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7. TOXICOLOGY OF PARTICULATE MATTER IN HUMANS AND LABORATORY ANIMALS

7.1 INTRODUCTION

The 1997 revisions to the U.S. PM NAAQS (Federal Register, 1997) were based, in large part, on newly emerging epidemiologic evidence showing associations between (a) ambient PM measured at community monitoring stations) and (b) increased risks for mortality and morbidity (especially cardiorespiratory-related) among human populations exposed to contemporary U.S. ambient concentrations. However, very little experimental toxicology data from controlled laboratory animal or human exposure studies were then available that provided more direct evidence supporting the plausibility of the PM-mortality/morbidity relationships observed at the relatively low ambient PM concentrations. The then-limited PM toxicologic data was assessed in Chapter 11 of the 1996 PM Air Quality Criteria Document (U.S. Environmental Protection Agency, 1996a) that provided scientific assessment inputs supporting the 1997 PM NAAQS decisions. Since completion of the 1996 PM Criteria Document, numerous hypotheses have been advanced and extensive new toxicologic evidence generated with regard to possible pathophysiological mechanisms by which PM exposures (even at ambient or near ambient concentrations) might induce increased morbidity and/or mortality.

The extensive new toxicological research on airborne particulate matter (PM) during the past five years or so has focused mainly on addressing several interrelated questions, such as: (1) what types of pathophysiological effects are exerted by ambient PM or constituent substances and what are the potential mechanisms underlying them; (2) what PM characteristics (size, chemical composition, etc.) cause or contribute to health effects; (3) what susceptible subgroups are at increased risk for PM health effects and what factors contribute to increased susceptibility; and (4) what types of interactive effects of particles and gaseous co-pollutants have been demonstrated?

Various research approaches have been and continue to be used to address these questions, including studies of human volunteers exposed to PM under controlled conditions; in vivo studies of laboratory animals including nonhuman primates, dogs, and rodent species; and in vitro studies of tissue, cellular, genetic, and biochemical systems. A wide variety of exposure

conditions have been employed, including whole body and nose-only inhalation exposures to laboratory-generated particles or concentrated ambient particles (CAPs), intratracheal instillation, and in vitro exposure to test materials in solution or suspension. These research approaches have been targeted mainly to test hypotheses to provide improved understanding of the role of PM in producing health effects identified by PM-related epidemiologic studies. Thus, many of the new toxicological studies have been designed to address the question of biologic plausibility of epidemiologically-demonstrated effects, rather than being explicitly aimed at providing dose-response quantification for experimentally-induced toxic effects.

Reflecting this, most of the toxicology studies assessed here have generally used exposure concentrations or doses that are high relative to concentrations commonly observed in ambient air. Given the relatively high concentrations used, much care should therefore be taken when attempting to interpret effects seen in these studies to provide insight into the biological plausibility and mechanisms of action for effects in humans under "real world" exposure conditions. Some of the responses might only be seen at the higher concentrations more typical of occupational and experimental laboratory exposures and not necessarily at (usually much lower) ambient particle exposure concentrations. On the other hand, there are substantial differences in the inhalability and deposition profiles of PM in humans and rodents (see Chapter 6 for details), which may make doses from experimental exposures more similar to those from ambient exposures.

To help place the toxicologically relevant concentrations/doses into context in relation to ambient conditions, EPA has carried out some illustrative analyses to provide comparisons between the high doses typically used in toxicological studies and doses typical of human exposures under ambient conditions. Building upon advances in dosimetric modeling (discussed in Chapter 6), these analyses compare PM doses delivered to a rat's lung from experimental exposures and PM doses to the human lung from exposures during normal activities. These analyses and their results (described in Appendix 7-A) provide context for the exposure concentrations used and results obtained in studies assessed in this chapter. The exposure/dose extrapolation modeling illustrated in Appendix 7A demonstrates reasonably good comparability (often within 2 to 10-fold, rather than orders of magnitude) between doses of PM deposited/retained in respiratory tract regions/tissues following high concentration controlled inhalation/instillation exposures and doses resulting from human exposures under ambient

conditions. Another important consideration is that healthy animals are most typically used in controlled-exposure toxicology studies, whereas epidemiologic findings often reflect ambient pollutant effects on susceptible or compromised humans (e.g., children or those with one or another chronic disease) that may have greater-than-average PM deposition/retention.

Particulate matter is a broad term that encompasses myriad physical and chemical species, some of which have been investigated in the controlled laboratory animal or human studies. However, a full discussion of all types of particles that have been studied is beyond the scope of this chapter (see Chapter 2). Thus, specific criteria were used to select topics for presentation. High priority was placed on studies that (a) may contribute to enhanced understanding of ambient PM epidemiologic study results and/or (b) elucidate mechanisms understanding health effects of ambient PM or its major common constituents. Diesel particulate matter (DPM) generally fits the above criteria; however, because it is discussed in great detail in other documents (Health Effects Institute, 1995; U.S. Environmental Protection Agency, 2002), only limited aspects (e.g., chronic animal studies, controlled human studies, and immune effects) are covered in this chapter. Individual particle species with high inherent toxicity that are of concern mostly because of occupational exposure (e.g., silica) that are discussed in detail in other documents and reports (e.g., U.S. Environmental Protection Agency, 1996b; Gift and Faust, 1997 for silica) are not assessed in detail in this chapter.

Because of the sparsity of toxicological data on ambient PM at the time of the 1996 PM Air Quality Criteria Document or "1996 PM AQCD" (U.S. Environmental Protection Agency, 1996a), the discussion of toxicologic effects of PM was organized there into specific chemical components of ambient PM or "surrogate" particles (e.g., acid aerosols, metals, ultrafine particles, bioaerosols, "other particle matter"). Many of the newer toxicological studies evaluate potential toxic effects of combustion-related particles. The main reason for this extensive interest in combustion particles is that these particles, along with materials adsorbed to these particles and secondary aerosols formed from them, are typically among the most dominant components represented in the fine fraction of ambient air PM.

This chapter is organized as follows. The cardiovascular and systemic effects of in vivo PM exposure are discussed first (Section 7.2), followed by discussion of respiratory effects of specific components of ambient PM or surrogate particles delivered by controlled in vivo exposures of humans or laboratory animals (Section 7.3). In vitro exposure studies are discussed

next (Section 7.4) and are valuable in providing information on potential hazardous constituents and mechanisms of PM injury. Studies of PM effects in laboratory animal models meant to mimic human disease are then discussed (Section 7.5) as providing information useful for characterizing factors affecting susceptibility to PM effects. Section 7.6 assesses controlled-exposure studies evaluating health effects of mixtures of ambient PM or specific PM constituents (surrogates) with gaseous pollutants. This organization provides the underlying information for interpretive summarization, in Section 7.7, of the extensive new findings assessed in the above sections with regard to PM-related effects on the cardiac, pulmonary, and nervous systems, all of which may individually contribute to and/or, through intricate linkages among them, combine to mediate ambient PM exposure effects.

