and irritants (i.e., endotoxin, chemical air pollutants) with respect to sensitization. Note that allergens are always water soluble, and must diffuse out of the allergen-bearing particle before inducing their effect. It is likely, then, that the larger the particle, the more slowly the allergen exposure, and hence the response, will occur.

11.7.1.3 Toxicoses

Microbial toxins are (essentially) chemicals that are produced by living organisms. The microbial toxicoses are basically similar to the comparable diseases caused by non-biological toxins. Microbial toxins are known that are mutagenic, teratogenic, tumorigenic, and cytotoxic. In addition, some (like endotoxin) have adjuvant activity (i.e., they stimulate the immune system).

Exposure/response relationships for biological toxins are poorly known with the possible exception of endotoxin. Endotoxin clearly affects pulmonary function and at high levels may cause serious disease (Burge, 1995). Organic dust toxic syndrome has been associated with massive exposure to endotoxins (along with mycotoxins and other components of grain dust). The incidence of the disease (the percent of the farm worker population with at least one attack) ranges from 1% in Sweden to up to 44% in the United States (Do Pico, 1992). Grain dust also causes a less acute disease with prolonged

exposures at relatively low exposure levels. Whether a component of the grain itself or of contaminating bacteria or fungi is actually the toxic agent remains unknown.

Mycotoxin-related lung disease remains poorly documented. There is some evidence that exposure to *Aspergillus flavus* aerosols containing aflatoxin B1 is a risk for lung and esophageal cancer in peanut handlers (Sorenson et al., 1984) and in farmers handling moldy corn (Baxter et al., 1981). Exposure to trichothecene toxins contained in *Stachybotrys atra* has been blamed for central nervous system symptoms, skin rashes, and pulmonary hemorrhages in specific cases, although in all cases, exposure was inferred rather than measured (Croft et al., 1986).

Particle sizes required for disease related to biological toxin exposure depend on the nature of the disease. Pulmonary effects of endotoxin probably require pulmonary deposition, while systemic effects could be precipitated by larger particles impacting in the upper airway. The fungal spores that have been blamed for mycotoxin-induced airway disease range from about 3 to 5 μm in diameter. The location of the mycotoxins in fungal spores is unknown. The toxins may not be present on the surface of particles, and, in some cases, must be released from the particle to be effective. Endotoxin is a part of the outer cell wall.

11.7.2 Ambient Bioaerosols

Ambient bioaerosols include fungal spores, pollen, bacteria, viruses, endotoxins, and animal and plant debris. Bacteria, viruses and endotoxins are mainly found attached to aerosol particles, while entities in the other categories are found as separate particles. Data for characterizing ambient concentrations and size distributions of bioaerosols are sparse. Matthias-Maser and Jaenicke (1994) found that bioaerosols constituted about 30% of the total number of particles in samples collected on a clean day in Mainz, Germany. The proportion of particles that were bioaerosols was higher in the fine size mode (as much as a third) and slightly lower in the coarse size mode. In Brisbane, Australia, Glikson et al. (1995) found that fungal spores dominate the bioaerosol count in the coarse fraction of PM_{10} and that the overall contribution of bioaerosols to total PM_{10} particulate mass was on the order of 5 to 10%. However, the cytoplasmic content of spores and pollen was often found to be adhered to particles emitted by motor vehicles and particles of crustal origin**.**

Fungal spores range in size from 1.5 µm to >100 µm, although most are 2 to 4 µm MMAD. They form the largest and most consistently present component of biological aerosols in ambient air. Levels vary seasonally, usually being lowest when snow is on the ground. Fungal spores often reach levels of 1000 to 10,000 spores/m^3 during the summer months (Lacey and Dutkiewicz, 1994; Madelin, 1994) and may be as high as 100,000/m^3 near some anthropogenic sources (agriculture activities, compost, etc.).

Asthma mortality has been associated with ambient levels of fungal spores, unadjusted OR of 2.16 (95% CI = 1.31 to 3.56) per increment of 1000 spores/m^3; controlling for time and pollen counts reduced the RR to 1.2 (95% CI = 1.07 to 1.34) (Targonski et al., 1995). Asthma mortality in Scotland shows a seasonal peak that follows the peak in ambient pollen levels (MacKay et al., 1992). Exposure to fungal spores has also been identified as a possible precipitating factor in respiratory arrest in asthmatics (O'Hollaren et al., 1991). Such exposure can lead to allergic alveolitis (hypersensitivity pneumonitis) or pulmonary mycoses such as coccidioidomycosis or histoplasmosis (Lacey and Dutkiewicz, 1994).

Bioaerosols can contribute to increased mortality and morbidity. Most commonly, bioaerosols appear to exacerbate allergic rhinitis and asthma. Induction of hypersensitivity generally requires exposure to concentrations that are substantially higher than in ambient air, although subsequent antigenic responses require much lower concentrations. Association of fungal and pollen spores with exacerbations of asthma or allergic rhinitis is well established (Ayres, 1986) and fungal spore levels may be associated with asthma mortality (Targonski et al., 1995). The incidence of many other diseases (e.g., coccidioidomycosis) induced by fungal spores is relatively low, although there is no doubt about the causal organisms (Lacey and Dutkiewicz, 1994). The potential for fungal induced diseases is much higher in immunocompromised patients and those with unusually high exposures, such as military personnel.

In addition to fungal spores and pollen, other bioaerosol material can exacerbate asthma and can also induce responses in nonasthmatics. For example, in grain workers who experience symptoms, spirometry decrements, and airway hyperresponsiveness in response to breathing grain dust, the severity of responses is associated with levels of endotoxin in the bioaerosol rather than the total dust concentration (Schwartz et al., 1995). A classic series of studies (Antó and Sunyer, 1990) proved that airborne dust from soybean husks was

responsible for asthma epidemics and increased emergency room visits in Barcelona, Spain. These studies indicate that airborne fragments of biological substances can produce severe health effects.

Bacterial aerosol counts may range as high as 30,000 bacteria/m^3 downwind of sewage treatment facilities, composting areas, waterfalls from polluted rivers, or certain agricultural activities. Typical levels in urban areas range from several hundred to several thousand bacteria/m^3 (Lighthart and Stetzenbach, 1994). Human pathogenic activity of such bacteria is not well understood or characterized. Infective potential of aerosolized bacteria depends on size (smaller are more effective), virulence, host immune status, and host species sensitivity (Salem and Gardner, 1994). Aerosolized bacteria can cause bacterial infections of the lung including tuberculosis and legionnaire's disease. The *Legionella pneumophila* bacterium is one of the few infectious agents known to reside outside an infected host and is commonly found in water, including lakes and streams. Levels of bioaerosols (fungi and bacteria) are generally higher in urban than in rural areas (Lighthart and Stetzenbach, 1994).

Exposures to bioaerosols of the above types, while clearly capable of producing serious health effects (especially at high concentrations often encountered in indoor environments) appear unlikely to account for observed ambient (outdoor) PM effects on human mortality and morbidity demonstrated by epidemiology studies reviewed in Chapter 12. This conclusion is based on (1) bioaerosols generally represent only a very small percentage (< 5 to 10%) of measured urban ambient PM mass; (2) they typically have even lower concentrations in ambient air during winter months, when notable ambient PM effects have been demonstrated; and they tend to be in the coarse fraction size range.

11.8 TOXICOLOGY OF OTHER PARTICULATE MATTER

11.8.1 Introduction

This section reviews the toxicology of other PM within the framework described in the introduction to the chapter. The particle classes chosen for inclusion here are those which may actually occur in ambient air or may be surrogates for these. For example, some of the particles discussed are considered to be models of "nuisance" or "inert" dusts (i.e., those having low intrinsic toxicity) and, as such, are likely to be representative of similar ambient

PM. In many instances, there are only a few studies examining the response on specific biological endpoints following inhalation exposure. In these cases, and where available, intratracheal instillation studies have been used to compare the toxicity of different particle types. While instillation may produce more severe pulmonary damage than would inhalation (largely due to differences in delivered doses and dose rates), the relative toxicities of different particles seem to be similar when given by either method (Driscoll et al., 1991). Thus, intracheal instillation studies can be used for comparative potency purposes, but it is not possible to quantitatively extrapolate the resulting exposure-response data to inhalation exposure-responses. In a number of cases, particles with low intrinsic toxicity have been used in instillation studies to delineate nonspecific particle effects from effects of known toxicants. Some of these studies are discussed herein, as they offer the only database for such materials.

11.8.2 Mortality

Examples of studies in which effects on mortality were reported using particles >1 μm in diameter are presented in Table 11-18; all of these studies involved repeated or chronic exposures to high concentrations of various PM, some of which are considered to be of low toxicity. While incomplete, the studies are of a variety of materials and indicate that essentially no treatment-related mortality was induced in any of the studies.

Recent interest has been focused on the inherent toxicity of a smaller size class of particles, namely the ultrafine particles which are discussed in Section 11.4. While the mass concentration of ultrafine particles in ambient air may be low, their number concentration may be quite high, as discussed previously.

11.8.3 Pulmonary Mechanical Function

Assessments of pulmonary mechanical function have generally been carried out with particles having some inherent toxicity, as well as other studies examining effects of other particles with low intrinsic toxicity (see Table 11-19). Wright et al. (1988) instilled rats (Sprague-Dawley; F; 200g) with 10,000 μg iron oxide (0.1 μm GMD, σg = 1.7) or silica (quartz) (1.3 μm, σg = 2.5). At 1 mo after exposure, they noted no changes in various indices of pulmonary mechanics (total lung capacity [TLC];

TABLE 11-18. EFFECTS OF PARTICULATE MATTER (≥1 μm) ON MORTALITY

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference [a]
TiO_2	Rat, M/F, F-344, 8 weeks	Whole body	5,000	1.1 (MMAD); 1.5	6 h/day, 5 days/week, 2 years	None	Muhle et al. (1991)
Toner	Rat, M/F, F-344, 8 weeks	Whole body	16,000	4 (MMAD)	6 h/days, 5 days/week, 2 years	None	Muhle et al. (1991)
Coal dust	Rat, M, Wistar, 18 weeks	Whole body	6,600, 14,900	2.1 (MMAD); 2.7	6 h/day, 5 days/week, 20 mo	None	Karagianes et al. (1981)
Petroleum coke (micronized)	Rat, M, SD	Whole body	10,000, 30,000	3.1 (AED); 1.9	6 h/day, 5 days/week, 2 years	None	Klonne et al. (1987)
Petroleum coke (micronized)	Monkey, adult, cynomologous	Whole body	10,000, 30,000	3.1 (AED); 1.9	6 h/day, 5 days/week, 2 years	None	Klonne et al. (1987)
Volcanic ash	Rat, M/F, F-344, 3 mo	Whole body	5,000, 50,000	Respirable (unspecified size)	6 h/day, 5 days/week, 2 years	None	Wehner et al. (1983)
TiO_2	Rat, M/F, CD	Whole body	10,000, 50,000, 250,000	1.5-1.7 (MMD)	6 h/day, 5 days/week, 2 years	None	Lee et al. (1985)
Fly ash (coal)	Rat, M, Wistar, 3 mo	Whole body	270,000	47% ≤ 3.75 μm	6 h/day, 15 days	None	Chauhan et al. (1987)
California road dust	Rat, F-344	Nose-only	300, 900	4 (MMAD); 2.2	4 h/day, 4 days/week, 8 weeks	None	Kleinman et al. (1995)
Talc	Rat, M/F, F-344	Whole body	6,000, 18,000	2.7-3.2 (MMAD); 1.9	6 h/day, 5 days/week, 2 years	None	National Toxicology Program (1993)

[a]Effect indicates "treatment related" mortality.

TABLE 11-19. EFFECTS OF INHALED PM ON PULMONARY MECHANICAL FUNCTION

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ	Exposure Duration	Observed Effect	Reference [a]
Volcanic ash	Rat, Sprague-Dawley, 40 days	Whole body	9,400	0.65 (MMAD); 1.78	2 h/days, 5 days	No changes (f, V_T, V_{insp}, V_{exp})	Raub et al. (1985)
Fly ash (coal) (Illinois # 6)	Guinea pig, Hartley, 250-320 g	Nose-only	5,800	0.21 (MMAD); 4.14	1 or 2 h	2 h: ↓TLC, VC, DL up to 96 h PE 1 h: no effect	Chen et al. (1990)
Fly ash (coal) (Montana lignite)	Guinea pig, Hartley, 250-320 g	Nose-only	5,800	0.21 (MMAD); 4.14	1 or 2 h	2 h: ↓TLC, VC; no change in DL_{co}	Chen et al. (1990)
Volcanic ash	Rat, M/F, F-344, 3 mo	Whole body	5,000, 50,000	Respirable	6 h/day, 5 days/week, 24 mo	↑f for 50,000 $\mu g/m^3$ by 8 mo; no change for 5,000 $\mu g/m^3$	Wehner et al. (1983)
Volcanic ash	Guinea pig, Hartley, 300-425 g	Head	9,400	0.65 (MMAD); 1.78	2 h	No change in R_{aw}, C_{dyn}, f, V_T, $\dot{V}_E$	Wiester et al. (1985)
Coal dust	Rat, Wistar, 200-300 g, conventional and germ free	Whole body	10,000	Geometric mean <5 μm	8 h/day, 120 days	↓ FEV, V (10%) (Germfree); only ↓ $\dot{V}_{max}$ (10%) conv.	Moorman et al. (1977)
TiO_2	Rat, F, F-344, 8 weeks	Whole body	5,000	—	6 h/day, 5 days/week, 24 mo	No changes (C, V_T, IC, VC, RV, TLC, DL_{co}, N_2 washout)	Heinrich et al. (1989b)

Key to abbreviations:
f: breathing frequency
V_T: tidal volume
V_{insp}: inspiratory flow
V_{exp}: expiratory flow
TLC: total lung capacity
VC: vital capacity
DL_{co}: carbon monoxide diffusing capacity
PE: post exposure
IC: inspiratory capacity
RV: residual volume
R_{aw}: airway resistance
C_{dyn}: dynamic compliance
V_I = max inspiratory flow
$\dot{V}_E$ = expiratory minute volume
FEV = forced expiratory volume (1 sec)
$\dot{V}_{max}$ (10%) = maximal flow at 10% FVC
FVC = forced vital capacity

functional residual capacity [FRC]; nitrogen [N_2] washout; FEV1; or peak expiratory flow [PEF]) in animals exposed to iron oxide, but silica exposure resulted in changes in the N_2 washout curve and decreased compliance. Bégin et al. (1985) instilled into sheep (Male; 25 to 45 kg BW) 100,000 μg latex beads (0.1 μm) or asbestos fibers. The latex produced no change in pulmonary function (TLC, residual volume [RV]; vital capacity [VC]; expiratory reserve volume [ERV]; pulmonary compliance [Cpulm]; pulmonary resistance [Rpulm]; FRC), while the asbestos produced a reduction in compliance, abnormalities in the N_2 washout curve, and changes in forced expiratory flow measurements.

There are a few studies of pulmonary function responses following inhalation exposures to PM. Chen et al. (1990) evaluated pulmonary function of guinea pigs exposed to coal fly ash (5.8 $\mu g/m^3$, MMAD = 0.21 μm) produced during combustion of Illinois no. 6 coal (high sulfur) or Montana lignite (low sulfur). Total lung capacity (TLC), vital capacity (VC), and diffusing capacity for carbon monoxide (DL_{CO}) were all significantly reduced below control values at 2h and 8h postexposure in guinea pigs exposed to Illinois no. 6 ash. The DL_{CO} was still 10% below control values 96h postexposure. Guinea pigs exposed to the Montana lignite fly ash at comparable concentration and particle size did not show alterations in diffusing capacity. The authors suggested that the different effects could be due to sulfuric acid produced during combustion of the two coals but neutralized by the high alkali content of the Montana lignite.

Wehner et al. (1983) exposed rats (F-344; M/F, 3mo) to 5,000 or 50,000 $\mu g/m^3$ volcanic ash (Mt. St. Helens) for 6 h/day, 5 days/week for up to 24 mo (Table 11-19). By 12 mo of exposure, no changes in lung volume were noted. By 8 mo of exposure, there was an increase in respiratory frequency in animals exposed at the higher concentration, but no change at the lower concentration.