7.2 CARDIOVASCULAR AND SYSTEMIC EFFECTS OF PM EXPOSURES IN HUMANS AND LABORATORY ANIMALS IN VIVO

A growing number of epidemiology studies are finding (a) associations between ambient PM and increases in cardiac-related deaths and/or morbidity indicators and (b) that the risk of PM-related cardiac effects may be as great or greater than those attributed to respiratory causes (see Chapter 8). Both acute and chronic PM exposures have been implicated in the observed cardiovascular morbidity and mortality effects. These effects, independent of the respiratory system, appear to be induced via direct particle uptake into the blood or via mediation by the nervous system. Figure 7-1 schematically illustrates hypothesized mechanisms thought to be involved in cardiovascular responses to PM exposure. Such effects may be especially deleterious to individuals compromised by disease states such as COPD, ischemic heart disease, and cardiac arrythmias.

As shown in Figure 7-1, the heart receives both parasympathetic and sympathetic inputs, which serve to decrease or increase heart rate, respectively. Vasoconstriction elicited by PM could cause increased blood pressure, which is detected by baroreceptors. Parasympathetic neural input may then be increased to the heart, lowering heart oxygen-carrying capacity of the blood (which is sensed by aortic and carotid chemoreceptors). These, in turn, may cause a sympathetic response, manifested by increased heart rate and contractile force, thus increasing cardiac output. This arrhythmogenesis and altered cardiac output in either direction can be life-threatening to susceptible individuals. Pathophysiological changes in cardiac function can be detected by electrocardiographic recordings, with certain ECG parameters (e.g., heart rate variability or HRV) recently gaining widening use as indicators of PM-induced cardiac effects.

Heart rate variability (HRV), a measure of the beat-to-beat change in heart rate, is a reflection of the overall automatic control of the heart. HRV has been used for many years as a research tool to study cardiovascular physiology and pharmacology. Its role as a clinical predictor of outcome for populations with heart disease has been extensively studied. HRV can be divided into time and frequency measures. Frequency measures of variability are more commonly used for mechanistic studies because they resolve parasympathetic and sympathetic influences on the heart better than do time domain measurements. It has been well established that the frequency analysis of heart rate variability is a robust method for measuring the autonomic modulation of heart rate. Under certain circumstances, HRV provides insight into

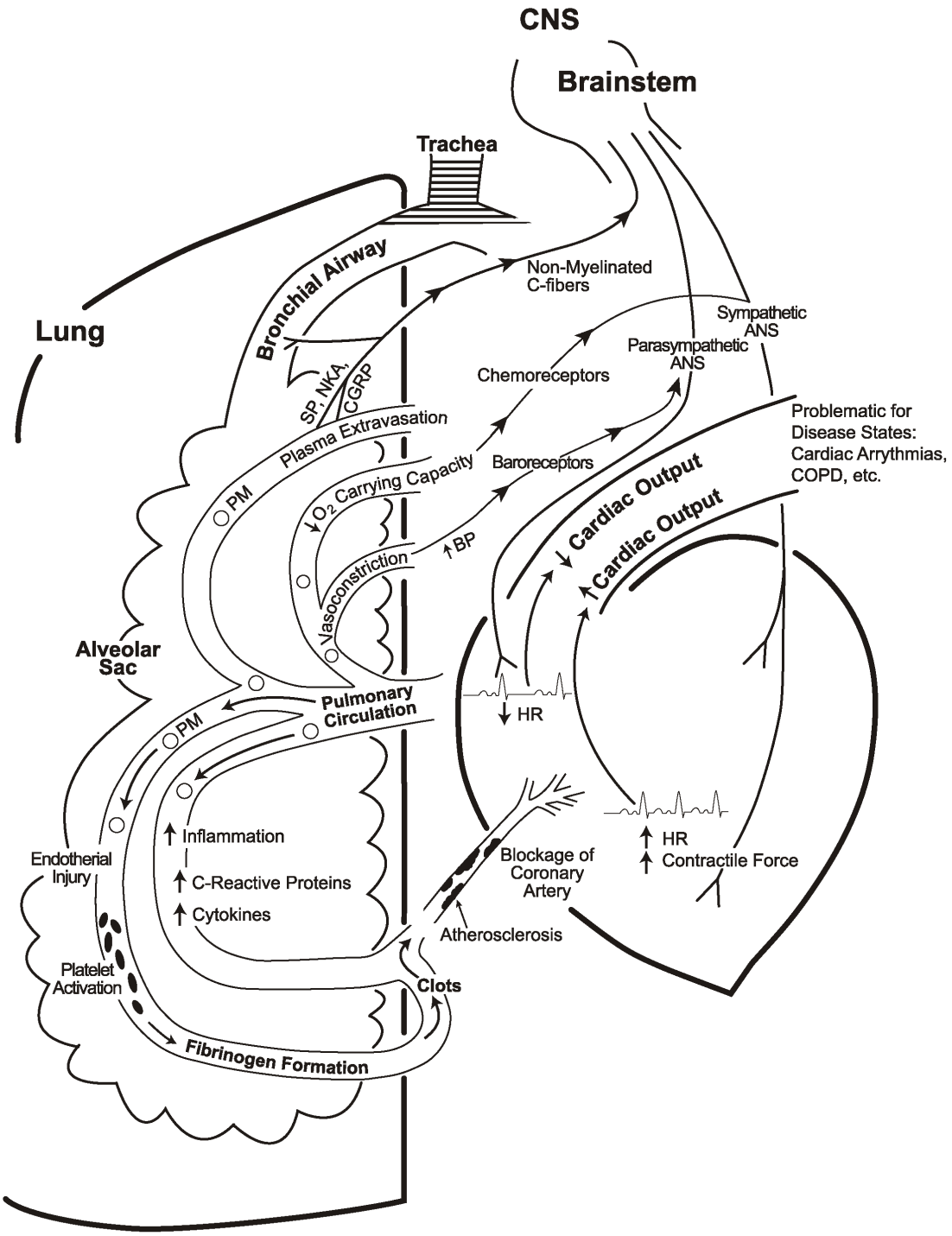

Figure 7-1. Schematic illustration of hypothesized pathways/mechanisms potentially underlying cardiovascular effects of PM.

sympathetic nervous activity, but more commonly it is a very good measurement of parasympathetic modulation. For prognostication in heart disease, both the time and frequency domain measures of heart rate variability seem equivalent in predicting events. Heart rate variability can be used to judge the relative influences of sympathetic and parasympathetic forces on the heart, as such short-term spectral parameters (i.e., measures averaged over five minute intervals) can vary as much as 4-fold during the course of a 1-hour period (Kleiger 1991). Despite the inherent variability of short-term HRV measures during route daily activity, long-term measures (i.e., measures averaged over 24 hours) show excellent day-to-day reproducibility. Given this inherent variability in the minute-to-minute spectral measurements, great care is required in the experimental design of studies utilizing HRV techniques and interpretation of HRV results. When appropriately designed and carefully interpreted, studies utilizing measures of HRV provide insight into the relationship between the perturbation of the internal or external environment and subsequent changes in the modulation of autonomic neural input to the heart.

Heart rate variability has been studied in multiple settings, using different parameters (both time and frequency domain) to determine prognosis in populations. This has been studied most frequently in coronary artery disease populations, particularly in the post-MI population. Most reports have dichotomized the study group by HRV parameters and then compared outcomes. To summarize those results, lower time domain as well as frequency domain variables are associated with an increase in cardiac and all-cause mortality. Those variables most closely correlated with parasympathetic tone appear to have the strongest predictive value in heart disease populations. Specifically, acute changes in RR-variability temporally precede and are predictive of increased long-term risk for the occurrence of ischemic sudden death and/or precipitating ventricular arrhythmias in individuals with established heart disease (see for example LaRovere et al., 2003). However, acute changes in HRV parameters do not necessarily occur immediately prior to sudden fatal ventricular arrhythmias (Levy, 1994). The heart rate variability itself is not the causative agent nor has it been implied to be a causative agent in any of the studies performed to date. Altered HRV is simply a marker for enhanced risk, as are such changes in HRV associated with exposure to PM.

Another route by which PM could exert deleterious cardiovascular effects may involve ambient PM effects on blood chemistry. In particular, as hypothesized by Seaton et al. (1995),

PM exposure could affect blood coagulation, possibly through endothelial injury that results in platelet activation. This then could initiate a cascade of effects, e.g., increased fibrinogen and fibrin formation, leading to increased formation of clots. Figure 7-2 (from Nadziejko, et al. 2002) nicely illustrates physiological events (and applicable timeframes) involved in the blood clotting cascade, as well as denoting important substances released at successive steps which, in turn, stimulate the next step in the clotting cascade and, ultimately, trigger clot lysing events that normally terminate the cascade. Various studies have measured such substances as a means to evaluate possible PM-induced effects on blood coagulation. Another significant effect of PM exposure could be release of C-reactive proteins and cytokines, which cause an inflammatory response that, on a chronic basis, can lead to atherosclerosis. In narrowed coronary arteries, the clots formed in the aforementioned cascade could easily block blood flow, resulting in acute myocardial infarction (MI).

Nadziejko et al. (2002) further note that small prothrombotic changes in blood coagulation parameters in a large population can have substantial effects on the incidence and prevalence of cardiovascular disease events (Di Minno and Mancini, 1990; Branwald, 1997; Lowe et al., 1997). In particular, altered coagulation can increase heart attack risk through formation of clots on atherosclerotic plaques in coronary arteries that cut off blood supply to the myocardium or induce ischemic strokes via clots forming or lodging in the carotid arteries blocking blood flow to cerebral arteries and brain tissue. Also, Nadziejko et al. note that (a) evidence exists for formation of small thrombi being common in persons with atherosclerosis (Meade et al., 1993) and (b) whether such thrombi lead to more serious effects (heart attack, stroke) depends in part on the balance between thrombogenic factors underlying blood clot formation and fibrinolytic factors that lyse clots. Also, they note that effects of small changes in coagulation on heart attack risk are reflected by the risk of sudden cardiac death being 70% higher between 6 a.m. and 9 a.m. than the average risk for the rest of the day (Willich, et al., 1987), likely due in part to the circadian rhythm of fibrinolytic factors that are at their lowest levels in the early morning (Andrews et al. 1996). Also, as stated by Nadziejko et al. (2002), sympathetic nervous system activity is increased by standing up after lying prone (Tofler, et al., 1987; Andrews et al., 1996), and increased sympathetic activity causes prothrombotic changes in blood coagulation parameters such that even small, homeostatic modulations of coagulation within a normal range could translate into significant increased risk for heart attack.

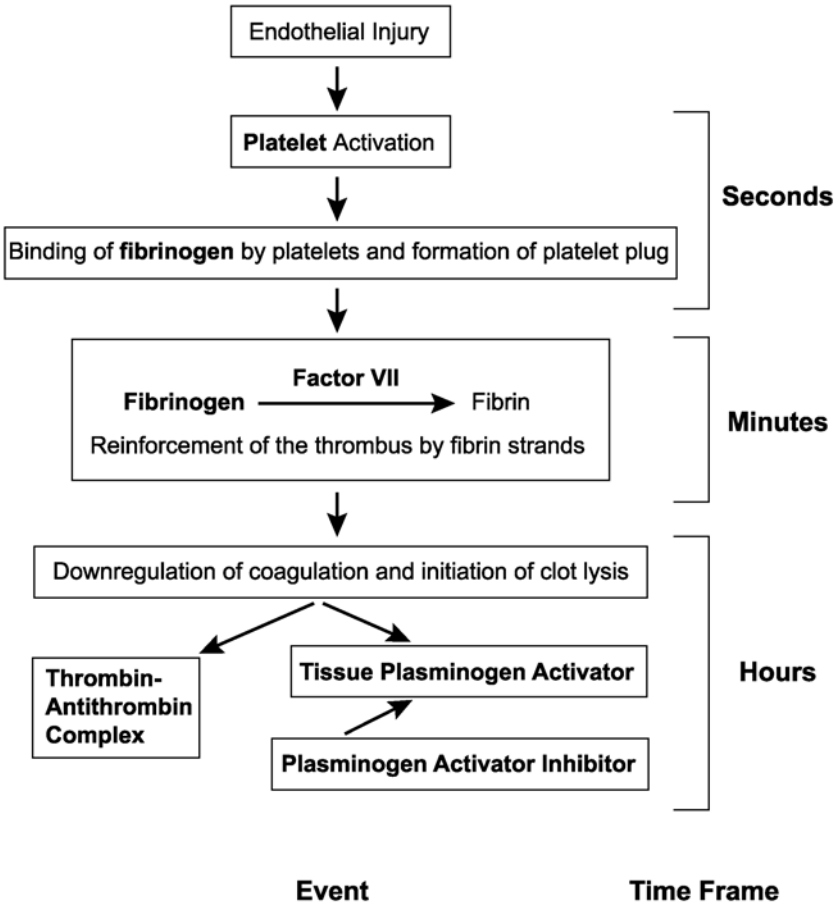

Figure 7-2. Simplified overview of blood coagulation system. The coagulation parameters often measured in the study of PM effects on blood coagulation are indicated by bold type. The relations of these selected parameters with the rest of the coagulation system are outlined.

Source: Nadziejko et al. (2002).

Thus, potentially dangerous alterations in cardiovascular functions due to PM exposures could be signaled by even small PM-related (a) changes in blood coagulation cascade indicators, e.g., increased blood platelet, fibrinogen, or Factor VII, or decreased tissue plasma activator (TPA) levels; (b) increased C-reactive protein, or cytokines contributing to increased atherosclerosis plaque formation and/or blood coagulation; (c) increased blood pressure; and/or (d) certain alterations in heart rate, heart rate variability, or other ECG indicators indicative of

deleterious shifts in parasympathetic/sympathetic neural inputs to the heart or other underlying cardiac pathophysiology.