Heinrich et al. (1989b) exposed rats for 6 h/day, 5 days/week up to 24 mo to titanium dioxide (TiO_2) at 5,000 $\mu g/m^3$ and silica at 1,000 $\mu g/m^3$. Exposure to silica produced a reduction in quasistatic lung compliance, tidal volume, (V_T), inspiratory capacity (IC), VC, RV, and TLC. Diffusion capacity for carbon monoxide (DLco) was also reduced, and the N_2 washout curve was altered; these changes indicate a functionally restrictive lung, a finding often noted in humans occupationally exposed to silicates. None of these variables were altered by exposure to TiO_2.

Acidic sulfates have been associated with alterations in bronchial responsiveness, but there are few studies with other particles which examined this response. Fedan et al. (1985) exposed rats (F344, whole body) for 7 h/day, 5 days/week for 2 years to coal dust (size described as respirable, but not specifically stated) at 2,000 $\mu g/m^3$, and examined the pharmacological response of isolated tracheal preparations to various agonists. The coal dust exposure increased the maximal contractile response of the tracheal smooth muscle to acetylcholine (a bronchoconstrictor), compared to air exposed control tissue, but did not alter the slope of the acetylcholine concentration-response curve nor sensitivity (i.e., EC50). No change in response to isoproterenol (a bronchodilator) was noted. Wiester et al. (1985) exposed guinea pigs for 2 h to 9,400 $\mu g/m^3$ of Mt. St. Helens volcanic ash (0.65 μm). No changes in pulmonary mechanics measured during exposure (airway resistance, dynamic compliance, breathing frequency, maximum inspiratory flow or expiratory minute volume) were noted. However, following exposure, airway hyporesponsiveness to histamine challenge was observed.

It should be noted that, as with acidic sulfates, changes in pulmonary function may not be the most sensitive marker of response to other PM. For example, inflammatory changes in sheep following the instillation of latex particles (100,000 μg in 100 ml fluid**)** were not associated with any changes in lung volumes, resistance, or compliance (Bégin et al., 1985).

11.8.4 Pulmonary Morphology and Biochemistry

A considerable amount of the information concerning morphologic alterations from inhaled particles has been obtained in studies of diesel exhaust, and this is discussed in this chapter and reviewed elsewhere (U.S. Environmental Protection Agency, 1994; Health Effects Institute, 1995). In addition, and as previously mentioned with acidic sulfate particles, markers in lung BAL have been used to assess damage following PM exposure.

The ability of ambient particles to affect lung morphology was strongly suggested by Böhm et al. (1989). They exposed rats (Wistar, F, 2.5 mo) for 6 mo to the ambient air of two cities in Brazil, namely São Paulo and Cubatao. Although characterization of air pollution levels was vague, pollution in the former appeared to be dominated by automobile exhaust gases, while that in the latter by industrially derived particulate matter. Rats exposed in Cubatao showed various responses, such as mucus hypersecretion and epithelial

hyperplasia, in both the upper and lower bronchial tree, while those exposed in São Paulo showed effects generally limited to the upper bronchial tree. Particle concentrations (PM_{10}) were as high as 164 $\mu g/m^3$ in Cubatao. Thus, high PM levels were suggested to be responsible for the observed effects, although the contribution of other components of the pollutant mix could not be discounted.

Some intratracheal instillation studies have compared morphological effects resulting from exposure to different particles. Wright et al. (1988) instilled 10,000 μg iron oxide (Fe_2O_3; 0.1 μm GMD, $\sigma g = 1.7$) or 10,000 μg quartz (1.3 μm GMD, $\sigma g = 2.5$) into rats, and examined the lungs 30 days following each exposure. The iron oxide did not produce any histological or morphometric changes, while the quartz exposure resulted in aggregations of PMNs and AMs around small airways, alveolar proteinosis, increased alveolar distances, airspace enlargement, and increased thickness of respiratory bronchiolar walls.

Another example of an instillation study which may be used to compare effects from different types of particles is that of Sanders et al. (1982), who instilled rats (F-344, female, young adult) with 40,000 μg of either soil (sandy loam, 1.6 μm CMD), volcanic ash (Mt. St. Helens, 0.5 to 1.5 μm CMD), or crystalline quartz (1.5 μm CMD). Mononuclear cell infiltration was noted with both the soil and ash particles in regions of high particle aggregation. There was also some Type 2 epithelial cell hyperplasia 7 to 37 days following ash or soil instillation. However, the ash produced a fibrotic response to a greater extent than did the soil, with indications from the former of a simple pneumoconiosis and moderate lipoproteinosis. Some foci of particle-laden macrophages were noted in the mediastinal lymph nodes of soil exposed animals, but the ash-exposed animals showed reactive lymphoid hyperplasia. Quartz resulted in production of granulomas, deposition of collagen, widespread lipoproproteinosis, and fibrosis in regional lymph nodes.

The comparative fibrogenic potential of a number of particle types was examined by Schreider et al. (1985). Male Sprague-Dawley rats were exposed by intratracheal instillation to 5,000, 15,000, or 45,000 μg of Montmorillonite clay (0.84 μm CMD), quartz (1.1 μm), Mt. St. Helens volcanic ash (1.2 μm), stack-collected coal fly ash (1.5 μm) or hopper-collected fly ash (1.9 μm), or to 5,000 or 15,000 μg of a coal-oil ash mixture (3.9 μm). Lung histology was assessed at 90 days post instillation. Neutrophils were noted in alveoli only with quartz (all concentrations), stack ash (at high concentration), and

volcanic ash (low and mid concentrations). Some fibrosis was produced by all of the particles, although there were qualitative and quantitative differences among the different exposure groups. The order of fibrosis potential, from greatest to least, was as follows: quartz > clay > volcanic ash > hopper coal ash > stack coal ash > oil-coal ash mixture.

Bégin et al. (1985) instilled 100,000 μg of 0.1 μm latex beads or asbestos fibers into the lungs of sheep (25 to 45 kg) and examined lavage fluid at 1 to 60 days post instillation. The latex produced only transient alveolitis and transient increases in the number of AMs and PMNs in lavage beginning at day 1, whereas the asbestos-exposed animals had a persistent inflammatory response and more severe damage. Callis et al. (1985) instilled silica or latex particles (0.9 μm) into the lungs of mice. While the latter produced some increase in protein and cell number in lavage, the response to the former was much greater. Finally, Lindenschmidt et al. (1990) instilled rats with either of two inert dusts, (Al_2O_3; 5.3 μm) and TiO_2 (2.2 μm) at 1,000 or 5,000 μg/100g body weight and examined the lungs up to 63 days post instillation. Both particle types produced similar increases in N-acetylglucosamine and total recovered cells in lavage, while a minimal Type 2 cell hyperplasia noted with Al_2O_3 was even less severe with TiO_2. However, when results were compared with those for instilled silica, any responses seen with the inert particles decreased towards control level during the 2-mo study period, while changes with silica progressed. This highlights the difference between the inert and fibrogenic materials. Thus, the instillation studies suggest that there may be some nonspecific particle effect, but clearly the chemical characteristics of the particle affects the ultimate biological response. In any case, levels of particles with low intrinsic toxicity are not associated with major nonspecific effects.

The effects of inhaled PM on pulmonary morphology are outlined in Table 11-20. Most of the studies used fly ash and volcanic ash; TiO_2 has also been used to assess effects of a "nuisance" (low intrinsic toxicity) type of particle. However, with the exception of the study of road dust by Kleinman et al. (1995), exposure concentrations ranged from very high to extremely high and likely caused overload with long-term exposures. Responses, when they did occur, were quite similar for the various particles, characterized by focal aggregates of particle-laden macrophages with evidence of an inflammatory response; the intensity of both effects was related to exposure duration and concentration. On the other

TABLE 11-20. EFFECTS OF PARTICULATE MATTER ON RESPIRATORY TRACT MORPHOLOGY

Particle	Species, Gender, Strain, Age or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
Coal dust (micronized bituminous)	Rat, M, Wistar, 18 weeks	Whole body	6,600, 14,900	2.1 (MMAD); 2.7	6 h/day, 5 days/week, 20 mo	Accumulation of aggregates of particles in AMs immed. after exposure; alveolar histocytosis, interstitial fibrosis and emphysema, indication of simple pneumoconiosis; no lesions in upper respiratory tract.	Karagianes et al. (1981)
Petroleum coke (micronized raw)	Rat, M/F, S-D; Monkey, cynomologous (mature)	Whole body	10,000, 30,000	3.1 (AED); 1.9	6 h/day, 5 days/week, 2 years	Rat: chronic pulmonary inflammation at 3, 6, 12, and 18 mo observation times at both conc; focal fibrosis; sclerosis; squamous alveolar metaplasia. Monkey: accumulation of particle-laden AMs; no inflammation	Klonne et al. (1987)
Fly ash (coal)	Rat, M/F, F-344, 10-13 mo	Whole body	36,000	3.6 (MMAD); 2	7 h/day for 3 days on week 1, 5 days/week next 3 weeks, 2 days in week 5	No exposure-related histopathology in large or small airways; but increased cell division; slight increase in number of hypertrophic Type 2 cells by 2 weeks; small areas of thickened alveolar walls and some perivenous inflammatory cell infiltration; by 4 weeks, aggregation of AMs with particles and greater alveolar wall thickening and inflammation; some resolution by 42 weeks in pathology.	Shami et al. (1984)
Volcanic ash (Mt. St. Helens)	Rat, M/F, F-344, 3 mo	Whole body	5,000, 50,000	Respirable (no size given)	6 h/day, 5 days/week, up to 24 mo	At 5,000 $\mu g/m^3$: small aggregations of particle-laden AMs at 4 mo and some thickening of alveolar septa. Aggregates of dust deposits at 8 mo, and some peribronchiolar lymphoid hyperplasia which increased by 12 mo. Enlargement of mediastinal nodes by 12 mo. At 50,000 $\mu g/m^3$: more severe lesions; low to moderate AM accumulation by 4 mo which increased by 8 mo and stabilized by 12 mo. Prominent peribronchial and mediastinal node reaction by 4 mo, which increased by 8 mo and stabilized by 12 mo; alveolar proteinosis by 8 mo.	Wehner et al. (1983)
TiO_2	Rat, F, F-344, 8 weeks	Whole body	5,000		6 h/day, 5 days/week, up to 24 mo	No fibrosis; no bronchiolar hyperplasia; no accumulation of AMs in lung tissue.	Heinrich et al. (1989b)

TABLE 11-20 (cont'd). EFFECTS OF PARTICULATE MATTER ON RESPIRATORY TRACT MORPHOLOGY

Particle	Species, Gender, Strain, Age or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
Fly ash (coal)	Mice, M, C57BL/6, 12 weeks	Nose-only	200,000	1.6-1.7 (MMAD); 1.4-1.5	100 min	Increased no. of AMs; no other lesions evident by light microscopy.	Fisher and Wilson (1980)
TiO_2	Guinea pig, F, Dunkin-Hartley, 300-350 g	Whole body	23,000	95% < 1.98 (MMAD)	20 h/day, 14 days	At 1 day PE: dust laden cells in bronchial lymph nodes and BALT; some thickening of alveolar septa in areas of high dust conc.; some degenerative changes in AMs; no PMNs. At 6 d PE: increased number of dust laden AMs.	Baskerville et al. (1988)
Volcanic ash	Rat, Sprague-Dawley, 40 days	Whole body	9,400	0.65 (MMAD); 1.78	2 h/day, 5 days	Slight peribronchial and perivascular mononuclear cell infiltration.	Raub et al. (1985)
California road dust	Rat, F-344	Nose-only	900	4 (MMAD); 2.2	4 h/day, 4 days/week, 8 weeks	↑ Alveolar septal wall thickness; ↓ Alveolar diameter	Kleinman et al. (1995)
TiO_2	Rat, M/F, CD	Whole body	10,000, 50,000, 250,000	1.5-1.7 (MMAD)	6 h/day, 5 days/week, 2 years	At 10,000 $\mu g/m^3$: slight alveolar epithelial hyperplasia. At 50,000 $\mu g/m^3$: marked alveolar epithelial hyperplasia; bronchioarization of alveoli adjacent to terminal bronchioles; alveolar proteinosis. At 250,000 $\mu g/m^3$: increased alveolar hyperplasia and bronchioarization; deposition of collagen fibers.	Lee et al. (1985)
Fly ash (fluidized bed coal combustion)	Rat, M/F, F-344, 12-16 weeks	Whole body	142,000	3 (MMAD); 2.6	6 h	No pathology, except accumulation of particles.	Hackett (1983)
Fly ash (coal)	Hamster, golden, 8 weeks	Whole body	2,000, 1,000, 2,000 20,000	2.3-2.4 (MMAD); 1.5	20 h/day, 7 days/week, 6 mo	Accumulation of particle-laden AMs in proximal alveoli in concentration/duration dependent fashion; ↑ PMNs at 20,000 $\mu g/m^3$ in peripheral alveoli.	Negishi (1994)

TABLE 11-20 (cont'd). EFFECTS OF PARTICULATE MATTER ON PULMONARY MORPHOLOGY

Particle	Species, Gender, Strain, Age or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
Carbon black	Rat, M, F-344, 14-15 weeks	Whole body	10,000	2.0/0.12 (MMAD) (bimodal distr. with 70% in smaller mode) 2.5/2.3	7 h/day, 5 days/week, 12 weeks	Mild hyperplasia of Type 2 cells; particle laden AMs in distal terminal bronchioles and proximal alveolar ducts.	Wolff et al. (1990)
Carbon black	Rat, F, Wistar 6 weeks		6,000	n/s	18 h/day, 5 days/week, 10 mo	Moderate to severe hyperplasia in bronchioalveolar region; some inflammation; alveolar lipoproteinosis	Nolte et al. (1994)
Fly ash (coal)	Rat, M, Wistar, 160-175 g	Whole body	270,000	47% <3.75 μm	6 h/day, 15 days	Mild infiltration of mononuclear cells and mild pneumonitis 45 days PE; numerous particle-laden AMs outside alveoli up to 105 days PE; ↑ lung weight by 30 days PE.	Chauhan et al. (1987)
Shale dust (raw or spent)	Monkey, cynomolgus, M/F, 2-4.5 kg Rat, M/F, F344, 90-95 g	Whole body	10,000, 30,000	3.9-4.5; (1.8-2.2)	6 h/day, 5 days/week, 2 years	Concentration-related accumulation of AMs; subacute bronchiolotis and alveolitis Concentration-related proliferative bronchiolitis and alveolitis, chronic inflammation with spent shale; no lymph node inflammation; accumulation of AMs	MacFarland et al. (1982)
Coal dust	Monkey cynomolgus, M Rat, M/F, F-344 Mice, M/F CD-1	Whole body	2,000	8.6 μm (MMAD)	7-h/day, 5 days/week, up to 2 years	Type II cell hyperplasia and pulmonary lipodosis in rats; increased phagocytosis. Mild obstructive airway disease in monkeys.	Lewis et al. (1989)

Key to abbreviations:

NS: Not specified
PE: Post-exposure
AM: Alveolar macrophage
PMNs: Polymorphonuclear leukocytes

hand, the Kleinman et al. (1995) study at relatively low particle concentrations showed a more diffuse pattern of morphological change and no inflammatory loci.

There is some evidence for interspecies differences in response to comparable exposure atmospheres (Klonne et al., 1987). In the study of Shami et al. (1984), increased proliferation of large and small airway epithelial cells occurred in the absence of overt histopathology following exposure to fly ash. The authors suggested that this may indicate some potential for the interaction of fly ash with carcinogens.

Clark et al. (1990) exposed dogs (mongrel, 15 to 20 kg) for 5 min to wood smoke (from fir plywood sawdust and kerosene; no specified particle size or exposure concentration) via an endotracheal tube. The lungs were examined for increased extravascular water around the pulmonary arteries, which was found to occur with smoke exposure but not in air sham controls. This response was suggested to be due to increased microvascular permeability without any increase in capillary pressure. A decrease in lung compliance was also noted with smoke exposure.

Table 11-21 outlines studies in which lavage fluid was analyzed following inhalation exposure to PM. As with morphology, most exposure concentrations were very high, but effects, when they occurred, indicated inflammation.

As mentioned earlier, eicosanoids are potent mediators of various biological functions, and alterations in arachidonic acid metabolism, which may be involved in lung pathology, can be assessed in lavage fluid. Exposure to coal dust (25,000 $\mu g/m^3$) produced decreases in prostaglandin E_2, and increases in thromboxane A_2 and leukotriene B_4, perhaps suggesting smooth muscle constriction, vasoconstriction and increased chemotactic activity of macrophages (Kuhn et al., 1990).

Table 11-22 outlines studies examining lung biochemistry following particle inhalation, mostly to fly ash. In some cases, effects on the xenobiotic metabolizing system of the lungs were examined. For example, van Bree et al. (1990) exposed rats to coal fly ash (10,000, 30,000, 100,000 $\mu g/m^3$) and examined cytosolic antioxidant enzymes and the microsomal P-450 linked mixed function oxidase system involved in lung metabolic defense against reactive oxygen species and xenobiotic compounds. They noted both exposure-related increases and decreases in different components of this system, which they ascribed to differential effects of organic and trace metal components of the ash. Srivastava et al. (1985)

TABLE 11-21. EFFECTS OF PARTICULATE MATTER ON MARKERS IN LAVAGE FLUID

Particle	Species, Gender, Strain, Age or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
Carbon black	Mouse, F, Swiss, 20-23 days	Nose-only	10,000	2.45 (MMAD); 2.54	4 h/day, 4 days	No change in total cell no. or differential counts; no change in albumin levels.	Jakab (1992, 1993)
Volcanic ash	Mouse, F, CD-1, 4-8 weeks	Whole body	9,400	0.65 (MMAD); 1.8	2 h	Increase in PMNs.	Grose et al. (1985)
TiO_2	Rat, M, F-344 180-200 g	Whole body	50,000	1 (MMAD); 2.6	6 h/day, 5 days	No change in: AMs, PMNs, lymphocytes; LDH; protein; to 63 days PE.	Driscoll et al. (1991)
TiO_2	Rat, HAN	Whole body	50,000		8 h/day, 5 days/week (up to 15 weeks)	Slight increase in PMNs at 15 weeks.	Brown et al. (1992)
Coal dust	Rat, HAN	Whole body	10,000, 50,000		8 h/day, 5 days/week (up to 15 weeks)	Increased PMNs (persistent).	Brown et al. (1992)
California road dust	Rat, F-344	Nose-only	300, 900	4 (MMAD); 2.2	4 h/day, 4 days/week, 8 weeks	↑ Albumin at 900 $\mu g/m^3$; no change in total cells or differential counts	Kleinman et al. (1995)
TiO_2	Rat, M/F, F-344, 8 weeks	Whole body	5,000	1.1 (MMAD); 1.6	6 h/day, 5 days/week, 24 mo	No change in total cell no. in lavage but ↑ AMs and ↓ PMNs some time points; no change in LDH, protein, ß-glucuronidase in lavage.	Muhle et al. (1991)
Fe_2O_3	Rat, M, Long-Evans, 225-250 g	Nose-only	18,000-24,000	1.45-1.7 (MMAD); 2.9-3	2 h	No change total cell no. or differential counts.	Lehnert and Morrow (1985)
Carbon black	Rat, M, F-344, 14-15 weeks	Whole body	10,000	2.0/0.12 (MMAD) (bimodal distr. with 70% in smaller mode); 2.5/2.3	7 h/day, 5 days/week, 12 weeks	↑ PMNs in lavage; ↑ acid proteinase in lavage.	Wolff et al. (1990)
Carbonyl iron	Rat, M Crl:CDBR, 8 weeks	Nose-only	100,000	3.6 (MMAD); 1.7	6 h; 6 h/day, 3 days	No change in total cell no, protein, or LDH.	Warheit et al. (1991a)
Carbon black	Mouse, F, Swiss 20-23 g	Nose-only	10,000	2.4 (MMD); 2.75	4 h	No change in total cell no. or differential count at 20 h PE.	Jakab and Hemenway (1993)
TiO_2	Guinea pig, M/F, 400 g	Whole body	24,000	85% < 2 μm	8 h/day, 5 days/week, 3 weeks	No change in LDH, AP, AG, Cathepsin D at 4-24 h PE.	Kuhn et al. (1990)
Coal dust	Rat, F, F-344, 180 g	Whole body	25,000	4-5	16 h/day, 7 days/week, 2 weeks	↑ TxA_2, LTB_4, protein; ↓ PGE_2 at 1 day PE; TxA_2, and LTB_4 change persistent for 2 weeks.	Sjöstrand and Rylander (1984)

TABLE 11-21 (cont'd). EFFECTS OF PARTICULATE MATTER ON MARKERS IN LAVAGE FLUID

Particle	Species, Gender, Strain, Age or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
TiO_2	Guinea pig, M/F, 400 g	Whole body	24,000	Most between 0.5-2 (GMD)	8 h/day, 5 days/week, 3 week	No change PMNs; ↑ no. AMs, eosinophils by 16 weeks PE.	Fogelmark et al. (1983)

Key to abbreviations:
LDH: lactate dehydrogenase
AP: acid phosphatase
AG: N-acetyl-ß-d-glucosaminidase
TxA_2: thromboxane A_2
LTB_4: Leukotrine B_4
PGE_2: Prostaglandin E_2
AM: alveolar macrophage
PE: post-exposure
PMN: polymorphonuclear leukocyte
↑: increase
↓: decrease

TABLE 11-22. EFFECTS OF PARTICULATE MATTER ON LUNG BIOCHEMISTRY

Particle	Species, Gender, Strain, Age or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
Fly ash (coal)	Rat, M, Wistar, 5 weeks	Whole body	10,000, 30,000, 100,000	80-95% mass was ≤42 μm (AED)	6 h/day, 5 days/week, 4 weeks	↑ Cytosolic $GSHP_x$, protein at 30,000 100,000; ↑ G6PDH at 100,000; ↑ lung microsomal protein, ↓ microsomal BROD at 30,000/100,000; no change microsomal P-450 content; induction of EROD activity at all conc. (all in lung tissue).	van Bree et al. (1990)
Carbon black	Rat, M, F-344, 200-250 g	Whole body	6,000	0.22 (MMAD)	20 h/day, 1-14 days	No change in synthesis of lung total DNA; no change in DNA synthesis of Type 2 cells.	Wright (1986)
Fly ash (fluidized bed coal)	Rat, M/F, F-344	Whole body	142,000	3 (MMAD); 2.6	6 h	↑ Labeling of Type 2 cells; ↑ incorporation of thymidine in AM DNA, persisting 4 days PE; ↑ labeling airway epithelial cells, persistent up to 4 days PE.	Hackett (1983)
Carbonyl iron	Rat, M, Crl:CD BR, 8 weeks	Nose-only	100,000	3.6 (MMAD); 2.6	6 h/day, 3 days	No effect on labeling index of lung parenchymal or airway cells.	Warheit et al. (1991a)
Fly ash (fluidized bed coal combustion)	Rat, M/F, F-344, 10-13 weeks	Whole body	36,000	3.6 (MMAD); 2	7 h/day, 3 days week 1; 5 days/week week 2-4; 2 days week 5	↑ Labeling index of large airway basal cells and bronchiolar Clara cells at 2 weeks, resolved by 2 weeks PE; ↑ labeling index of Type 2 cells by 4 weeks, resolved by 2 weeks PE.	Shami et al. (1984)
Fly ash (coal)	Rat, M, Wistar, 160-175 g	Whole body	270,000	47% < 3.75 μm	6 h/day, 15 days	↑ P-450 content; ↑ activity of aryl hydrocarbon hydroxylase, glutathione S-transferase, δ-amino levulinic acid synthetase; inhibition of hemeoxygenase.	Chauhan et al. (1989)
Fly ash (coal)	Rat, M, Wistar, 160-170 g	Whole body	270,000	47% <3.75 μm	6 h/day, 15 days	↑ Total lung phospholipids; ↑ phosphatidylcholine up to 45 days PE.	Chauhan and Misra (1991)

Key to abbreviations:
$GSHP_x$ = glutathione peroxidase
G6PDH = glucose 6 phosphate dehydrogenase
BROD = benzoxyresorufin 0-dearylase
EROD = NADPH-mediated ethoxyresorufin 0-deethylase
↑: increase
↓: decrease
PE = post exposure

also found that the effects of fly ash were likely due to chemicals adsorbed onto, or that were part of, the fly ash particle, rather than to some nonspecific particle effect. This was because the activity of the lung mixed function oxidase system was induced in rats by instillation of coal fly ash (<0.5 μm), but not by instillation of glass beads.

There is some evidence that fly ash exposure can initiate cell division and DNA synthesis in the lungs (Hackett, 1983; Shami et al., 1984), but exposure levels were very high (>30,000 $\mu g/m^3$).

11.8.5 Pulmonary Defenses

11.8.5.1 Clearance Function

Mucociliary Transport

Grose et al. (1985) exposed (whole-body) rats (Sprague-Dawley CD, M, 60 to 70 days) to volcanic ash from Mt. St. Helens (0.65 μm, σg=1.8) at 9,400 $\mu g/m^3$ for 2 h. At 24 h post exposure, a depression in ciliary beat frequency in excised tracheas was noted. Whether this would contribute to any change in mucociliary transport function in the intact animal is unknown.

Pulmonary Region Clearance and Alveolar Macrophage Function

A number of studies have examined particle retention following exposure to high concentrations of inhaled particles, some of which have low intrinsic toxicity. Such exposures resulted in a phenomenon known as overload, in which the effectiveness of lung clearance mechanisms is significantly reduced. This response, which is nonspecific to a wide range of particles, is discussed in detail in Chapter 10.

While there are no studies of effects of exposure to nonacidic sulfate particles on alveolar region clearance, there have been several studies examining AM function following inhalation exposures (Table 11-23) or with in vitro exposure. High exposure concentrations of various particles can depress the phagocytic activity of AMs following inhalation.

To examine the effects of different fly ashes, Garrett et al. (1981b) incubated rabbit AMs with $\leq$1,000 μg of either conventional coal combustion fly ash or fluidized bed combustion fly ash at >3 and <3 μm, for 20 h. While all exposures caused reductions in cell viability and cell ATP levels, conventional coal fly ash <3μm produced the greatest

TABLE 11-23. EFFECTS OF PARTICULATE MATTER ON ALVEOLAR MACROPHAGE FUNCTION

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
Carbon black	Mouse, F, Swiss, 20-23 g	Nose-only	10,000	2.45 (MMAD); 2.54	4 h/day, 4 days	No change in F -mediated AM phagocytic activity up to 40 days PE.	Jakab (1992, 1993); Jakab and Hemenway (1993)
Volcanic ash	Mouse, F, CD-1, 4-8 weeks	Whole body	9,400	0.65 (MMAD); 1.8	2 h	No change in viability of recovered cells; no effect on AM phagocytosis at 0 or 24 h PE.	Grose et al. (1985)
TiO_2	Rat, M, F-344 180-200 g	Whole body	50,000	1 (MMAD); 2.6	6 h/day, 5 days	No change in spontaneous/ stimulated release of IL-1 by AMs up to 63 days PE.	Driscoll et al. (1991)
Fly ash (coal)	Mouse, F, BALB/C; C57BL; 6-8 weeks	Whole body	535 (fine particle fraction < 2.1 μm)	32 % < 2.1 μm (by wt)	148 days	↓ AM phagocytic activity by 21 days of exposure.	Zarkower et al. (1982)
TiO_2	Rat, HAN	Whole body	50,000	"respirable fraction"	8 h/day, 5 days/week	No change in chemotactic activity of AM.	Brown et al. (1992)
Coal dust	Rat, HAN	Whole body	10,000, 50,000	"respirable fraction"	8 h/day, 5 days/week	Decreased AM chemotactic activity.	Brown et al. (1992)
California road dust	Rat, F-344	Nose-only	300, 900	4 (MMAD)	4 h/day, 4 days/week, 8 weeks	↓ Production of superoxide at high concentration; no change in F_c receptor mediated phagocytic activity.	Kleinman et al. (1995)
Iron oxide (Fe_2O_3)	Rat, M, Long-Evans, 225-250 g	Nose-only	18,000-24,000	1.45-1.7 (MMAD); 2.9-3	2 h	No change in AM adherence; ↑ phagocytic activity of AM (F_c-mediated) up to 20 days PE.	Lehnert and Morrow (1985)
Carbonyl iron	Rat, M, Crl:CDBR, 8 weeks	Nose-only	100,000	3.6 (MMAD); 1.7	6 h; 6 h/day, 3 days	No change in AM chemotactic activity; cell viability ; slight ↑ AM phagocytic activity for single exp.	Warheit et al. (1991a)
Carbon black	Mouse, F, Swiss, 20-23 g	Nose only	10,000	2.4 (MMD); 2.75	4 h	No change in F -receptor mediated AM phagocytic activity.	Jakab and Hemenway (1993)
TiO_2	Guinea pig, M/F 400g	Whole body	24,000	Most between 0.5-2 (GMD)	8 h/d, 5 days/week, 3 weeks	No change in AM phagocytic activity.	Fogelmark et al. (1983)

effect. These results suggest toxicity somewhat dependent on size, as observed previously with other endpoints.

There is little available data on complex mixtures of other PM. Fick et al. (1984) exposed rabbits (NZW, 1.5 to 2 kg) for 0.2 to 2 h to the pyrolysis products derived from Douglas fir wood (exposure concentrations and particle size were not stated). They noted an increase in the total number of cells recovered by lavage immediately postexposure, and the magnitude of this increase was related to the exposure duration. The ratio of AMs, PMNs and lymphocytes was constant at all exposure durations except for the longest, in which case lymphocyte numbers increased. A depression in the uptake and intracellular killing of *Pseudomonas aeruginosa* was found in AMs obtained from the smoke-exposed animals compared to cells from air controls. Furthermore, cells from the smoke-exposed animals were smaller, and had reduced surface adherence.

To examine for a nonspecific particle effect on phagocytosis, Finch et al. (1987) exposed bovine AMs in vitro to TiO_2 (1.57 μm MMD, σg=2.3) or to glass beads (2.1 μm, σg=1.8), the former at 2.3 or 5 μg/ml, and the latter at 5 or 8.4 μg/ml. Neither exposure altered phagocytic activity, but TiO_2 did produce some decrease in cell viability.

Macrophages may contact particles via chemotactic-directed movement. Constituents of lung fluid having high chemotactic activity are components of complement, and particles which activate complement tend to show greater chemoattractant activity for macrophage accumulation at sites of particle deposition (Warheit et al., 1988). For example, in an in vitro study, iron-coated asbestos and carbonyl iron particles activated chemotactic activity in rat serum and concentrated rat lavage proteins, while volcanic ash did not. When the rats were exposed by inhalation to 10,000 to 20,000 $\mu g/m^3$ of these particles, only the volcanic ash failed to produce an increased number of macrophages on the first alveolar duct bifurcations, the primary deposition site for these particles and fibers. Complement proteins on alveolar surfaces are likely to be derived primarily from normal transudation of serum components from the pulmonary vasculature (Warheit et al., 1986). The generation of chemotactic factors at particle deposition sites may facilitate clearance for some particle types, but not for others, such as silica (Warheit et al., 1988, 1991a).

In a somewhat related study, Hill et al. (1982) examined the interaction with complement of coal combustion fly ash particles (2 to 3 μm MMAD) from different sites,

using serum from dogs. In addition to releasing peptides that are chemotactic for macrophages and other inflammatory cells, fly ash also induced release of lysosomal enzymes and increased vascular permeability, all processes involved in inflammation. While the authors noted that some fly ash samples activated complement, while others did not, they were not able to determine which component on or in the ash was responsible for this action. A possibility was suggested to be some metals, such as Mn, which are potent activators of the complement cascade (Lew et al., 1975).

Thorén (1992) examined the metabolic activity of AMs by measuring heat exchange rates after exposing cell monolayers to TiO_2 or manganese dioxide (MnO_2) at $0.6 - 4 \times 10^6$ particles/ml. The former affected metabolism only at the highest concentration used, while the latter caused changes at lower concentrations as well.

The response of AMs to PM is influenced by both physical and chemical characteristics of the particles with which they come into contact. Shanbhag et al. (1994) exposed a macrophage cell line (P388D1) to particles of two different composition (TiO_2 or latex) at comparable sizes, 0.15 and 0.45 μm for the former, and 0.11 and 0.49 for the latter. They also used pure titanium at 1.76 μm for comparison to latex at 1.61 μm. Titanium dioxide decreased cellular proliferation, depending upon both size and concentration. Similar sizes and concentrations of latex produced lesser responses. In addition, cells incubated with latex released factors, into the medium, which produced fibroblast proliferation to a greater extent than did cells incubated with TiO_2 of a similar size and concentration.