Another cardiovascular-related effect of PM exposure could be plasma extravasation from post-capillary venules. The mechanisms by which this occurs are thought to include the release of peptides such as neurokinin A, substance P, and calcitonin-gene-related peptide from unmyelinated sensory nerves, near to or on the blood vessels. These peptides bind to receptors on the endothelial cells of vessels and create gaps, allowing leakage of plasma, which is one component of neurogenic inflammation.

Tables 7-1a and 7-1b summarize newly-available studies (since the 1996 PM AQCD) that have evaluated cardiovascular effects of ambient PM or surrogate PM in response to controlled exposures of humans or laboratory animals via intratracheal instillation or inhalation, respectively. In vitro exposure studies of cardiovascular effects are discussed in Section 7.4.

The toxicological consequences of inhaled particles on the cardiovascular system had not been extensively investigated prior to 1996. Since then, Costa and colleagues (e.g., Costa and Dreher, 1997) have demonstrated that intratracheal instillation of high levels of ambient particles can increase or accelerate death in an animal model of cardiorespiratory disease induced by monocrotaline (MCT) administration in rats (see Table 7-1a). These deaths did not occur with all types of ambient particles tested. Some dusts, such as volcanic ash from Mount Saint Helens, were relatively inert; whereas other ambient dusts, including those from urban sites, were toxic. These early observations suggested that particle composition plays an important role in the adverse health effects associated with episodic exposure to ambient PM, despite the "general particle" effect implied by epidemiologic observations of ambient PM exposure associations with increased mortality and morbidity in many regions of the United States with varying particle composition. Studies evaluating possible increased susceptibility to the adverse effects of PM in compromised animal models of human pathophysiology provide a potentially important link to epidemiologic observations and are among those discussed below.

To date, studies examining the systemic and cardiovascular effects of particles have used a number of compromised animal models, largely rodent models. Two studies in normal or compromised dogs (Godleski et al., 2000; Muggenburg et al., 2000a) also have been published as well as the preliminary results from studies in which human subjects were exposed to concentrated ambient PM (see Section 7.4.1). Muggenburg et al. (2000b) described several

TABLE 7-1a. CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INSTILLED AMBIENT AND COMBUSTION-RELATED PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiovascular Effects	Reference
Rats, male, S-D, 60 days old, healthy and MCT-treated	Emission source PM	Instillation	Total mass: 2.5 mg/rat	Emission PM: 1.78-4.17 µm	Analysis at 24 and 96 h following instillation	ROFA alone induced some mild arrhythmias; MCT-ROFA showed enhanced neutrophilic inflammation.	Costa and Dreher (1997)
	Ambient airshed PM ROFA		Total transition metal: 46 µg/rat	Ambient PM: 3.27-4.09 µm		MCT-ROFA animals showed more numerous and severe arrhythmias including S-T segment inversions and A-V block.	
Rats, male, S-D, 60 days old, MCT-treated and healthy, n = 64	ROFA	Instillation	0.0, 0.25, 1.0, and 2.5 mg/rat	1.95 µm	Analysis at 96 h post-exposure	Dose-related hypothermia and bradycardia in healthy rats, potentiated by compromised models at 2.5 mg dose..	Campen et al. (2000)
Rats, male, SD, 60 days old, healthy and MCT-treated.	$Fe_2(SO_4)_3$ $NiSO_4$ VSO_4	Intratracheal instillation	105 µg 263 µg 245 µg		Analysis at 96 h post-exposure	V caused bradycardia, arrhythmogenesis and hypothermia immediately. Ni caused delayed bradycardia, arrhythmogenesis and hypothermia. Fe had little effect.	Campen et al. (2002)
MCT-treated							
	$Fe_2(SO_4)_3$ +VSO_4		105 µg 245 µg			Ni exacerbated the immediate effects of V. Fe attenuated them	
	$Fe_2(SO_4)_3$ + $NiSO_4$		105 µg 263 µg				
	$NiSO_4$ + VSO_4		263 µg 245 µg				
	VSO_4 + $Fe_2(SO_4)_3$ + $NiSO_4$		245 µg 105 µg 263 µg				

TABLE 7-1a (cont'd). CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INSTILLED AMBIENT AND COMBUSTION-RELATED PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiovascular Effects	Reference
Rats, male, S-D; 60 days old	ROFA	Instillation	0.3, 1.7, or 8.3 mg/kg	1.95 µm σg = 2.19	Analysis at 24 h	Increased plasma fibrinogen at 8.3 mg/kg only.	Gardner et al. (2000)
Rats, male, SD, 60 days old	ROFA classified by soluble metals (As, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn, and sulfate)	Intratracheal instillation	0.833, 3.33 or 8.33 mg/kg	< 3.0 µm MADD	Anaylsis at 24 h post-exposure	Dose-dependent increase in BAL protein, LDH, hemoglobin and NAG activity (only high dose data shown). ROFA containing highest concentration of water-leachable Fe, V, and Ni or V and Ni caused largest increase. ROFA with highest V content induced greatest increase in BAL neutrophils. AM chemiluminescence was greatest with ROFA containing primarily soluble V and less with Ni + V.	Kodavanti et al. (1998a)
Rat, SD, 60 d old; 250-300 g healthy or MCT-treated	ROFA	Instilled	0.83 or 3.33 mg/kg	1.95 µm MMAD, σg = 2.19	Analysis at 24 and 96 h postexposure	Increases in BAL markers of lung injury and inflammation; 58% of MCT rats exposed to ROFA died by 96 h regardless of the dose.	Kodavanti et al. (1999)
Rats, male SH and WKY; 12-13 weeks old	ROFA from a precipitator of an oil-burning power plant	Intratracheal instillation	1 and 5 mg/kg	1.5 µm σ_g = 1.5	Analysis at 1, 2, and 4 days	Exposure increased plasma fibrinogen and decreased peripheral lymphocytes in both SH and WKY rats at 5.0 mg/kg dose.	Kodavanti et al. (2002)
Rats, male, S-D, MCT-treated	ROFA	Instillation	0.25, 1.0, or 2.5 mg in 0.3 mL saline	1.95 µm MMAD σg = 2.19	Monitored for 96 h after instillation of ROFA particles	Dose-related increases in incidence and duration of serious arrhythmic events in normal rats. Incidence and severity of arrythmias increased greatly in MCT rats. Changes occurred at all doses ranging from modest effects at the lowest to more serious disturbances at the higher doses. Deaths seen at each instillation level in MCT rats only (6/12 died after MCT + ROFA).	Watkinson et al. (1998)