11.8.5.2 Resistance to Infectious Disease

Susceptibility of mice to challenge with several infectious agents has been used to assess effects of various inhaled particles on microbial defense of the lungs (Table 11-24). The study of Jakab (1993) is of particular interest because the infectious agents used were selected based upon differences in the antimicrobial defense mechanism most effective in eliminating each organism. Thus, *Staphylococcus aureus* defense depends primarily upon the integrity of AMs, while that for *Proteus mirabilis* involves both AMs and PMNs. *Listeria monocytogenes* defenses involve specific acquired immunity, namely the integrity of the lymphokine-mediated components of the cell- mediated immune response (e.g., AMs and lymphocytes). A number of host defenses play a role in defense against influenza, including

TABLE 11-24. EFFECTS OF PARTICULATE MATTER ON MICROBIAL INFECTIVITY

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
Carbon black	Mouse, F, Swiss, 20-23 g	Nose-only	4,700-6,100	2.45 (MMAD); 2.54	4 h/day, 4 days	No effect on susceptibility to infection from *S. aureus* administered 1 day PE; no effect on intrapulmonary killing of bacteria by AM.	Jakab (1992)
Carbon black	Mouse, F, Swiss, 20-23 g	Nose-only	10,000	2.4 (MMAD); 2.75	4 h/day, 4 days	No change in no. of *S. aureus* or *P. mirabilis* recovered in lung after bacterial challenge or on intrapulmonary killing of bacteria administered 1 d PE; no effect on proliferation of *L. monocytogenes*; no effect on proliferation or elimination of influenza A virus; no change in albumin level in lavage 4 h after bacterial challenge; no change in PMN in lavage 4 h after challenge.	Jakab (1993)
TiO_2	Guinea pig, F, Dunkin-Hartley 300-350 g	Whole body	23,000	95% < 1.98 μm (MMAD)	20 h/day, 14 days	No change in susceptibility to *Legionella pneumophila* administered 1-6 days PE but AM with heavy particle burden did not ingest bacteria.	Baskerville et al. (1988)
Coal dust	Mouse, F, Swiss CD-1, 20-24 g	Whole body	2,000	80% <10 μm; 50% <5 μm	7 h/day, 5 days/week, 6 mo	No change in susceptibility to influenza virus administered after 1, 3 and 6 mo exposure; decrease in interferon level in lung at 3 mo; no change in inflammatory response to virus.	Hahon et al. (1985)
Volcanic ash	Mouse, F, CD-1, 4-8 weeks	Whole body	9,400	0.65 (MMAD); 1.8	2 h	No change in susceptibility to bacteria (*Streptococcus)* or virus administered 0 or 24 h PE; no change in lymphocyte response to mitogens.	Grose et al. (1985)
TiO_2	Mouse, Harlan-Olac, 8 weeks	Whole body	2,000, 20,000	95% <1.98 μm (UDS)	20 h/day, 2 or 4 weeks	↓ Clearance of *P. haemolytica* administered after exposure in proportion to exposure duration at 20,000 $\mu g/m^3$ only.	Gilmour et al. (1989a)
TiO_2	Mouse, Harlan-Olac, 8 weeks	Whole body	20,000	95% <1.98 μm (UDS)	20 h/day, 10 days	↓ Clearance of *P. haemolytica*, persistent up to 10 days PE.	Gilmour et al. (1989a)

TABLE 11-24 (cont'd). EFFECTS OF PARTICULATE MATTER ON MICROBIAL INFECTIVITY

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
TiO_2	Mouse, Harlan-Olac, 8 weeks	Whole body	20,000	95% <1.98 μm (UDS)	20 h/day, 7 days	↓ Response to bacterial antigens of mediastinal lymph node lymphocytes from mice inoculated with *P. haemolytica* after exposure.	Gilmour et al. (1989b)

Key to abbreviations:
↓: decrease
PE: post-exposure

specific cytotoxic lymphocytes. However, repeated exposure to 10,000 $\mu g/m^3$ carbon black did not alter any of these antimicrobial defense systems.

Particles of low intrinsic toxicity may impair mechanisms involved in the clearance of bacteria, perhaps increasing their persistence and resulting in increased infectivity. To examine this possibility, a study was aimed at determining whether animals (guinea pigs) in which phagocytic activity was impaired by exposure to a high concentration (23,000 $\mu g/m^3$) of an "inert" dust (TiO_2) were more susceptible to bacterial infection, in this case due to *Legionella pneumophila* (Baskerville et al., 1988). While those AMs having heavy burdens of TiO_2 particles did not phagocytize the bacteria, there was no increase in infectivity in particle-exposed compared to air-exposed control animals; this was suggested to be due to the recruitment of monocytes into the lungs of the TiO_2-exposed animals, and these cells were able to phagocytize the bacteria.

The studies presented in Table 11-24 indicate that particles inhaled even at high concentrations did not reduce resistance to microbial infections. However, some changes were noted in an instillation study. Hatch et al. (1985) examined various particles administered by intratracheal instillation for their ability to alter infectivity in mice subsequently exposed to a bacterium *(Streptococcus sp)*. The specific particle types and their sizes (VMD) were as follows: conventional coal combustion fly ash from various sources (0.5 μm); various samples of fluidized bed combustion coal fly ash (0.4 to 1.3 μm); various samples of oil combustion fly ash (0.8-1.3μm); volcanic ash (1.4 and 2.3μm); latex (0.5 and 5 μm); and urban air particles (0.4 μm) from Dusseldorf, Germany, Washington, DC, and St. Louis, MO. The instillation dose was 100 μg particles/mouse. An increase in infectivity was found with all oil fly ash samples, some of the combustion and fluidized bed coal fly ash samples, ambient air particles from Dusseldorf and Washington, latex, and also from carbon and ferric oxide particles of unstated size. Exposure to volcanic ash, St. Louis ambient particles, and other coal fly ash samples did not have an effect. It was postulated that the activity of the fly ash reflected either the speculated presence of metals or the ability of the ash to alter the pH of airway fluid. In a corollary to the above study, rabbit AMs were incubated for 20 h with the various particles and cell viability assessed. Viability was reduced by all oil fly ash samples, coal fly ash, ambient particles from all three sites,

volcanic ash and latex. These results did not totally correlate with the response following in vivo exposures.

To examine effects of particles on nonimmunological antiviral defense, Hahon et al. (1983) exposed monolayers of mammalian cells (rhesus monkey kidney cell line) to coal combustion fly ash (2.5 μm) at 500 to 5,000 μg/10 ml medium and assessed effects on interferon. Induction of interferon due to infection with influenza and parainfluenza virus was reduced when the cells were pretreated with the fly ash. This was suggested to be due to either the matrix itself, or to some surface component which was not extractable with either polar or nonpolar solvents.

One study examined the effect of two larger particles on infectivity. Grose et al. (1985) instilled (42 μg/animal) mice (CD-1, F, 4 to 8 weeks) with two sizes of volcanic ash from Mt. St. Helens, namely coarse mode (12.1 μm MMAD, σg=2.3) and fine mode (2.2 μm MMAD, σg=1.9), followed by challenge with bacteria (*Streptococcus sp.*) immediately or 24 h postexposure. No particle size related difference was noted in susceptibility to bacterial infection, with both sizes producing a similar increase in infection following bacterial challenge at 24 h, but not immediately, after pollutant exposure. However, inhalation exposure to 9,400 $\mu g/m^3$ volcanic ash (0.65 μm) for 2 h produced no change in infectivity (Table 11-24).

11.8.5.3 Immunologic Defense

The few studies on effects of inhaled particles on respiratory tract immune function are shown in Table 11-25. Particles may affect some aspects of immune defense and not others. For example, fly ash did not produce any change in the cellular immune response, namely delayed hypersensitivity, but did depress the ability of macrophages to enhance T-cell mitogenesis (Zarkower et al., 1982).

11.8.6 Systemic Effects

A few studies have examined systemic effects of inhaled particles. One assessed the ability of particles to affect systemic immune responses (Eskew et al., 1982). Mice (F, BALB/C) were continuously exposed for various times to coal combustion fly ash (32% by wt <2.1 μm), and the antigenic response of spleen cells to protein derivatives after

TABLE 11-25. EFFECTS OF PARTICULATE MATTER ON RESPIRATORY TRACT IMMUNE FUNCTION

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Technique	Mass Concentration ($\mu g/m^3$)	Particle Characteristics: Size (μm); σ_g	Exposure Duration	Observed Effect	Reference
Fly ash (fluidized bed coal combustion)	Rat, M/F, F-344, 12 weeks	Whole body	36,000	3.6 (MMAD); 2.0	7 h/day, 5 days/week, 4 weeks	No effect on humoral immune function.	Bice et al. (1987)
Fly ash (pulverized coal combustion)	Rat, M/F, F-344, 12 weeks	Whole body	37,000	2.7 (MMAD); 2.1	7 h/day, 5 days/week, 4 weeks	↓ Antibody response at 48 weeks PE.	Bice et al. (1987)
Fly ash (coal)	Mouse, F BALB/C; C57BL 6-8 weeks	Whole body	760 (fine particle fraction, <2.1 μm)	32% < 2.1 μm (by wt)	28 days (continuous)	↓ Ability of AMs to stimulate PHA-induced T-lymphocyte mitogenesis.	Zarkower et al. (1982)
					160 days (continuous)	No change in ability of animals sensitized with BCG during exposure to respond to purified protein derivative challenge (delayed hypersensitivity cellular immune response).	
			2,200 (fine particle fraction, <2.1 μm)				

Key to abbreviations:
AM: macrophage
PE: post-exposure
IL = interleukin
↑: increase
↓: decrease

sensitization with BCG (delayed hypersensitivity reaction) was examined, as was the mitogenic response of spleen cells to concanavalin A or lipopolysaccharide (LPS). Exposure for 1 to 8 weeks to 1,150 $\mu g/m^3$ reduced the mitogenic response of spleen cells after 3 weeks of exposure, but not after 5 or 8 weeks and only for concanavalin A. Exposure for 5 mo to 2,220 $\mu g/m^3$ increased thymidine incorporation into spleen cells from BCG-sensitized mice. Finally, exposure for 5 weeks to 871 $\mu g/m^3$ reduced the number of antibody plaque forming cells in the spleen and the hemagglutinin titer. These results suggest that fly ash has little effect on the cellular immune response, but depresses the humoral response. The implications of the increase in thymidine incorporation into the spleen of BCG-sensitized mice was not clear, but may indicate an increase in resistance to infection.

In another study of systemic immunity, Mentnech et al. (1984) exposed rats (F344, M, whole body) to 2,000 $\mu g/m^3$ coal dust (40% <7μm) for 7 h/day, 5 days/week for 12 or 24 mo. The number of antibody-producing cells in the spleen 4 days after immunization with sheep red blood cells was used as a test of effects on humoral immunity, while the proliferative response of splenic T-lymphocytes to the mitogens concanavalin A and phytohemagglutin was used to assess cellular immunity. No changes were found.

11.8.7 Toxicological Interactions of Other Particulate Matter Mixtures

11.8.7.1 Laboratory Animal Toxicology Studies of Particulate Matter Mixtures

Toxicological interactions with PM may be antagonistic, additive, or synergistic (Mauderly, 1993). The presence and nature of any interaction seems to depend upon the concentration of pollutants in the mixture, the exposure duration, and the endpoint being examined, and it is not possible to predict a priori from the presence of certain pollutants whether there will be any interaction.

Mechanisms responsible for the various forms of interaction are generally not known. The greatest hazard in terms of potential health effects from pollutant interaction is the possibility of synergism, especially if effects occur at all with mixtures which do not occur at all when the individual constituents are inhaled. Various broad mechanisms may underly synergism. One is physical, the result of adsorption or absorption of one material on a particle and subsequent transport to more sensitive sites, or sites where this material would not normally deposit in toxic amounts. This may explain the interaction found in studies of

mixtures of carbon black and formaldehyde, or carbon black and acrolein (Jakab, 1992, 1993), especially since formaldehye has been shown to be absorbed onto particles (Rothenberg et al., 1989).

Somewhat related to this hypothesis is the possibility of reactions on particle surfaces, forming some secondary products which may be more toxicologically active than the primary material and which is then carried to some sensitive site. This may explain the results of the Jakab and Hemenway (1993) study, wherein mice were exposed to carbon black either prior to or after exposure to O_3, and then to both materials simultaneously. Simultaneous exposure produced evidence of interaction, while exposure to carbon black either before or after O_3 did not produce responses which were different from that due to exposure to O_3 alone. The authors' suggested that this was due to a reaction of O_3 on the surface of the carbon black particles in the presence of adsorbed water, producing surface bound, highly toxicologically active reactive oxygen species. Production of these species would not occur when the exposures were sequential.

Another mechanism may involve a pollutant-induced change in the local microenvironment of the lung, enhancing the effects of the co-inhalant. Thus, the observed synergism in rats between O_3 and acidic sulfates was suggested to be due to a shift in the local microenvironmental pH of the lung following deposition of acid, enhancing the effects of O_3 by producing a change in the reactivity or residence time of reactants, such as radicals, involved in O_3-induced tissue injury (Last et al., 1984). This hypothesis was examined in a series of studies (Last et al., 1983, 1984, 1986; Last and Cross, 1978; Warren and Last, 1987; Warren et al., 1986) in which rats were exposed to various sulfur oxide aerosols [H_2SO_4, $(NH_4)_2SO_4$, Na_2SO_4] with and without oxidant gases (O_3 or NO_2), and various biochemical endpoints examined. Acidic sulfate aerosols alone did not produce any response at concentrations that caused a response in conjunction with O_3 or NO_2. Further evidence that the synergism was due to H^+ was the finding that neither Na_2SO_4 nor NaCl was synergistic with O_3 (Last et al., 1986). But if this was the only explanation for acid/O_3 interaction, then the effects of ozone should be consistently enhanced by the presence of acid in an exposure atmosphere regardless of endpoint examined. However, in the study of Schlesinger et al. (1992b), in which rabbits were exposed for 3 h to combinations of 0.1, 0.3, and 0.6 ppm O_3 with 50, 75, and 125 $\mu g/m^3$ H_2SO_4 (0.3 μm), antagonism was noted

when evaluating stimulated production of superoxide anion by AMs harvested by lavage immediately after exposure to 0.1 or 0.3 ppm ozone in combination with 75 or 125 $\mu g/m^3$ H_2SO_4, and also for AM phagocytic activity at all of the ozone/acid combinations; there was no change in cell viability compared to air control.

The database for binary mixtures containing PM other than acid sulfates is quite sparse. But as with acidic sulfates, interaction depends upon pollutant combinations, exposure regimen and biological endpoints (see Table 11-26). Some interaction was noted following exposure of mice to mixtures of 9,400 $\mu g/m^3$ volcanic ash and 2.5 ppm SO_2 (Grose et al., 1985), in that synergism was suggested in terms of immune cell activity and numbers but no interaction was found with overall bacterial infectivity. On the other hand, exposure of mice to various concentrations of carbon black and formaldehyde (HCHO) produced no evidence of interaction in terms of bacterial infectivity but possible synergism in terms of macrophage phagocytic activity (Jakab, 1992).

The infectivity study of Jakab (1993), in which mice were exposed to acrolein and carbon black (Table 11-26), is of interest because, as mentioned earlier, the microbial agents were selected on the basis of the defense mechanisms they elicited. The results indicated that while particle or acrolein exposure alone did not alter infectivity from any of the microbes, exposure to the mixture did, and also suggested differential effects on different aspects of antimicrobial defense. For example, the increase in intracellular killing of *P. mirabilis* was ascribed to the increase in PMN levels after bacterial challenge. The reduced effectiveness for *L. monocytogenes* and influenza virus were somewhat more persistent, which led the authors to suggest that the particle/gas mixture had a greater impact upon acquired immune defenses than on innate defense mediated by AMs and PMNs, this being the major defense against *S. aureus* and *P. mirabilis*.

Another complex mixture examined was a combination of gaseous sulfur (IV), particulate sulfur (IV) and particulate sulfur (VI). A series of studies involved exposures (whole body) of Beagle dogs (M, 34 mo old) for 22.5 h/day, 7 days/week for up to 290 days to such an atmosphere, in which respirable sulfur IV (0.6 μm MMAD, σg=2) was maintained at a concentration of 300 $\mu g/m^3$ (Heyder et al., 1992; Maier et al., 1992; Kreyling et al., 1992; Schulz et al., 1992; Takenaka et al., 1992). Various biological endpoints were examined, and responses included reductions in nonspecific defense

TABLE 11-26. TOXICOLOGIC INTERACTIONS TO MIXTURES CONTAINING NON-ACID AEROSOL PARTICLES

Co-pollutant			Particle								
Chemical	μg/m	ppm [3]	Chemical	μg/m (μm)	Exposure Regime	$_3$ Exposure Conditions	Species, Gender Strain, Age or Body Weight	Endpoints	Response to Mixture	Interaction	Reference
SO_2	2,500	—	Volcanic ash	9,400 (0.65 μm, MMAD, σg=1.8)	2 h	Whole body	Mouse, F, CD-1, 4-8 weeks	Infectivity to Group C *Streptococcus* or virus given 0 or 24 h after exposure	No change in susceptibility to infection	None	Grose et al. (1985)
SO_2	2,500	—	Volcanic ash	9,400 (0.65 μm, MMAD, σg=1.8)	2 h	Whole body	Rat, M, Sprague-Dawley, 60-70 days	Lavaged cell nos. at 0 or 24 h PE	↑ PMN; ↑ lymphocytes; ↓ AM (no change in total cell no.)	Possible at 0 h: effect greater than either pollutant alone; similar to SO_2 alone at 24 h	Grose et al. (1985)
SO_2	2,500	—	Volcanic ash	9,400 (0.65 μm, MMAD, σg=1.8)	2 h	Whole body	Rat, M, Sprague-Dawley, 60-70 days	AM phagocytosis at 0 or 24 h PE	↓ phagocytic activity	Possible at 0 hr: effect greater than either pollutant alone; at 24 h: similar to SO_2 alone	Grose et al. (1985)
SO_2	2,500	—	Volcanic ash	9,400 (0.65 μm, MMAD, σg=1.8)	2 h/day, 5 days	Whole body	Rat, M, Sprague-Dawley, 60-70 days	Splenic lymphocyte response to mitogen (phytohemogglutinin)	Decrease	Possible synergism: no effect with either pollutant alone	Grose et al. (1985)
HCHO	1,000;	2.4-3	C black	1,000; 2,400-6,800 (2.45 μm, MMAD, σg=2.54)	4 h	Nose-only	Mouse, F, Swiss, 20-23 g	Infectivity of *S. aureus* administered prior to pollutant; differential counts in lavage	None	None	Jakab (1992)
HCHO	—	4.1-5	C black	4,800-13,200	4 h	Nose-only	Mouse, F, Swiss, 20-23 g	Infectivity of *S. aureus* administered prior to pollutant; differential counts in lavage	None	None	Jakab (1992)
SO_2	2,500	—	Volcanic ash	9,400 (0.65 μm, MMAD, σg=1.8)	2 h	Whole body	Rat, M, Sprague-Dawley, 60-70 days	Tracheal ciliary beat frequency at 0, 24, 72 h PE	Decrease	None: same as ash alone	Grose et al. (1985)
HCHO	—	2.4-3	C black	2,400-6,800 (2.45 μm, MMAD, σg=2.54)	4 h	Nose-only	Mouse, F, Swiss, 20-23 g	Infectivity of *S. aureus* administered prior to pollutant; differential counts in lavage	None	None	Jakab (1992)

TABLE 11-26 (cont'd). TOXICOLOGIC INTERACTIONS TO MIXTURES CONTAINING NON-ACID AEROSOL PARTICLES

Co-pollutant			Particle				Species, Gender Strain, Age or Body Weight	Endpoints	Response to Mixture	Interaction	Reference
Chemical	$\mu g/m^3$	ppm	Chemical	$\mu g/m$ (μm)	Exposure Regime	[3] Exposure Conditions					
HCHO	—	1	C black	1,000; and 2,400-6,800 (2.45μmMMAD, σg = 2.54)	4 h	Nose-only	Mouse, F, Swiss, 20-23 g	Infectivity of *S. aureus* administered prior to pollutant; differential counts in lavage	None	None	Jakab (1992)
HCHO	—	4.1-5	C black	4,800-13,200 (2.45 μm, MMAD, σg=2.54)	4 h	Nose-only	Mouse, F, Swiss, 20-23 g	Infectivity of *S. aureus* administered prior to pollutant; differential counts in lavage	None	None	Jakab (1992)
HCHO	—	1.8-2.8; 5	C black	4,700-6,100; 10,000	4 h/day, 4 days	Nose-only	Mouse, F, Swiss, 20-23 g	Infectivity of *S. aureus* administered 1 day after last pollutant exposure; differential counts in lavage	None	None	Jakab (1992)
HCHO	—	5	C black	10,000	4 h/day, 4 days	Nose-only	Mouse, F, Swiss, 20-23 g	F_c-receptor mediated Mø phagocytosis up to 40 days PE	↓ Phagocytic activity from day 25 PE, return to normal by day 40 PE	Possible synergism: no 3-day effect of C black or HCHO alone	Jakab (1992)
Acrolein	—	2.5	C black	10,000 (2.4 μm, MMAD, σ=2.75)	4 h/day, 4 days	Nose-only	Mouse, F, Swiss, 20-23 g	Infectivity to *S. aureus*, *P. mirabilis*, *L. monocytogenes*; influenza A virus administered 1 day PE	↓ Elimination of virus; ↓ killing of *L. monocytogenes*; ↓ killing of *S. aureus*; ↑ killing of *P. mirabilis* ↑ PMN count 4 h after *P. mirabilis* challenge; No change total cell no. by lavage after *S. aureus*	Possible synergism: no effect of either alone Possible: no effect of C black Possible: greater than either alone None	Jakab (1993)

TABLE 11-26 (cont'd). TOXICOLOGIC INTERACTIONS TO MIXTURES CONTAINING NON-ACID AEROSOL PARTICLES

Co-pollutant			Particle								
Chemical	$\mu g/m^3$	ppm	Chemical	$\mu g/m$ (μm)	Exposure Regime	Exposure Conditions	Species, Gender Strain, Age or Body Weight	Endpoints	Response to Mixture	Interaction	Reference
SO_2	2,700	—	Volcanic ash	9,400 (0.65, MMAD, σg=1.78)	2 h/day, 5 days	Whole body	Rat, Sprague-Dawley(40 days)	Pulmonary mechanics	Reduced tidal volume and peak expiratory flow; no effect on breathing frequency	None: effect due to SO_2	Raub et al. (1985)

capabilities of AMs such as phagocytosis and production of reactive oxygen species; increases in protein and β-N-acetylglucosaminidase in lavage fluid; increased rate of clearance of test particles from lungs to blood (suggesting a change in the permeability of the epithelium); minor changes in pulmonary function; and some histopathological effects, such as hyperplasia of respiratory epithelium of the posterior nasal passages and a slight (but not statistically significant) decrease in the volume density of alveolar septa. The exact role played by specific components of this mixture could not be determined because responses to individual components were not examined.

11.8.7.2 Human Studies of Particulate Matter Mixtures Other Than Acid Aerosols

Few studies have examined the effects of particles other than acid aerosols, despite the fact that ambient particulate matter consists of a mixture of soluble and insoluble material of varying chemical composition. Human safety considerations limit experimental exposures to particles considered to be essentially inert and non-carcinogenic. As reviewed in the 1982 Criteria Document (U.S. Environmental Protection Agency, 1982), Andersen et al. (1979) examined effects on healthy subjects of exposure to Xerox toner at concentrations ranging from 2,000 to 25,000 $\mu g/m^3$. These concentrations are not relevant to outdoor environmental exposures. Nevertheless, the studies were remarkable for the virtual absence of symptomatic or lung functional responses.

Utell et al. (1980) exposed healthy young subjects with acute influenza to a $NaNO_3$ aerosol (0.5 μm) or NaCl (control), and observed significant reductions in specific airway conductance in response to the $NaNO_3$ aerosol, but not to NaCl aerosol, for up to 1 week following the acute illness. These studies suggested that individuals with acute viral illness may experience bronchoconstriction from particulate nitrate pollutants that do not have effects on healthy subjects. However, the concentration of particles in these experiments was $\approx$7,000 $\mu g/m^3$, more than 100 times greater than peak ambient concentrations.

Three more recent studies have attempted to examine effects of exposure to carbon black particles, either alone or in combination with other pollutants (see Table 11-27). First, Kulle et al. (1986) exposed 20 healthy nonsmokers (10 males and 10 females) to air, 0.99 ppm SO_2, 517 $\mu g/m^3$ activated carbon aerosol (MMAD = 1.5 μm, GSD = 1.5), and SO_2 + activated carbon for four hours in an environmental chamber. Two 15-minute

TABLE 11-27. CONTROLLED HUMAN EXPOSURE STUDIES OF PARTICULATE MATTER MIXTURES OTHER THAN ACID AEROSOLS

Ref.	Subjects	Exposures	MMAD[2] (μm)	GSD[3] (μm)	Duration	Exercise	Temp (°C)	RH (%)	Symptoms	Lung Function	Other Effects	Comments
Green et al. (1989)	24 healthy 18 to 35 yrs	Air; activated carbon 510 μg/m^3; HCHO 3.01 ppm; carbon 510 μg/m^3 + HCHO 3.01 ppm	1.4	1.8	2 h	15 of each 30 min., 57 L/min	22	65	Increased cough with carbon + HCHO	No direct effects of carbon. Additive effects of carbon + HCHO on FVC, FEV_3, peak flow; decrements less than 5%.		
Kulle et al. (1986)	20 healthy 20 to 35 yrs	Air; activated carbon 517 μg/m^3; SO_2 0.99 ppm; carbon 517 μg/m^3 + SO_2 0.99 ppm.	1.5	1.5	4 h	15 min × 2, 35 L/min	22	60	No symptoms related to carbon exposure	No direct or additive effects of carbon exposure		
Yang and Yang (1994)	30 healthy 25 asthmatic 23 to 48 yrs	Mouthpiece: Bagged polluted air, TSP = 202 μg/m^3			30 min	At rest				Healthy subjects: no change Asthmatics: ↓FEV_1 ≈7%	Increased airway responsiveness in asthmatics reported; no allowance for change in airway caliber	No control exposure

exercise periods ($\dot{V}_E$ = 35 L/min) were included in the exposure. The exposure days were separated by one week and were bracketed by control air exposures on the day prior to and the day following the experimental exposure. Measurements included respiratory symptoms, spirometry, lung volumes, and airway responsiveness to methacholine. The carbon aerosol exposure resulted in no significant effects on symptoms or lung function, and exposure to carbon + SO_2 did not enhance the very small effects on lung function seen with SO_2 alone. Results of methacholine challenge testing were not provided.

Second, a separate report from the same laboratory (Green et al., 1989) examined potential interactions between formaldehyde (HCHO) and carbon exposure. Twenty-four healthy nonsmokers without airway hyperresponsiveness were exposed for two hours to air, 3 ppm HCHO, 510 $\mu g/m^3$ activated carbon aerosol (MMAD = 1.4 μm, GSD = 1.8) and HCHO + carbon. Exposures incorporated exercise ($\dot{V}_E$ = 57 L/min) for 15 of each 30 minutes. The exposures were separated by one week. Measurements included symptoms, spirometry, lung volumes, and serial measurements of peak flow. There were no significant effects on symptoms or decrements in lung function with exposure to carbon alone. The combination of carbon and HCHO increased cough at 20 and 80 minutes of exposure when compared to either pollutant alone. There were also small (less than 5%) but statistically significant decrements in FVC, FEV_3, and peak flow with carbon + HCHO, compared with either pollutant alone. The authors speculated that the enhancement of cough with carbon + HCHO resulted from increased delivery of HCHO adsorbed to carbon.

Finally, the studies by Anderson et al. (1992), summarized previously, were designed to test the hypothesis that inert particles in ambient air may become coated with acid, thereby delivering increased concentrations of acid sulfates to "sensitive" areas of the respiratory tract. Carbon black particles (MMAD $\approx$1 μm, GSD $\approx$2 μm) were coated with H_2SO_4 using fuming H_2SO_4. Electron microscopy findings suggested successful coating of the particles. Fifteen healthy and 15 asthmatic subjects were exposed for 1 h to acid-coated carbon, with a total suspended particulate concentration of 358 $\mu g/m^3$ for asthmatic subjects and 505 $\mu g/m^3$ for healthy subjects. On separate occasions, subjects were also exposed to carbon black alone ($\approx$200 $\mu g/m^3$, estimated as the difference between total suspended particulate and non-carbon particulate concentrations), H_2SO_4 alone ($\approx$100 $\mu g/m^3$), and air.

No adverse effects of particle exposure on lung function or airway responsiveness were observed for either study group.

Clinical studies of single particulate pollutants or simple mixtures may not be representative of effects that occur in response to complex ambient mixtures. In an attempt to examine effects of an ambient air pollution atmosphere under controlled laboratory conditions, Yang and Yang (1994) exposed 25 asthmatic and 30 healthy subjects to polluted air collected in a motor vehicle tunnel in Taiwan. This compressed air sample contained 202 $\mu g/m^3$ particles as well as 0.488 ppm NO_2, 0.112 ppm SO_2, and 3.4 ppm carbon monoxide (CO). The chemical and size characteristics of the particles were not provided. Mouthpiece exposure to polluted air was performed at rest for 30 min, and lung function and methacholine responsiveness were assessed after exposure. Small but significant decrements in FEV_1 and FVC were observed in asthmatic, but not healthy subjects when compared with baseline measurements. However, no control exposure to air was performed, which seriously limits interpretation of these results. The small decrements in lung function could have resulted from exposure conditions other than the pollutants, such as humidity or temperature of the inhaled air, which were not specified.

Thus, few studies have examined effects of particles other than acid aerosols on lung function, although available data suggest inert particles in the respirable range have little or no acute effects at levels well above ambient concentrations. Other than the studies of Rudell et al. on diesel exhaust discussed in Section 11.5.1, no studies have examined effects on mucociliary clearance, epithelial inflammation, or host defense functions of the distal respiratory tract in humans.

11.9 PHYSICOCHEMICAL AND HOST FACTORS INFLUENCING PARTICULA MATTER TOXICITY

11.9.1 Physicochemical Factors Affecting Particulate Matter Toxicity

The physicochemical factors modulating biological responses to PM are not always clear. However, the available toxicological database does allow for some speculation as to factors which may influence biological responses to diverse types of PM. For example, the toxic potency of inorganic particles may be related to certain physicochemical characteristics.

While the bulk chemical makeup of a particle would clearly influence its toxicity, responses may also be driven by chemical species adsorbed onto the particle surface, even for those particles considered to have low intrinsic toxicity. Furthermore, certain physical properties of particles, such as size or surface area, and of aerosols, such as number concentration, may be factors in determining reponses to PM. This section provides an overview of current hypotheses concerning particle characteristics which may relate to toxicity.

Particle Acidity: It should be clear from discussions in Section 11.2 that the deposition of acidic particles in the respiratory tract can result in various biological effects. The bulk of the toxicologic database on acidic PM involves sulfate particles, primarily H_2SO_4 and the available evidence indicates that the observed responses to these are likely due to the H^+, rather than to the $SO_4^=$. Thus, effects observed for this pollutant likely apply to any acidic particle having a similar deposition pattern in the respiratory tract, although the specific chemical composition of different acids may be a factor mediating the quantitative response (Fine et al., 1987a). In terms of H^+, the irritant potency of an acid aerosol may be related more to the total available H^+ concentration (i.e., titratable acidity in lung fluids following deposition) rather than to the free H^+ concentration as measured by pH (Fine et al., 1987b). In any case, the response to acidic particles appears to be due to a direct irritant action and/or the subsequent release of humoral mediators.

Acidic particles exert their action throughout the respiratory tract, with the response and location of effect dependent upon particle size and mass concentration. They have been shown to alter bronchial responsiveness, mucociliary transport, clearance from the pulmonary region, regulation of internal cellular pH, production of cytokines and reactive oxygen species, pulmonary mechanical function, and airway morphology.

Particles do not have to be pure acid droplets to elicit health effects. The acid may be associated with another particle type. For example, in the study of Chen et al. (1990), guinea pigs were exposed to two different fly ashes, one derived from a low sulfur coal and one from a high sulfur coal (Table 11-19). Levels of acidic sulfates associated with the fly ash were found to be proportional to the coal sulfur content, and greater effects on pulmonary functional endpoints were noted for the high sulfur fly ash than for the low sulfur fly ash.

Particle Surface Coatings: The presence of surface coatings may make certain particles more toxic than expected based solely upon particle core composition. This was noted in studies of acid-coated metal oxides (Section 11.2.3) and is discussed in greater detail in Section 11.3.8. Certain surface metals may be especially important in this regard, and because trace metal species vary geographically, this may account to some extent for particles in different areas having different toxic potentials.

Particle Size: Studies which have examined PM-induced mortality seem to suggest some inherent potential toxicity of inhaled ultrafine particles (Section 11.4), and other endpoints appear to show this as well. This is especially important when considering particles which may have low inherent toxicity at one size, yet greater potency at another. However, the mechanism which underlies a size-related difference in toxicity is not known at this time.