TABLE 7-1a (cont'd). CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INSTILLED AMBIENT AND COMBUSTION-RELATED PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiovascular Effects	Reference
(1) Rats, S-D healthy and MCT, cold-stressed, and ozone-treated	ROFA	Intratracheal instillation	0.0, 0.25, 1.0, or 2.5 mg/rat	1.95 µm σg = 2.19	Monitored for 96 h after instillation	(1) Healthy rats exposed IT to ROFA demonstrated dose-related hypothermia, bradycardia, and increased arrhythmias at 2.5 mg dose. Similar response pattern seen at 0.25 and 1.0 mg, but reduced in magnitude and duration. Compromised rats showed exaggerated hypothermia and cardiac responses to IT ROFA at all doses. Mortality was seen only in the MCT-treated rats exposed to ROFA by IT.	Watkinson et al. (2000a,b); Watkinson et al. (2001)
(2) Rats, SH, 15-mo-old	OTT ROFA MSH	Intratracheal instillation	2.5 mg 0.5 mg 2.5 mg			(2) Older rats exposed IT to OTT showed a pronounced biphasic hypothermia and a severe drop in HR accompanied by increased arrhythmias. Exposure to ROFA caused less pronounced, but similar effects. No cardiac effects seen with MSH exposure.	Watkinson et al. (2000a,b); Watkinson et al. (2001)
(3) Rats, S-D MCT-treated	$Fe_2(SO_4)_3$ VSO_4 $NiSO_2$	Intratracheal instillation	105 µg 245 µg 262.5 µg			(3) Ni and V showed the greatest toxicity; Fe-exposed rats did not differ from controls.	
Hamsters, 100-150 g	polystyrene particles unmodified	Intratracheal instillation	5, 500, 5000 µg/kg	60 nm		Instillation of 5 mg/kg of unmodified and carboxylate-polystyrene particles did not significantly modify the intensity of the thrombus formed.	Nemmar et al. (2002)
	carboxylate-modified		50, 100, 500 µg/kg				
	amine-modified		5, 50, 500 µg/kg			Administration of 500 µg/kg of amine-polystyrene particles induced a significant increase in thrombus formation.	

TABLE 7-1a (cont'd). CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INSTILLED AMBIENT AND COMBUSTION-RELATED PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiovascular Effects	Reference
Rabbits, female, New Zealand White, 1.8 to 2.4 kg	Colloidal carbon	Instillation	2 mL of 1% colloidal carbon (20 mg)	< 1 µm	Examined for 24 to 192 h after instillation	Colloidal carbon stimulated the release of BRDU-labeled PMNs from bone marrow. The supernatant of alveolar macrophages treated with colloidal carbon in vitro also stimulated release of PMNs from bone marrow, likely via cytokines.	Terashima et al. (1997)
Rabbits, female, New Zealand, 2.2 to 3.0 kg	PM_{10} (EHC-93)	Intrapharyngeal instillation	5mg/dose	4-5 µm mass median diameter	5mg twice/wk for 3 wk	PM_{10} increased circulating band cells and shortened transit time of PMN through postmitotic pool in marrow. Increased bone marrow pool of PNM, esp. in mitotic pool.	Mukae et al. (2001)
Rabbits, female, Watanabe heritable hyperlipidemic 3.2 ± 0,1 kg	OTT, PM_{10} EHC-93	Intrapharyngeal instillation	5 mg in 1 mL saline	0.8 ± 0.4 µm	5mg 2 times per week for 4 weeks	Increased circulating PMN band cell counts and size of bone marrow mitotic pools of PMNs. Progression of atherosclerotic lesions. Increase in plaque cell turnover, extracellular lipid pools, and total lipids in aortic lesions.	Suwa et al. (2002)

[a]ROFA = Residual oil fly ash
OTT = Ottawa dust
$Fe_2(SO_4)_3$ = Iron sulfate
MSH = Mt. St. Helen's volcanic ash
VSO_4 = Vanadium sulfate
$NiSO_2$ = Nickel sulfate

TABLE 7-1b. CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INHALED AMBIENT AND COMBUSTION-RELATED PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiovascular Effects	Reference
Humans, healthy nonsmokers, 18 to 40 years old	CAPs	Inhalation	23.1 to 311.1 μg/m^3	0.65 μm σg = 2.35	2 h, analysis at 18 h	Increased blood fibrinogen. PM concentration in chamber varied with ambient air PM level. Estimated total dose of 1200 μg.	Ghio et al. (2000a)
Dogs, female mongrel, 14 to 17 kg	CAPs	Inhalation via tracheostomy	3-360 μg/m^3	0.2 to 0.3 μm	6 h/day for 3 days	Peripheral blood parameters were related to specific particle constituents. Factor analysis from paired and crossover experiments showed that hematologic changes were not associated with increases in total CAP mass concentration.	Clarke et al. (2000a)
Dogs, mongrel, some with balloon occluded LAD coronary artery, n = 14	CAPs	Inhalation via tracheostomy	69-828 μg/m^3	0.23 to 0.34 μm σg = 0.2 to 2.9	6 h/day for 3 days	Decreased time to ST segment elevation and increased magnitude in compromised dogs. Decreased heart and respiratory rate and increased lavage fluid neutrophils in normal dogs. PM concentration varied depending on ambient PM level and concentrator operation. No dose-response.	Godleski et al. (2000)
Dogs, beagles, 10.5-year- old, healthy, n = 4	ROFA	Oral inhalation	3 mg/m^3	2.22 μm MMAD σg = 2.71	3 h/day for 3 days	No consistent changes in ST segment, the form or amplitude of the T wave, or arrhythmias; slight bradycardia during exposure.	Muggenburg et al. (2000a)
Rats	CAPs	Nose-only inhalation	110-350 μg/m^3	N/A	3 h	Small but consistent increase in HR; no pulmonary injury was found; increased peripheral blood neutrophils and decreased lymphocytes. Concentration to chamber varied from 132 to 199 μg/m^3.	Gordon et al. (1998)
Rats, male, F-344, MCT-treated Hamsters, 6-8 mo old; Bio TO-2	CAPs	Inhalation	132-919 μg/m^3	0.2-1.2 μm σg = 0.2-3.9	3 h, evaluated at 3 and 24 h	No increase in cardiac arrhythmias; PM associated increases in HR and blood cell differential counts, and atrial conduction time of rats were inconsistent. No adverse cardiac or pulmonary effects in hamsters.	Gordon et al. (2000)
Rats, male, F-344; 200-250 g	OTT	Nose-only Inhalation	40 mg/m^3	4 to 5 μm MMAD	4 h	Increased plasma levels of endothelin-1. No acute lung injury; however, lung NO production decreased and macrophage inflammatory protein-2 from lung lavage cells increased after exposure.	Bouthillier et al. (1998)
Rats, S-D, MCT-treated, 250 g	ROFA	Inhalation	580 ± 110 μg/m^3	2.06 μm MMAD σg = 1.57	6 h/day for 3 days	Increased expression of the proinflammatory chemokine MP-2 in the lung and heart of MCT-treated rats; less in healthy rats. Significant mortality only in MCT-treated rats.	Killingsworth et al. (1997)