To compare toxic potency of particles of different sizes, intratracheal instillation has often been used. This technique allows the delivery of equivalent doses of different materials and avoids differences in deposition which would occur if particles of different sizes were inhaled. While this approach may highlight inherent similarities and differences in responses to particles of various sizes, in reality, there would be greater deposition of singlet ultrafine particles (in the size range used in the toxicology studies described) in the lungs, especially within the alveolar region, than for the larger fine or coarse mode particles.

The release of proinflammatory mediators may be involved in lung disease, and their levels may be increased with exposure to ultrafine particles. For example, Driscoll and Maurer (1991) compared effects of instilled fine (0.3 μm) or ultrafine (0.02 μm) TiO_2, in rat (F344) lungs. Concentrations were 10,000 μg particles/kg BW. Lavage was performed up to 28 days post-exposure, and pathology was assessed at this 28-day time point. While both size modes produced an increase in the number of AMs and PMNs in lavage, the increase was greater and more persistent with the ultrafine particles. The release of another monokine, tumor necrosis factor (TNF), by AMs was stimulated with both sizes, but again the response was greater and more persistent for the ultrafines. A similar response was noted for fibronectin produced by AMs. Finally, fine particle exposure resulted in a minimally increased prominence of

particle-laden macrophages associated with alveolar ducts, while ultrafine particle exposures produced somewhat of a greater prominence of macrophages, some necrosis of macrophages and slight interstitial inflammation associated with the alveolar duct region. In addition, increased collagen occurred only with ultrafine particle exposure.

Oberdörster et al. (1992) instilled rats with 500 μg TiO_2 in either fine (0.25 μm) or ultrafine (0.02 μm) sizes, and performed lavage 24 h later. Various indicators of acute inflammation were altered with the ultrafine particles; this included an increase in the number of total cells recovered, a decrease in percentage of AMs and increase in percentage of PMNs, and an increase in protein. On the other hand, instillation of the fine particles did not cause statistically significant effects. Thus, the ultrafine particles had greater pulmonary inflammatory potency than did the larger size particles of this material. The investigators attributed enhanced toxicity to greater interaction of the ultrafine particles, with their large surface area, with alveolar and interstitial macrophages, resulting in enhanced release of inflammatory mediators. They suggested that ultrafine particles of materials of low in vivo solubility appear to enter the interstitium more readily than do larger size particles of the same material, which accounted for the increased contact with macrophages in this compartment of the lung. In support of these results, Driscoll and Maurer (1991) noted that the pulmonary retention of ultrafine TiO_2 particles instilled into rat lungs was greater than for the same mass of fine mode TiO_2 particles.

Not all ultrafine particles will enter the interstitium to the same extent, and this may influence toxicity. For example, both TiO_2 (~20 nm) and carbon black (~20 nm) elicit an inflammatory response, yet much less of the latter appears to enter the interstitium after exposure (Oberdörster et al., 1992). Since different particles may induce chemotactic factors to different extents, it is possible that less chemotoxis with TiO_2 results in less contact with and phagocytosis by macrophages, a longer residence time at the area of initial deposition, and a resultant greater translocation into the interstitium. Similarly, Brown et al. (1992; Table 11-23) noted following inhalation exposure of rats to TiO_2 or coal mine dust that the former did not affect macrophage chemotaxis, while the latter reduced it; the coal dust also produced a greater inflammatory response than did the TiO_2. This is consistent with less interaction of coal dust with AMs and greater movement into the interstitium.

The above studies appear to support the concept of some inherent toxicity of ultrafine particles compared to larger ones. Both particle size and the resultant surface area of a unit

mass of particles likely influences toxic potential. Surface area is important because, as noted above, adsorption of certain chemical species on particles may enhance their toxicity, and this could be an even greater factor for ultrafine particles with their larger surface area per unit mass.

Other studies have compared effects following exposures to larger than ultrafine particle sizes, and the results ranged from none detectable to some particle size-related differences. Raub et al. (1985) instilled into rats coarse mode (12.2 μm) and fine mode (2.2 μm) volcanic ash at two dose levels, 50,000 or 300 μg particles/animal. The coarse mode produced a change in end expiratory volume, but no changes in other pulmonary function endpoints (i.e., frequency, V_T, peak inspiratory and expiratory flows, VC, RV, TLC). When lungs were examined 6 mo after instillation, animals exposed to the low dose of either size fraction showed no changes in lung weight or hydroxyproline content compared to control, while those exposed to the high concentration of coarse mode ash showed increased lung weight. In terms of histopathology, both size modes produced some focal alveolitis. Thus, there were essentially no differences in responses between the two size modes, especially at the low exposure dose. In a similar study, Grose et al. (1985) instilled mice with 42 μg/animal of volcanic ash in the same two size fractions as above, coarse and fine, 24 h prior to challenge with bacteria *(Streptococcus sp.)*. A small, but similar, increase in susceptibility to infection was noted with both particle sizes.

Shanbhag et al. (1994) exposed a mouse macrophage cell line (P388D1) to particles of two different composition (TiO_2 or latex) at comparable sizes, 0.15 and 0.45 μm for the former, and 0.11 and 0.49 for the latter. They also used pure titanium at 1.76 μm for comparison to latex at 1.61 μm. In order to examine effects of particle surface area, the cells were exposed to a constant surface area of particles, expressed in terms of mm^2 per unit number of cells. This was obtained based upon particle size and density and, therefore, the weight percentage was greater for larger particles than for smaller ones for the same surface area. Furthermore, because of particle density differences, the weight percentage for similarly sized particles of different materials to obtain the same surface area also differed. The authors noted that at a constant total particle surface area to cell ratio, the 0.15 and 0.45 μm particles were likely to be less inflammatory than were the 1.76 μm particles, in that the smaller particles produced lower elicited levels of interleukin-1 and less cell

proliferation. These results indicate that the larger particles had greater toxicity than the smaller ones in this experimental system. Thus, the exact relationship between particle size and toxicity is not resolved. It may differ for different size modes and may also depend on the specific experimental system used.

Particle Number Concentration: The number concentration of particles within an aerosol will increase as the size of the constituent particles decrease. Thus, for a given mass concentration of a material, there would be greater particle numbers in an ultrafine aerosol than in a fine aerosol. As previously discussed (Section 11.2.3), studies have shown various biological responses, such as reductions in lung volumes and diffusion capacity, alterations in biochemical markers, and changes in lung tissue morphology, in guinea pigs following exposure to ultrafine ZnO having a surface layer of H_2SO_4. These responses were much greater than were found following exposure to H_2SO_4 aerosols in pure droplet form yet having a similar mass concentration.

A possible contribution to this differential response is that the number concentration of particles in the exposure atmospheres were different, resulting in different numbers of particles deposited at target sites. At an equal total sulfate mass concentration, H_2SO_4 existed on many more particles when layered on the ZnO carrier particles than when dissolved into aqueous droplets (i.e., pure acid aerosol); this was because the particle size distribution of the former aerosol was smaller than that of the latter. Therefore, it is possible that the greater the number of particles containing H_2SO_4, the greater will be the number of cells affected after these particles deposit in the lungs, and the more severe will be the overall biological response. While differences in particle size distributions between the coated and pure acid particles may have influenced the results to some extent, a recent study (Chen et al., 1995) confirmed that the number of particles in the exposure atmosphere, not just total mass concentration, is an important factor in biological responses following acidic sulfate particle inhalation when aerosols having the same size distribution were compared.

11.9.2 Host Factors Affecting Particulate Matter Toxicity

Not only do the differences in particle chemistry and morphology influence responses to inhalation of particulate matter, but also various factors related to host susceptibility. One obvious example is the differences associated with species susceptibility as well as differences

in dosimetry related to animal mass and lung structure and geometry. Host health status, specifically the presence of pulmonary inflammation or bacterial or viral infection or nutritional status also may markedly alter responses to PM. The presence of chronic pulmonary disease is also a factor in both animals and humans. Age of the animal, especially very young or very old, can influence susceptibility**.**

Host Health Status: Epidemiological studies suggest there may be subsegments of the population that are especially susceptible to effects from inhaled particles (see Chapter 12). One particular group may be those having lungs compromised by respiratory disease. However, most toxicology studies have used healthy adult animals, and there are very few data to allow examination of the effects of different disease states upon the biological response to PM. A number of studies have examined the effects of lung disease on deposition and/or clearance of inhaled aerosols, and these are discussed in Chapter 10. Alterations in deposition sites and clearance rates/pathways due to concurrent disease may impact upon dose delivered from inhaled particles, and thus influence ultimate toxicity.

Some work has been performed with sulfate and nitrate aerosols using models of compromised hosts. Rats and guinea pigs with elastase-induced emphysema were examined to assess whether repeated exposures (6 h/day, 5 days/week, 20 days) to $(NH_4)_2SO_4$ (1,000 $\mu g/m^3$, 0.4 μm MMAD) or NH_4NO_3 (1,000 $\mu g/m^3$, 0.6 μm MMAD) would alter pulmonary function compared to saline-treated controls (Loscutoff et al., 1985). Similarly, dogs having lungs impaired by exposure to NO_2 were treated with H_2SO_4 (889 $\mu g/m^3$, 0.5 μm, 21 h/day, 620 days) (Lewis et al., 1973). Results of both of these studies indicated that the specific induced disease state did not enhance the effect of acidic sulfate aerosols in altering pulmonary function; in some cases, there were actually fewer functional changes in the diseased lungs than in the unimpaired animals. It is possible, however, that other types of disease states could result in enhanced response to inhaled acidic aerosols; as mentioned, asthma is a likely one, but there are no data to evaluate whether effects are enhanced in animal models of human asthma.

Few studies have examined effects of other particles in health compromised host models. Mauderly et al. (1990) exposed young rats having elastase-induced emphysema to whole diesel exhaust (3,500 μg soot/m^3) for 24 mo (7 h/day, 5 days/week). Various endpoints were examined after exposure, including pulmonary function (e.g., respiratory

pattern, lung compliance, DLco), biochemical components of BAL (e.g., enzymes, protein, collagen), and histopathology and morphometry. There was no evidence that the diseased lungs were more susceptible to the diesel exhaust than were normal lungs. In fact, in some cases, there seemed to be a reduced effect of the diesel exhaust in the emphysematous lungs. But this could be due to a reduced lung burden in the diseased lungs, resulting from differences in deposition and/or clearance compared to normal lungs.

Rats having elastase-induced emphysema were exposed to 9,400 $\mu g/m^3$ (0.65 μm) Mt. St. Helens volcanic ash for 2 h/day for 5 days (Raub et al., 1985; Table 11-19), with and without 2,700 $\mu g/m^3$ SO_2. Effects on pulmonary mechanics were similar to those noted in normal animals exposed to the same atmospheres.

Raabe et al. (1994) exposed rats with elastase-induced emphysema to two particle atmospheres, a California-type aerosol and a London-type aerosol. The former consisted of 1.1 to 1.5 μm (MMAD; σg = 1.7 to 2.4) particles of graphitic carbon, natural clay, NH_4HSO_4, $(NH_4)_2SO_4$, NH_4NO_3, and trace amounts of metals ($PbSO_4$, $VOSO_4$, $MnSO_4$, and $NiSO_4$). The latter consisted of 0.8 to 0.9 μm particles (σg = 1.7 to 1.8) of NH_4HSO_4, $(NH_4)_2SO_4$, coal fly ash, and lamp black carbon. The elastase treated rats showed increased lung DNA and RNA, a general marker for repair of cell damage. Exposure for 3 days (23 h/day) to the London aerosol produced a further increase not seen in exposed normal rats. A 30-day exposure to the California aerosol enhanced small airway lesions in the elastase-treated animals. These preliminary results suggest that the California aerosol and the London aerosol both caused significant responses in animals with elastase-induced emphysema, but clarification of these responses must await a more comprehensive treatment of these data.

Thus, the available toxicological database indicates only limited evidence of enhanced susceptibility to PM of "compromised" hosts. However, these studies were restricted to emphysema models and it is not known whether other simulated pulmonary diseases would enhance susceptibility to PM in laboratory animals.

***Species Differences**:* The effects of asbestos-free talc at 6,000 or 18,000 $\mu g/m^3$ (2.7-3.2 μm) were studied in male and female F344 rats and B6C3F1 mice exposed 6 hours/day 5 days/week for 24 mo (National Toxicology Program, 1993). In rats and mice exposed to the higher concentration for 24 mo the specific talc lung burdens (mg/g lung)

were nearly identical. Rats had a greater increase than mice in lung weight as well as greater elevations of neutrophils, enzymes and protein in BALF. The histopathology of rats, including accumulations of talc-filled macrophages, inflammation, epithelial hyperplasia and squamous metaplasia, and focal fibrosis was identical to that described for other dusts. The histopathology differed in that the epithelial hyperplasia and metaplasia, and focal fibrosis observed in rats was absent in mice. These findings illustrate that differences between the responses of rats and mice persist across a wide range of different types of inhaled dusts.

There are a few reports comparing the responses of other species to chronic dust inhalation (Mauderly, 1994a). Alarie et al. (1973, 1975) studied the response of cynomolgus monkeys and guinea pigs chronically exposed to coal combustion fly ash in combination with H_2SO_4. In the study (Alarie et al., 1973), monkeys and guinea pigs were exposed 23+ hours/day 7 days/week for either 52 weeks (guinea pigs) or 78 weeks (monkeys) to approximately 500 μg ash/m^3 (MMAD $\approx$2.6μm) in combination with 0.1 to 5.0 ppm sulfur dioxide. Although particles accumulated in the lungs in both species (including bronchial and alveolar deposition) and caused slight inflammation, type II cell proliferation was observed in guinea pigs but not monkeys. In the second study (Alarie et al., 1975), guinea pigs and monkeys were exposed 23+ hours/day 7 days/week for 18 mo to approximately 500 μg ash/m^3 (MMAD $\approx$4-5μm) in combination with 100 or 1,000 μg sulfuric acid mist/m^3. The effects attributed to fly ash were similar to those described in the first study. Comparison between guinea pigs and monkeys in this series of studies is complicated because the concentrations of co-pollutants and fly ash were not always equivalent and the deposition pattern of the 2.6-5.3 μm fly ash particles is undoubtedly different in monkeys than in guinea pigs.

Comparison of Human and Laboratory Animal Response: There are limited data allowing direct comparisons of responses of humans and laboratory animals to ambient particulate matter constituents. Chronic occupational exposures to high concentrations of mineral dusts cause pneumoconioses in human lungs, consisting primarily of fibrotic responses with many features similar to those observed in animals. Exposure to silica and dusts with high quartz content causes granulomatous lesions in both human and animal lungs. Merchant et al. (1986) provided a comprehensive review of the pulmonary responses to coal dust in coal workers. The focal collections of dust (macules) and the progressive focal

fibrosis have many 1989). features similar to the responses of rats (Martin et al., 1977; Lewis et al., Although little information is available on the effects of coal dust in other animals, Heppleston (1954) reported dust accumulations and responses in the lungs of rabbits and ponies that were similar to the responses seen in humans. Emphysema, a common feature of pneumoconiosis even in nonsmoking coal workers (Green et al., 1992) is not a prevalent finding in other species and is usually found only in association with large scars in rats (Mauderly et al., 1988). Other features including the epithelial hyperplasia of rodents and squamous metaplasia of rats are not seen in coal workers' pneumoconiosis.

There are obviously similarities and differences between animals and humans and among animals in their responses to chronic dust inhalation. It is not yet clear which, if any, animal species is a good model for predicting noncancer pulmonary responses of humans to chronic dust exposure. The most common bioassay species, rats and mice, clearly differ in their responses, but it is not clear which best represents humans.

Age of Animals: There is limited information on the effects of inhaled particles as a function of changes occurring with age in laboratory animals Mauderly (1989). Mauderly et al. (1987c) exposed rats for 6 mo to diluted, whole diesel exhaust containing 3500 $\mu g/m^3$ (MMAD $\approx 0.25 \mu m$) soot particles. Effects in rats conceived and born in the exposure chambers and exposed up to 6 mo of age were compared to those of rats exposed between 6 and 12 mo of age. Soot accumulated in similar amounts in the lungs of both the young and adult groups, but soot-laden macrophages formed more intraalveolar aggregates in the adults. Tissue responses adjacent to the aggregated macrophages were greater in the adults than in the young rats. Lung weight and the cellularity of pulmonary lymph nodes increased and particle clearance was delayed in the older group, but not in the younger group. Exposure throughout the period of lung development did not cause differences between the lung morphology or respiratory function of exposed and sham-exposed young rats after they reached adulthood (6 mo of age). These results indicate that rats with developing lungs may be less susceptible than adults to the effects of diesel exhaust.

Mauderly (1989) indicates that there is insufficient information on the influence of age on the effects of inhaled particles. It is therefore inappropriate to draw conclusions regarding age-related susceptibility at the present time.

11.10 POTENTIAL PATHOPHYSIOLOGICAL MECHANISMS FOR THE EFFECT CONCENTRATIONS OF PARTICULATE POLLUTION

11.10.1 Physiological Mechanisms

The pathophysiologic mechanisms by which low level ambient particle concentrations may increase morbidity and mortality are not clear. Potential mechanisms might be posited through examining hypotheses considering the pathological mechanisms by which inhaled particles might alter normal physiological, immunological, and biochemical processes in the lung.

In the healthy person, air is drawn into the respiratory tract through a branching airway network. Although the large airways of the tracheobronchial region continuously branch into narrower airways the increase in total cross sectional area makes resistance to airflow low. The inspired air ultimately enters the alveolar or gas exchange region of the lung where the area available for the diffusion of gases is large and the distances for diffusion across the respiratory membrane are minimal.

In the healthy person, the pulmonary circulation is a low resistance system requiring only about 1/5 of the pressure required to pump blood through the high resistance systemic circuit. Any changes in the pulmonary vasculature that increase the resistance to blood flow through the lungs will impose an additional work load on the right ventricle which, if severe enough in a compromised individual, could result in right heart failure.

In considering the potential mechanisms by which increases in ambient PM might affect morbidity and mortality, it is important to consider the physiological characteristics of the population most affected. In general, the population most susceptible to elevations in ambient PM is older (see chapter 12) and may have preexisting respiratory disease. As the healthy older population (Folkow and Svanborg, 1993; Dice, 1993; Lakatta, 1993) ages, cardiorespiratory function, including lung volumes, FEV_1, and cardiac output reserve (Kenney, 1989) decline. Many of the decrements in physiological function associated with the aging process also may be associated with pathological changes caused by disease or other environmental stressors impacting a person over their lifespan.

There is little information on the extent to which an elderly population might be more susceptible to the effects of particulate pollution in the ambient environment (Cooper et al., 1991). The elderly might be expected to be more susceptible to particulate pollution because

of numerous changes in the body's protective mechanisms. While young and healthy animals might be more adaptable, older animals and those with chronic illness have a more limited ability to adapt to environmental stressors.

11.10.2 Physiological-Particle Interaction

Particles inhaled into the respiratory tract deposit at a variety of sites depending on their size, shape, and the pulmonary ventilation characteristics of the organism. Once deposited, the particles may be cleared by from the lung, sequestered in the lymphatics, metabolized or otherwise transformed by mechanisms described in Chapter 10.

If the particle mass inhaled into the lung is so excessive that the normal pulmonary clearance mechanisms are overwhelmed, or if repeated insult from toxic particles has somehow reduced the ability of normal mechanisms to clear particles, then particles, their degradation products, and metabolic products associated with the clearance process may accumulate and present an additional stress to the organism. This stress may affect the entire organism and not just the respiratory tract. While a young healthy organism may tolerate or adapt to the consequences of an excessive particle load, an older organism or one with chronic respiratory disease or one rendered more susceptible by other stressors (dietary, crowding, thermal, etc.) may become sicker or may die. Thus, it is possible that death of an organism may be the result of an accumulation of lifetime stressors (or, the response to these stressors) that is exacerbated by the addition of an incremental particle load on the system.

Cardiorespiratory system function may be compromised and become less efficient in older people or as a result of disease. Inhaled particles could, conceivably, further compromise the functional status in such individuals. Because a small increase in environmental particle concentrations would not be lethal to most subjects, the terminal event(s) must presumably result from a triggering or exacerbating of a lethal failing of a critical function, such as ventilation, gas exchange, pulmonary circulation, lung fluid balance, or cardiovascular function in subjects already approaching the limits of tolerance due to preexisting conditions.

11.10.3 Pathophysiologic Mechanisms

It is conceivable that inhaled particles, their reaction products, or the physiological response to deposited particles may further impair ventilation in the chronically ill individual. Inhaled particles may induce further bronchoconstriction and increase resistance to air flow by activating airways smooth muscle, as in asthmatics. Inhaled particles may also influence various airway secretions that could add to and thicken the mucous blanket leading to mucus plugging or decreased mucociliary clearance. Increases in airways resistance would increase the work of breathing and, in turn, the increased effort would require a greater proportion of the inhaled oxygen for the respiratory muscles and increase the potential risk of respiratory failure.

Inhaled particles or their pathophysiological reaction products could also act at the alveolar capillary membrane. At this site, inhaled particles could decrease the diffusing capacity of the lungs by increasing diffusion distances across the respiratory membrane (by increasing the thickness of the respiratory membrane) and causing abnormal ventilation-perfusion ratios in parts of the lung by altering ventilation distribution.

Inhaled particles, especially ultrafine particles could also act at the level of the pulmonary vasculature. Inhaled particles or the pathophysiological reaction to inhaled particles could elicit changes in pulmonary vascular resistance that could further alter ventilation perfusion abnormalities in people with respiratory disease. Particles could also cause alteration of the distribution of ventilation by causing changes in airway resistance. Diseases such as emphysema destroy alveolar walls as well as the pulmonary capillaries they contain. This causes a progressive increases in pulmonary vascular resistance and elevates pulmonary blood pressure. The generalized systemic hypoxia could result in further pulmonary hypertension and interstitial edema that would impose an increased workload on the heart.

Potential mechanisms which might be evoked to explain the phenomenon of particle related mortality have been considered by Utell and Frampton (1995). Mechanisms which could conceivably account for the particle-related mortality include: (1) "premature" death, that is the hastening of death for individuals already near death (i.e., hastening of an already certain death by hours or days); (2) increased susceptibility to infectious disease; and (3) exacerbation of chronic underlying cardiac or pulmonary disease.

Particulate pollution could contribute to daily mortality rates by affecting those at greatest risk of dying; those individuals for whom death is already imminent. Elevated concentrations of particulate matter, which might be only a minor irritant to healthy people, could be the "last straw" that tips over the precariously balanced physiology of a dying patient. In developing this possibility, Utell and Frampton (1995) have compared the effect associated with particulate matter with that associated with temperature deviations. Time-series analyses have shown relationships between temperature changes, regardless of the direction change, and increasing mortality of a magnitude similar to that described for air pollution (Kunst et al., 1993). While there are a few deaths that can be attributed to hyperthermia and hypothermia, the excess mortality due to moderate temperature deviations is associated primarily with the chronically and terminally ill. It is this excess mortality that is likely caused by further stress on over-burdened compensatory mechanisms.

However, if particulate air pollution simply represents a physiological stress similar to thermal stress, and the excess mortality is occurring among individuals who would have died within days or weeks, one would expect to see a "harvesting effect". That is, following the increase in mortality associated with an increase in particulate pollution mortality should fall below baseline, because some of those at risk will have already died. Although Kunst et al. (1993) have reported such an effect with temperature-related mortality, it has not been evident in epidemiology studies of ambient particulate exposure. It is possible however, that epidemiologic studies may not be sensitive enough to detect a harvesting effect because the overall changes in mortality are small. However, even in the 1952 London Fog episode, there was no decline in mortality following the peak in excess deaths; instead, increased mortality appeared to remain somewhat elevated in the days after pollution levels had returned to baseline (Logan, 1953).

Other studies suggest that the effect of particles on mortality cannot be explained solely by death-bed effects. In longitudinal studies, Dockery et al. (1993) and Pope et al. (1995) found a strong association between particulate air pollution and mortality in U.S. cities after adjusting for cigarette smoking and other risk factors. Moreover, mortality and respiratory illness in the Utah Valley have been linked with particulate exposure associated with a steel mill. These findings indicate an effect on annual mortality rates that cannot be explained by hastening death for individuals already near death.

Particle exposure could increase susceptibility to infection with bacteria or respiratory viruses, leading to an increased incidence of, and death from, pneumonia in susceptible members of the population. Potential mechanisms could include effects on mucociliary clearance, alveolar macrophage function, adherence of microorganisms to epithelia, and other specific or nonspecific effects on the immune response. However, pneumonia rarely results in death within 24 h of onset; serious infections of the lower respiratory tract generally take days or weeks to evolve. This would potentially contribute to morbidity effects from PM that are lagged by several days or weeks (Chapter 12). If pollutant exposure increased susceptibility to infectious disease, it should be possible to detect differences in the incidence of such diseases in communities with low vs. high particulate concentrations. It might be expected that emergency room visits and hospitalizations for pneumonia caused by the relevant agent should be measurably higher on days with elevated ambient particle concentrations. Examples of this are evident in data from several cities (see Chapter 12). Laboratory animal studies indicate that PM exposure can impair host defenses. Exposure to acidic aerosols has been linked with alterations in mucociliary clearance and macrophage function. However, bacterial infectivity studies with exposure to non-acidic aerosols and other particulate species have not been shown experimentally to cause increased infection.

What chronic disease processes are most likely to be affected by inhaled particulate matter? To explain the daily mortality statistics, there must be common conditions that contribute significantly to overall mortality from respiratory causes. The most likely candidates are the chronic airways diseases, particularly chronic obstructive pulmonary disease (COPD). COPD is the fourth leading cause of death in the US, and is the most common cause of non-malignant respiratory deaths, accounting for more than 84,000 deaths in 1989 (U.S. Bureau of the Census, 1992). This group of diseases encompasses both emphysema and chronic bronchitis, however, information on death certificates does not allow differentiation between these diagnoses. The pathophysiology includes chronic inflammation of the distal airways as well as destruction of the lung parenchyma. There is loss of supportive elastic tissue, so that the airways collapse more easily during expiration, obstructing flow. Processes that enhance airway inflammation or edema, increase smooth muscle contraction in the conducting airways, or slow mucociliary clearance could adversely affect gas exchange and host defense. Moreover, the uneven ventilation-perfusion matching

characteristics of this disease, with dependence on fewer functioning airways and alveoli for gas exchange, means inhaled particles may be directed to the few functioning lung units in higher concentration than in normal lungs (Bates, 1992)

Asthma is a common chronic respiratory disease that may be exacerbated by air pollution. Mortality from asthma (about 3% of all respiratory deaths) has been rising in the last 15 years (Gergen and Weiss, 1992), and air pollution has been implicated as a potential causative factor. Atmospheric particle levels have been linked with increased hospital admissions for asthma, worsening of symptoms, decrements in lung function, and increased medication use. The incidence of asthma is higher among children and young adults. Although asthma deaths are rare below the age of 35, asthma is the leading cause of non-infectious respiratory mortality below the age of 55. Nevertheless, approximately 70% of all asthma-related deaths occur after age 55 (National Center for Health Statistics, 1993). Death due to asthma may contribute to overall PM-related mortality but it is doubtful that asthma is a leading cause.

Particulate pollutants have been associated with increases in cardiovascular mortality both in the major air pollution episodes and in the more recent time-series analysis. Bates (1992) has postulated three ways in which pollutants could affect cardiovascular mortality statistics. These include: acute airways disease misdiagnosed as pulmonary edema; increased lung permeability, leading to pulmonary edema in people with underlying heart disease and increased left atrial pressure; and, acute bronchiolitis or pneumonia induced by air pollutants precipitating congestive heart failure in those with pre-existing heart disease. Moreover, the pathophysiology of many lung diseases is closely intertwined with cardiac function. Many individuals with COPD also have cardiovascular disease caused by: smoking; aging; or pulmonary hypertension accompanying COPD. Terminal events in patients with end-stage COPD are often cardiac, and may therefore be misclassified as cardiovascular deaths. Hypoxemia associated with abnormal gas exchange can precipitate cardiac arrhythmias and sudden death.

In comparison to healthy people, individuals with respiratory disease have greater deposition of inhaled aerosols in the fine ($PM_{2.5}$) mode. The deposition of particles in the lungs of a COPD patient may be as much as three-fold greater than in a healthy adult. Thus, the potential for greater target tissue dose in susceptible patients is present. The lungs of

individuals with chronic lung diseases, such as asthma, bronchitis, emphysema, etc. are often in a chronic state of inflammation. In addition to the fact that particles can induce an inflammatory response in the respiratory region, the influence of particles on generation of proinflammatory cytokines may be enhanced by the prior existence of inflammation. Phagocytosis by alveolar macrophages is down-regulated both by inflammation and the increased levels of ingested particles. Therefore, people with lung disease not only have greater particle deposition, but the conditions that exist in their lungs prior to exposure are conducive to amplification of the effects of particles and depression of their clearance.

11.11 SUMMARY AND CONCLUSIONS

11.11.1 Acid Aerosols

The results of human studies indicate that healthy subjects do not experience decrements in lung function following single exposures to H_2SO_4 at levels up to 2,000 $\mu g/m^3$ for 1 h, even with exercise and use of acidic gargles to minimize neutralization by oral ammonia. Mild lower respiratory symptoms (cough, irritation, dyspnea) occur at exposure concentrations in the mg/m^3 range. Acid aerosols alter mucociliary clearance in healthy subjects, with effects dependent on exposure concentration and the region of the lung being studied.

Asthmatic subjects appear to be more sensitive than healthy subjects to the effects of acid aerosols on lung function, but the effective concentration differs widely among studies. Adolescent asthmatics may be more sensitive than adults, and may experience small decrements in lung function in response to H_2SO_4 at exposure levels only slightly above peak ambient levels. Although the reasons for the inconsistency among studies remain largely unclear, subject selection and acid neutralization by NH_3 may be important factors. Even in studies reporting an overall absence of effects on lung function, occasional asthmatic subjects appear to demonstrate clinically important effects. Two studies from different laboratories have suggested that responsiveness to acid aerosols may correlate with degree of baseline airway hyperresponsiveness. There is a need to identify determinants of responsiveness to H_2SO_4 exposure in asthmatic subjects. In very limited studies, elderly and individuals with

chronic obstructive pulmonary disease do not appear to be particularly susceptible to the effects of acid aerosols on lung function.

Two recent studies have examined the effects of exposure to both H_2SO_4 and ozone on lung function in healthy and asthmatic subjects. In contrast with previous studies, both studies found evidence that 100 $\mu g/m^3$ H_2SO_4 may potentiate the response to ozone.

Human studies of particles other than acid aerosols provide insufficient data to draw conclusions regarding health effects. However, available data suggest that inhalation of inert particles in the respirable range, including three studies of carbon particles, have little or no effect on symptoms or lung function in healthy subjects at levels above peak ambient concentrations.

The bulk of the laboratory animal toxicologic data base on PM involves sulfur oxide particles, primarily H_2SO_4, and the available evidence indicates that the observed responses to these are likely due to H^+ rather than to $SO_4^=$.

Acidic sulfates exert their action throughout the respiratory tract, with the response and location of effect dependent upon particle size and mass and number concentration. At very high concentrations that are not environmentally realistic, mortality will occur following acute exposure, due primarily to laryngospasm or bronchoconstriction; larger particles are more effective in this regard than are smaller ones. Extensive pulmonary damage, including edema, hemorrhage, epithelial desquamation, and atelectasis can also cause mortality, but even in the most sensitive animal species, concentrations causing mortality are quite high, at least a thousand-fold greater than current ambient levels.

Both acute and chronic exposure to H_2SO_4 at levels well below lethal ones will produce functional changes in the respiratory tract. The pathological significance of some of these are greater than for others. Acute exposure will alter pulmonary function, largely due to bronchoconstrictive action. However, attempts to produce changes in airway resistance in healthy animals at levels below 1,000 $\mu g/m^3$ have been largely unsuccessful, except when the guinea pig has been used. The lowest effective level of H_2SO_4 producing a small transient change in airway resistance in the guinea pig is 100 $\mu g/m^3$ (1-h exposure). In general, the smaller size droplets (submicron) were more effective in altering pulmonary function, especially at low concentrations. Very low concentrations (< 100 $\mu g/m^3$) of acid-coated ultrafine particles are associated with lung function and diffusion decrements, as well as

airway hyperresponsiveness. Yet even in the guinea pig, there are inconsistencies in the type of response exhibited towards acid aerosols. Chronic exposure to H_2SO_4 is also associated with alterations in pulmonary function (e.g., changes in the distribution of ventilation and in respiratory rate in monkeys). But, in these cases, the effective concentrations are ≥ 500 $\mu g/m^3$. Hyperresponsive airways have been induced with repeated exposures to 250 $\mu g/m^3$ H_2SO_4 in rabbits, and have been suggested to occur following single exposures at 75 $\mu g/m^3$.