TABLE 7-1b (cont'd). CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INHALED AMBIENT AND COMBUSTION-RELATED PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiovascular Effects	Reference
Rats, Wistar	Ottawa ambient (EHC-93)	Inhalation (nose only)	48 mg/m^3	36, 56, 80, 100, and 300 µm	4 h	EHC-93 elevated blood pressure and ET-1 and ET-3 levels. EHC-93 L had no effect on blood pressure, transient effect on ET-1, -2, -3 levels. DPM had no effect on blood pressure, but elevated ET-3 levels. CB no effect.	Vincent et al. (2001)
	(ECH-93L)		49 mg/m^3				
	Diesel soot (DPM)		5 mg/m^3				
	Carbon black (CB)		5 mg/m^3				
Rats, S-D, SH rats, WKY rats, healthy and MCT-treated	ROFA	Inhalation	15 mg/m^3	1.95 µm MMAD	6 h/day for 3 days	Pulmonary hypertensive (MCT-treated S-D) and systemically hypertensive (SH) rats exposed to ROFA by inhalation demonstrated similar effects, but of diminished amplitude. There were no lethalities by the inhalation route.	Watkinson et al. (2000a,b)
Rats, male WKY and SH, 12 to 13-week-old	ROFA	Nose-only inhalation	15 mg/m^3	N/A	6 h/day for 3 days	Cardiomyopathy and monocytic cell infiltration, along with increased cytokine expression, was found in left ventricle of SH rats because of underlying cardiovascular disease. ECG showed exacerbated ST segment depression caused by ROFA.	Kodavanti et al. (2000b)
Rats, male, SH and WKY; 12 to 13 weeks old	ROFA from a precipitator of an oil burning power plant	Inhalation	15 mg/m^3	1.5 µm $\sigma g = 1.5$	6 h/d, 3 d/wk for 1, 2, or 4 wk	One week exposure increased plasma fibrinogen in SH rats only; longer exposure caused pulmonary injury but no changes in fibrinogen.	Kodavanti et al. (2002)
Rats, male, S-D, WKY and SH	Oil-combustion derived emission PM (EPM)	Inhalation (nose only)	2, 5, 10 mg/m^3		6 h/d for 4 consec. days.	No cardiovascular effects see in SD or SH rats with acute or chronic exposure. Cardiac lesions (chronic active inflammatory multifocal myocardial degeneration, fibrosis, decreased number granulated mast cells) seen for WKY rats with chronic (16 wk) exposures.	Kodavanti et al. (2003)
			10 mg/m^3		6 h/d 1 d/wk, 4 or 16 wks		
Rats, male, S-D, healthy and MI	Boston ROFA	Inhalation	3 mg/m^3	1.81 µm	1 h	ROFA increased arrhythmia frequency in animals with preexisting premature ventricular complexes and decreased heart rate variability. Other exposed groups not affected.	Wellenius et al. (2002)
	Carbon black			0.95 µm			

[a]ROFA = Residual oil fly ash
OTT = Ottawa dust
MSH = Mt. St. Helen's volcanic ash
$Fe_2(SO_4)_3$ = Iron sulfate
VSO_4 = Vanadium sulfate
$NiSO_2$ = Nickel sulfate
MI - Myocardial infraction

potential animal models of cardiac disease (monocrotaline-induced pulmonary hypertension, dilated cardiomyopathy, viral and mycoplasmal myocarditis, and ischemic heart disease) and discussed advantages and disadvantages associated with the use of animal models to study cardiac disease and air pollution. Pulmonary hypertension in humans may result from airway and vascular effects due to COPD, asthma, and cystic fibrosis. The MCT-induced vascular disease model exhibits common features of COPD in humans. The mechanism of injury includes selective pulmonary endothelial damage and progressive pulmonary arterial muscularization. Pulmonary hypertension develops as the blood flow is impeded and it induces compensatory right ventricular hypertrophy. To produce pulmonary hypertension, animals are injected subcutaneously with 50-60 mg/kg monocrotaline. Within two weeks following treatment, experimental animals, primarily rats, develop pulmonary hypertension (Kodavanti et al., 1998a). Many of the newer animal studies examining the systemic effects of PM have used metal-laden ROFA as a source particle, but a growing number of studies have also used collected and stored ambient PM or real-time generated concentrated ambient particles (CAPs) drawn from various airsheds (e.g., Boston, New York City, etc.). The following discussion of the systemic effects of PM first describes the studies using ROFA and then compares those findings with the ambient PM studies.

Killingsworth et al. (1997), using the MCT model of cardiorespiratory disease, examined adverse effects of one irritant particle mix (Boston area fuel oil fly ash). They observed 42% mortality in MCT rats exposed to ~580 $\mu g/m^3$ fly ash for 6 h/day for 3 consecutive days but no deaths among MCT rats exposed to filtered air or saline-treated healthy rats exposed to fly ash. The increase in MCT/fly ash group deaths was accompanied by (a) increased neutrophils in lavage fluid and (b) increased immunostaining of macrophage inflammatory protein (MIP-2), from among several proinflammatory chemokines evaluated, in the lungs and hearts of the MCT/fly ash animals. Cardiac immunohistochemical analysis indicated increased MIP-2 in cardiac macrophages. The fly ash-induced deaths did not result from a change in pulmonary arterial pressure, and the cause of death was not identified. The results suggest that MCT treatment and PM exposure produce significant lung inflammation and possible increases in proinflammatory signals in the heart.

In a similar experimental model, Watkinson et al. (1998) examined the effects of intratracheally instilled ROFA (0.0, 0.25, 1.0, 2.5 mg in 0.3 mL saline) on ECG measurements in

healthy control and MCT rats. They observed a dose-related increase in the incidence and duration of arrhythmic events in control animals exposed to ROFA particles, and these effects appeared to be exacerbated in the MCT animals (the strength of these conclusions and determination of lowest observed effective dose levels being limited due to lack of statistical analyses). Similar to the results of Killingsworth et al. (1997), healthy animals treated with ROFA suffered no deaths, but there were 1, 3, and 2 deaths in the low-, medium-, and high-dose MCT groups, respectively. Further, given that the observed rhythm disturbances were mimicked by infusion of acetylcholine, increased vagal (parasympathetic) input may have contributed to the PM-induced increased occurrence of arrythmias. Thus, ROFA PM may be linked to conductive and hypoxemic arrhythmias in rats having MCT-induced pulmonary hypertension. However, the specific data and analyses in this study do not establish that relationship with certainty. Such small sampling frequency as was used here does not allow any extrapolation in terms of the total frequency of arrhythmia because of the inherent variability of arrhythmia frequency. Also, since the increased arrhythmia reported by these investigators in this animal model is almost entirely dropped beats, these findings have questionable bearing on the mechanism of potential increased risk of cardiac mortality in humans exposed to PM. Also, it is possible that the reported mortalities were simply related to the MCT-induced pulmonary hypertension.

To examine the biological relevance of intratracheal instillation of ROFA particles, Kodavanti et al. (1999) exposed MCT rats to ROFA by either instillation (0.83 or 3.33 mg/kg) or nose-only inhalation (15 mg/m^3, 6 h/day for 3 consecutive days). Similar to Watkinson et al. (1998), intratracheal instillation of ROFA in MCT rats resulted in ≈50% mortality. Notably, no mortality occurred in MCT rats exposed to ROFA by the inhalation route despite the high exposure concentration (15 mg/m^3). In addition, no mortality occurred in healthy rats exposed to ROFA or in MCT rats exposed to clean air. Despite the fact that mortality was not associated with ROFA inhalation exposure of MCT rats, exacerbation of lung lesions and pulmonary inflammatory cytokine gene expression, as well as ECG abnormalities, clearly were evident.