Severe morphologic alterations in the respiratory tract will occur at high (» 1,000 $\mu g/m^3$) acid levels. At low (> 100 $\mu g/m^3$) levels and with chronic exposure, the main response seems to be hypertrophy and/or hyperplasia of mucus secretory cells in the epithelium; these alterations may extend to the small bronchi and bronchioles, where secretory cells are normally rare or absent.

The lungs have an array of defense mechanisms to detoxify and physically remove inhaled material, and available evidence indicates that certain of these defenses may be altered by exposure to H_2SO_4 levels <1,000 $\mu g/m^3$. Defenses such as resistance to bacterial infection may be altered even by acute exposure to concentrations of H_2SO_4 around 1,000 $\mu g/m^3$. However, the bronchial mucociliary clearance system is very sensitive to inhaled acids; fairly low levels of H_2SO_4 produce alterations in mucociliary transport rates in healthy animals. The lowest level shown to have such an effect, 100 $\mu g/m^3$ with repeated exposures, is well below that which results in other physiological changes in most experimental animals. Furthermore, exposures to somewhat higher levels that also alter clearance have been associated with various morphometric changes in the bronchial tree indicative of mucus hypersecretion.

Limited data also suggest that exposure to acid aerosols may affect the functioning of AMs. The lowest level examined in this regard to date is 500 $\mu g/m^3$ H_2SO_4. Alveolar region particle clearance is affected by repeated H_2SO_4 exposures to as low as 125 $\mu g/m^3$ (Schlesinger et al., 1992a).

The assessment of the toxicology of acid aerosols requires some examination of potential interactions with other air pollutants. Such interactions may be antagonistic, additive, or synergistic. Evidence for interactive effects may depend upon the sequence of exposure as well as on the endpoint examined. Low levels of H_2SO_4 (100 $\mu g/m^3$) have been

shown to react synergistically with O_3 in simultaneous exposures using biochemical endpoints (Warren and Last, 1987). In this case, the H_2SO_4 enhanced the damage due to the O_3. The most realistic exposures are to multicomponent atmospheres, but the results of these are often difficult to assess due to chemical interactions of components and a resultant lack of precise control over the composition of the exposure environment.

11.11.2 Metals

Data from occupational studies and laboratory animal studies indicate that acute exposures to high levels or chronic exposures to low levels (albeit high compared to ambient levels) of metal particulate can have an effect on the respiratory tract. However, it is doubtful that the metals at concentrations present in the ambient atmosphere (1 to 14 $\mu g/m^3$) could have a significant acute effect in healthy individuals.

The toxicity data on inhalation exposures to arsenic are limited in humans and laboratory animals. Acute data are largely lacking for this route of exposure. In humans, inhalation exposure data, primarily limited to long-term occupational exposure of smelter workers, indicate that chronic exposure leads to lung cancer. In laboratory animals, intratracheal administration of arsenic compounds in the lungs have not indicated tumor development in rats and mice, but insufficient exposure duration may have been used in these studies. However, respiratory tract tumors occurred in hamsters exposed to intratracheal doses of arsenic when a charcoal carbon carrier dust was used to increase arsenic retention in the lungs.

Chronic inhalation exposure to arsenic has also been shown to cause both skin changes (such as hyperpigmentation and hyperkeratosis) and peripheral nerve damage in workers; however, the available inhalation studies in laboratory animals have not evaluated these endpoints. The laboratory animal inhalation data are limited and thus do not allow a thorough comparison of the toxicological and carcinogenic potential of arsenic with the human data. Species differences in dosimetry, absorption, clearance, and elimination of arsenic (i.e., strong affinity to rat hemoglobin) exist between rats and other animal species, including humans, which complicate comparisons of quantitative toxicity.

The kidney is clearly the primary target of chronic inhalation exposure to cadmium in the human; toxicity is dependent on cumulative exposure. Tubular proteinuria occurs after kidney levels of cadmium accumulate to a certain level, estimated at 200 µg/g kidney weight.

The respiratory system is also a target of inhaled cadmium in humans and animals. Intense irritation occurs following high-level exposure in humans and more mild effects on pulmonary function (dyspnea, decreased forced vital capacity) occur following chronic low-level exposure. These effects and their mechanism have been investigated to a greater degree in laboratory animals, although spirometry has not been conducted in animals. The observed effects (increased lung weight, inhibition of macrophages and edema) are consistent with the irritation observed in human studies. In humans, symptoms reverse with cessation or lessening of exposure; laboratory animal studies have reported no progression or slight reversal with continued exposure.

Rat studies show that several forms of cadmium (cadmium chloride, cadmium oxide dust or fume, cadmium sulfide, or cadmium sulfate) can cause lung cancer. There is some evidence that lung cancer has been observed in humans following high occupational exposure, although confounding exposures were present. Because animal cancer studies only examined the lung, they did not address the suggestive evidence of cadmium-related prostate cancer found in several occupational studies.

Although both human and laboratory animal data are limited, both data bases support the respiratory system as a major target of inhaled copper and copper compounds, including copper sulfate and copper chloride. In humans, the data are limited primarily to subjective reporting of respiratory symptoms following acute and chronic inhalation exposures to copper fumes or dust supported with radiographic evidence of pulmonary involvement. The human data do not include pulmonary function tests or histopathology of the respiratory tract. In laboratory animal studies, supporting evidence exists for the involvement of the respiratory system after copper inhalation exposure. Respiratory tract abnormalities in mice repeatedly exposed to copper sulfate aerosols, and decreased tracheal cilia beating frequency in singly exposed hamsters have been reported. Respiratory effects, although minor, have also been observed in rabbits; these included a slight increase in amount of lamellated cytoplasmic inclusions in alveolar macrophages, and a slight increase in volume density of alveolar Type 2 cells. Although respiratory effects were observed in both human and

laboratory animal studies, direct comparisons are not possible since different parameters were examined in the different species for which limited data exist. Immunological effects have been investigated in only one animal study. In the one study addressing the issue, immunotoxic effects observed included: decreased survival time after simultaneous *S. zooepidemicus* aerosol challenge, and decreased bactericidal activity after simultaneous *K. pneumonia* aerosol exposure.

There is limited information on iron toxicity, with human data primarily from chronic occupational exposures. Both human and laboratory animal data, mostly qualitative information, do demonstrate that the respiratory system is the primary target organ for iron oxides following inhalation exposure. However, the differences in toxicity (if any) for different particle sizes or valence states of iron have not been well studied. In humans, respiratory effects (coughing, siderosis) have been reported in workers chronically exposed to iron dust. In laboratory animals, hyperplasia and alveolar fibrosis have been reported after inhalation or intratracheal administration of iron oxide. The lack of information on the histopathological changes in the lungs of exposed workers precludes direct comparison with animal data. Brief exposure to relatively high concentrations of large iron oxide particles in humans have not been associated with adverse responses. The available human and laboratory animal studies are limited and do not provide conclusive evidence regarding the respiratory carcinogenicity of iron oxide.

Human and laboratory animal data confirm the respiratory tract as the primary target of inhaled vanadium compounds. Laboratory animal data suggest that vanadium compounds damage alveolar macrophages, and that toxicity is related to compound solubility and valence. Because of the difficulty in obtaining clinical signs of respiratory distress in laboratory animals, most reported animal data consisted of histological findings (increased leukocytes and lung weights, perivascular edema, alveolar proteinosis, and capillary congestion). Human occupational case studies and epidemiological studies generally emphasize clinical symptoms of respiratory distress, including wheezing, chest pain, bronchitis, rhinitis, productive cough, and fatigue including the possibility of vanadium induced asthma. No human data were found describing histopathology following oral or inhalation exposure.

No major differences in the pharmacokinetics of zinc in humans and laboratory animals were evident. Both human and laboratory animal data demonstrate that the respiratory system is the primary target organ for zinc following inhalation exposure; the toxic compounds most studied are zinc chloride and zinc oxide. In humans, the development of metal fume fever, characterized by respiratory symptoms and pulmonary dysfunction, was observed in workers and experimental subjects during acute exposures to zinc oxide. An immunological component is believed to be responsible for these respiratory responses. Quantitative data on chronic exposures in humans are not available. Inflammation with altered macrophage function, morphological changes in the lungs, and impaired pulmonary function (decreased compliance, total lung capacity, decreased diffusing capacity) were observed in guinea pigs. Rats also showed altered macrophage function in the lungs. At subchronic durations, histopathological changes in the lungs (increased macrophages) were observed in rats, mice, and guinea pigs exposed to zinc chloride. It is clear that zinc can produce inflammatory response in both human and animal species. Alveologenic carcinomas have been observed in mice exposed to zinc chloride for 20 weeks; however, human studies have shown no evidence of increased tumor incidences at exposure levels found in occupational settings. Zinc compounds are soluble in lung fluids and do not appear to accumulate in the respiratory tract.

Studies examining the potential for the transition metals to cause lung injury by the generation of ROS have been conducted in vitro and in animals by intratracheal instillation. While these studies are interesting, the results thus far are of limited value.

11.11.3 Ultrafine Particles

There are only limited data available from human studies or laboratory animal studies on ultrafine aerosols. They are present in the ambient environment as singlet particles but represent an extremely small portion of the mass. However, ultrafine particles are present in high numbers and have a high collective surface area. There are in vitro studies that show ultrafine particles have the capacity to cause injury to cells of the respiratory tract. High levels of ultrafine particles, as metal or polymer "fume", are associated with toxic respiratory responses in humans and other mammals. Such exposures are associated with cough, dyspnea, pulmonary edema, and acute inflammation. Presence of ultrafine particles,

especially the metals Cd, V, Ti, Fe, in human alveolar macrophages indicates widespread exposure to ultrafines as single particles in ambient air. At concentrations less than 50 $\mu g/m^3$, freshly generated insoluble ultrafine particles can be severely toxic to the lung. There are also studies on a number of ultrafine particles (diesel, carbon black, acidic aerosols) where the particles are not present in the exposure atmosphere as singlet particles. Insufficient information is available at the present time to determine whether ambient ultrafine particles may play a role in PM-induced mortality.

11.11.4 Diesel Emissions

Acute toxic effects caused by exposure to diesel exhaust are mainly attributable to the gaseous components (i.e., mortality from carbon monoxide intoxication and lung injury from respiratory irritants). When the exhaust is diluted to limit the concentrations of these gases, acute effects are not seen.

The focus of the long-term (> 1 year) animal inhalation studies of diesel engine emissions studies has been on the respiratory tract effects in the alveolar region. Effects in the upper respiratory tract and in other organs were not found consistently in chronic animal exposures. Several of these studies are derived from research programs on the toxicology of diesel emissions that consisted of large-scale chronic exposures, which are represented by multiple published accounts of results from various aspects of the overall research program. The respiratory system response has been well characterized in terms of histopathology, biochemistry, cytology, pulmonary function, and respiratory tract clearance. The pathogenic sequence following the inhalation of diesel exhaust as determined histopathologically and biochemically begins with the phagocytosis of diesel particles by AMs. These activated macrophages release chemotactic factors that attract neutrophils and additional AMs. As the lung burden of diesel particles increases, there is an aggregation of particle-laden AMs in alveoli adjacent to terminal bronchioles, increases in the number of Type 2 cells lining particle-laden alveoli, and the presence of particles within alveolar and peribronchial interstitial tissues and associated lymph nodes. The PMNs and macrophages release mediators of inflammation and oxygen radicals and particle-laden macrophages are functionally altered resulting in decreased viability and impaired phagocytosis and clearance of particles. There is a substantial body of evidence for an impairment of particulate

clearance from the bronchioalveolar region of rats following exposure to diesel exhaust. The latter series of events may result in the presence of pulmonary inflammatory, fibrotic, or emphysematous lesions. The noncancer toxicity of diesel emissions is considered to be due to the particle rather than the gas phase, since the long-term effects seen with whole diesel are not found or are found to a much lesser extent in animals exposed to similar dilutions of diesel exhaust filtered to remove most of the particles. Chronic studies in rodents have demonstrated pulmonary effects at 200 to 700 $\mu g/m^3$ (expressed as equivalent continuous exposure to adjust for protocol differences). A range of no adverse effect levels has been estimated as from 200 to 400 $\mu g/m^3$.

Several epidemiologic studies have evaluated the effects of chronic exposure to diesel exhaust on occupationally exposed workers. None of these studies are useful for a quantitative evaluation of noncancer toxicity because of inadequate exposure characterization, either because exposures were not well defined or because the possible confounding effects of concurrent exposures could not be evaluated.

11.11.5 Silica

Emissions of silica into the environment can arise from natural, industrial, and farming activities. There are only limited data on ambient air concentrations of amorphous or crystalline silica, principally because existing measurement methods are not well suited for distinguishing silica from other particulate matter. Using available data on the quartz fraction of coarse dust (Davis et al., 1984) and average annual arithmetic mean PM_{10} measurements for 17 U.S. metropolitan areas, annual average and high U.S. ambient quartz levels of 3 and 8 $\mu g/m^3$, respectively, have been estimated (U.S. Environmental Protection Agency, 1996). Davis et al. (1984) found that most of the quartz was in the fraction between 2.5 to 15 μm MMAD.

Silica can occur in two chemical forms, amorphous and crystalline. Crystalline forms include quartz, which is the most prevalent; cristobalite, tridymite, and a few other rare forms. Freshly fractured crystalline silica is more toxicologically reactive than aged forms of crystalline silica. Amorphous silica is less well studied but is considered less potent than crystalline silica. Occupational studies show that chronic exposure to crystalline silica causes inflammation of the lung which can progress to fibrosis and silicosis, a human fibrotic

disease, which can lead to early mortality. Some occupational studies also show a concurrent incidence of lung cancer. The role, if any, of silica-induced lung inflammation, fibrosis, and silicosis in the development of lung cancer is postulated but not adequately demonstrated. Crystalline silica interaction with DNA has been shown under in vitro conditions. Chronic exposure studies in rats also show a similar pattern of lung inflammation, fibrosis, and lung cancer. The International Agency for Research on Cancer (1987) classified crystalline silica as a "possible" human carcinogen owing to a sufficient level of evidence in animal studies, but with inadequate evidence in human studies. The health statistics of the U.S. do not reveal a general population increase in the incidence of these silica-related disease, although there is an increase within segments of the occupational work force.

These effective occupational exposures are greater and the particle sizes smaller than those likely to be experienced by the general public, including susceptible populations. Information gaps still exist for the exposure-response relationship for levels of exposure within the general population.

11.11.6 Bioaerosols

Ambient bioaerosols include fungal spores, pollen, bacteria, viruses, endotoxins, and plant and animal debris. Such biological aerosols can produce three general classes of health effects: infections, hypersensitivity reactions, and toxicoses. Bioaerosols present in the ambient environment have the potential to cause disease in humans under certain conditions. However, it is improbable that bioaerosols, at the concentrations present in the ambient environment, could account for the observed effects of particulate matter on human mortality and morbidity reported in PM epidemiological studies. Moreover, bioaerosols generally represent a rather small fraction of the measured urban ambient PM mass and are typically present even at lower concentrations during the winter months when notable ambient PM effects have been demonstrated. Bioaerosols also tend to be in the coarse fraction of PM.

11.11.7 "Other Particulate Matter"

Toxicologic studies of other particulate matter species besides acid aerosols, metals, ultrafine particles, diesel emissions, silica, and bioaerosols were discussed in this chapter.

These studies included exposure to fly ash, volcanic ash, coal dust, carbon black, TiO_2, and miscellaneous other particles, either alone or in mixtures.

A number of studies of the effects of "Other PM" examined effects of up to 50,000 $\mu g/m^3$ of respirable particles with inherently low toxicity on mortality and found no effects. Some mild pulmonary function effects of 5,000 to 10,000 $\mu g/m^3$ of similar particles were observed in rats and guinea pigs. Lung morphology studies revealed focal inflammatory responses, some epithelial hyperplasia, and fibrotic responses to exposure generally >5,000 $\mu g/m^3$. Changes in macrophage clearance after exposure to >10,000 $\mu g/m^3$ were equivocal (no infectivity effects). In studies of mixtures of particles and other pollutants, effects were variable depending on the toxicity of the associated pollutant. In humans, associated particles may increase responses to formaldehyde but not to acid aerosol. None of the "other" particles mentioned above are present in ambient air in more than trace quantities. The relevance of any of these studies to ambient particulate standard setting is extremely limited.

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