Watkinson and colleagues further examined the effect of instilled ROFA in rodents previously exposed to ozone or housed in the cold (Watkinson et al., 2000a,b; Watkinson et al., 2001; Campen et al., 2000). The effect of ozone-induced pulmonary inflammation (preexposure to 1 ppm ozone for 6 h) or housing in the cold (10 °C) on the response to instilled ROFA in rats

was similar to that produced with MCT. Bradycardia, arrhythmias, and hypothermic changes were consistently observed in the ozone-exposed and hypothermic animals treated with ROFA (0.25, 1.0, or 2.5 mg/rat); but, unlike in the MCT animals, no deaths occurred. Thus, in rodents with cardiopulmonary disease/stress, instillation of 0.25 mg or more of ROFA can produce systemic changes that may be used to study potential mechanisms of toxicity that are consistent with the epidemiology and panel studies showing cardiopulmonary effects in humans.

While studies of instilled ROFA demonstrated immediate and delayed responses, consisting of bradycardia, hypothermia, and arrhythmogenesis in conscious, unrestrained rats (Watkinson et al., 1998; Campen et al., 2000), further study of instilled ROFA-associated transition metals showed that vanadium (V) induced the immediate responses, while nickel (Ni) was responsible for the delayed effects (Campen et al., 2002). Moreover, Ni, when administered concomitantly, potentiated the immediate effects caused by V.

In another study, Campen et al. (2001) examined the responses to these metals in conscious rats by whole-body inhalation exposure. The authors attempted to ensure valid dosimetric comparisons with the instillation studies, by using concentrations of V and Ni ranging from 0.3-2.4 mg/m^3. The concentrations used in this study incorporated estimates of total inhalation dose derived using different ventilatory parameters. Heart rate (HR), core temperature (T[CO]), and electrocardiographic (ECG) data were measured continuously throughout the exposure. Animals were exposed to aerosolized Ni, V, or Ni + V for 6 h per day for 4 days, after which serum and bronchoalveolar lavage samples were taken. While Ni caused delayed bradycardia, hypothermia, and arrhythmogenesis at concentrations > 1.2 mg/m^3, V failed to induce any significant change in HR or T (CO), even at the highest concentration. When combined, Ni and V produced observable delayed bradycardia and hypothermia at 0.5 mg/m^3 and potentiated these responses at 1.3 mg/m^3, to a greater degree than were produced by the highest concentration of Ni (2.1 mg/m^3) alone. Although these studies were performed at metal concentrations that were orders of magnitude greater than ambient concentrations, the results indicate a possible synergistic relationship between inhaled Ni and V.

Watkinson et al. (2000a,b) also sought to examine the relative toxicity of different particles on the cardiovascular system of spontaneously hypertensive rats. They instilled 2.5 mg of representative particles from ambient (Ottawa) or natural (Mount Saint Helens volcanic ash) sources and compared the response to 0.5 mg ROFA. Instilled particles were either mass

equivalent dose or adjusted to produce equivalent metal dose. They observed adverse changes in ECG, heart rate, and arrhythmia incidence that were much greater in the Ottawa- and ROFA-treated rats than in the Mount Saint Helens-treated rats. The cardiovascular changes observed with the Ottawa particles were actually greater than with the ROFA particles. These experiments by Watkinson and colleagues clearly demonstrate: (a) that instillation of ambient air particles, albeit at a very high concentration, can produce cardiovascular effects; and (b) that exposures of equal mass dose to particle mixes of differing composition did not produce the same cardiovascular effects, suggesting that PM composition rather than just mass was responsible for the observed effects.

Kodavanti et al. (2000b) exposed spontaneously hypertensive (SH) and normotensive (WKY) rats to 15 mg/m^3 ROFA for 6 h/day for 3 days. The exposure concentration, while 100 times or more higher than usual current U.S. ambient air PM concentrations, was selected to produce a frank but non-lethal injury and to allow comparison to the intratracheal approaches. Exposure to ROFA produced alterations in the ECG waveform of spontaneously hypertensive (SH) but not normotensive rats. Although the ST segment area of the ECG was depressed in the SH rats exposed to air, further depressions in the ST segment were observed at the end of the 6-h exposure to ROFA on Days 1 and 2. The enhanced ST segment depression was not observed on the third day of exposure, suggesting that adaptation to the response had occurred. Thus, exposure to a very high concentration of ROFA exacerbated a defect in the electroconductivity pattern of the heart in an animal model of hypertension. This ROFA-induced alteration in the ECG waveform was not accompanied by an enhancement in the monocytic cell infiltration and cardiomyopathy that also develop in SH rats. Further work is necessary to determine the relevance of this ROFA study to PM at concentrations relevant to ambient exposures.

Godleski and colleagues (2000) have performed a series of experiments examining the cardiopulmonary effects of inhaled concentrated ambient PM (CAPs) on normal mongrel dogs and on dogs with coronary artery occlusion. Dogs were exposed by inhalation via a tracheostomy tube to Boston CAPs for 6 h/day for 3 consecutive days. The investigators found little biologically-relevant evidence of pulmonary inflammation or injury in normal dogs exposed to PM (daily range of mean concentrations was ~100 to 1,000 µg/m^3). The only statistically significant effect was a doubling of the percentage of neutrophils in lung lavage. Despite the absence of major pulmonary effects, a significant increase in heart rate variability

(an index of cardiac autonomic activity), a decrease in heart rate, and a decrease in T alternans (an index of vulnerability to ventricular fibrillation) were seen. Exposure assessment of particle composition yielded no indication of which specific components of the CAPs were correlated with the day-to-day variability in response. The significance of these effects is not yet clear, given that the effects did not occur on all exposure days (e.g., changes in heart rate variability were observed on only 10 of the 23 exposure days). Although the HRV increase and the decrease in t-wave alternans might suggest a reduction in cardiovascular risk in response to inhaled concentrated ambient PM, the clinical significance of this effect is unclear. However, the magnitude of the observed changes, while small, are clearly not consistent with increased risk for cardiovascular events.

The most important finding of Godleski et al. (2000) was the observation of a potential increase in ischemic stress of the cardiac tissue from repeated exposure to concentrated ambient PM. During coronary occlusion in four dogs exposed to PM, they observed (a) significantly more rapid development of ST elevation of the ECG waveform; and (b) greater peak ST-segment elevation after PM exposure. Together, these changes are not internally consistent with those noted above. That is, on one hand, the ST segment elevation timing suggests a lower ischemic threshold and higher risk for serious outcomes in the compromised dog model, but the HRV and T-wave alternans changes in the normal dogs suggest lower cardiac risk. Clearly, much further work in more dogs (and other species) will be necessary both to try to confirm such findings and to better understand their potential significance.

Contrary to Godleski's study, Muggenburg and colleagues (2000a) reported that inhalation exposure to high concentrations of ROFA produced no consistent changes in amplitude of the ST-segment, form of the T wave, or arrhythmias in dogs. In their studies, four beagle dogs were exposed to 3 mg/m^3 ROFA particles for 3 h/day for 3 consecutive days. They noted a slight but variable decrease in heart rate, but the changes were not statistically or biologically significant. The transition metal content of the ROFA used by Muggenburg was ~15% by mass, a value on the order of a magnitude higher than that found in ambient urban PM samples. Although the study did not specifically address the effect of metals, it suggests that inhalation of high concentrations of metals may have little effect on the cardiovascular system of a healthy individual. In a second study, Muggenburg et al. (2003) evaluated the effects of short-term inhalation exposure (oral inhalation for 3 h on each of 3 successive days) to aerosols of transition

metals. Heart rate and the electrocardiogram were studied in conscious beagle dogs inhaling respirable particles of oxide and sulfate forms of transition metals (manganese, nickel, vanadium, iron, and copper oxides, and nickel and vanadium sulfates at concentrations of 0.05 mg/m^3). No significant effects of exposure to the transition metal aerosols were observed. The discrepancy between the results of Muggenberg et al. and those of Godleski and colleagues leave open major questions about PM effects on the cardiovascular system of the dog.

Wellenius et al. (2002) have developed and tested a model for investigating the effects of inhaled PM on arrhythmias and heart rate variability (HRV) in rats with acute myocardial infarction. Left-ventricular MI was induced in Sprague-Dawley rats by thermocoagulation of the left coronary artery or control rats underwent sham surgery. Diazepam-sedated rats were exposed (1 h) to residual oil fly ash (ROFA), carbon black, or room air at 12-18 h after surgery. Each exposure was immediately preceded and followed by a 1-h exposure to room air (baseline and recovery periods, respectively). Lead-II electrocardiograms were recorded. In the MI group, 41% of rats exhibited one or more premature ventricular complexes (PVCs) during the baseline period. Exposure to ROFA, but not to carbon black or room air, increased arrhythmia frequency in animals with preexisting PVCs. Furthermore, MI rats exposed to ROFA, but not to carbon black or room air, had decreased HRV, but there was no difference in arrhythmia frequency or HRV among sham-operated animals. The limited statistical significance (one MI rat mainly exhibited the reported changes) of the reported results call into question the biological relevance of the change observed in arrhythmia frequency in this myocardial infarction model exposed to ROFA.

In a series of studies, (Gordon et al., 2000) examined rodent cardiovascular system responses to concentrated ambient PM (CAPs) derived from New York City air. Particles of 0.2 to 2.5 μm diameter were concentrated up to 10 times their levels in ambient air (≈130 to 900 μg/m^3) to maximize possible differences in effects between normal and cardiopulmonary-compromised laboratory animals. ECG changes were not detected in normal Fischer 344 rats or hamsters exposed by inhalation to the New York City CAPs for 1 to 3 days. Similarly, no deaths or ECG changes were seem in MCT rats or cardiomyopathic hamsters exposed to PM.
In contrast to the nonsignificant decrease in heart rate observed in dogs exposed to Boston CAPs (Godleski et al., 2000), statistically significantly heart rate increases (~5%) were observed by Gordon et al. in both normal and MCT rats exposed to PM, but not on all exposure days. Thus,

extrapolation of the heart rate changes in these animal studies to human health effects is difficult, although the increase in heart rate in rats is similar to that observed in some human population studies.

Gordon and colleagues (1998) have reported other cardiovascular effects in animals exposed to inhaled CAPs. Increases in peripheral blood platelets and neutrophils were observed in control and MCT rats at 3 h, but not 24 h, after exposure to 150 to 400 $\mu g/m^3$ concentrated ambient PM (CAP). This neutrophil effect did not appear to be dose-related and did not occur on all exposure days, suggesting that day-to-day changes in particle composition may play an important role in the systemic effects of inhaled particles. The number of studies reported was small; and, it is therefore not possible to statistically determine if the day-to-day variability was truly due to differences in particle composition or even to determine the size of this effect.

Nadziejko et al. (2002) exposed healthy rats to concentrated ambient PM from New York City air at a concentration range of 95-341 $\mu g/m^3$ for six hours and sampled blood at 0, 12, and 24 hours post-exposure. They found no consistent differences in counts of platelets, blood cells, or in levels of proteins in the blood coagulation system that included fibrinogen, thrombin-anti-thrombin complex, tissue plasminogen activator, plasminogen activator inhibitor, and factor VII. Nadziejko et al. (2002) present a thorough discussion of the blood coagulation system, demonstrating its complexity and further discuss limitations of the study that include particle composition and size, the possible blunted response seen in rats compared to humans, the healthy status of the animals compared to a cardiovascular compromised model, and the endpoints chosen.

Terashima et al. (1997) also examined the effect of particles on circulating neutrophils. They instilled rabbits with 20 mg colloidal carbon, a relatively inert particle (< 1 μm), and observed a stimulation of the release of 5'-bromo-2'deoxyuridine (BrdU)-labeled PMNs from the bone marrow at 2 to 3 days after instillation. Because the instilled supernatant from rabbit AMs treated in vitro with colloidal carbon also stimulated the release of PMNs from the bone marrow, the authors hypothesized that cytokines released from activated macrophages could be responsible for this systemic effect. The same research group (Tan et al., 2000) looked for increased white blood cell counts as a marker for bone marrow PMN precursor release in humans exposed to very high levels of carbon from biomass burning during the 1997 Southeast Asian smoke-haze episodes. They found a significant association between PM_{10} (1-day lag) and

elevated band neutrophil counts expressed as a percentage of total PMNs. The biological relevance of this latter study to more usual urban PM exposure-induced systemic effects is unclear; however, because of the high dose of carbon particles.

The results of epidemiology studies suggest that homeostatic changes in the vascular system can occur after episodic exposure to ambient PM. Studies by Vincent et al. (2001) indicate that urban particles from Ottawa (48 mg/m^3) administered by nose-only inhalation to laboratory rats can affect blood levels of endothelin and cause a vasopressor response without causing acute lung injury. Moreover, the potency to influence hemodynamic changes can be modified by removing the polar organic compounds and soluble elements from the particles. Exposure to DPM (5 mg/m^3) had no effect on blood pressure, but caused elevated endothelin levels, whereas a comparable exposure to 5 mg/m^3 carbon black had no effects.

Frampton (2001) exposed healthy, nonsmoking subjects (18 to 55 years old) to 10 $\mu g/m^3$ ultrafine carbon while resting. Subjects were exposed to the ultrafine carbon through a mouthpiece for 2 h, with a ten minute break between each hour exposure. The exposure concentration (10 $\mu g/m^3$) corresponded to 2×10^6 particles/cm^3. Subjects were assessed for respiratory symptoms, spirometry, blood pressure, pulse-oximetry, blood markers, and exhaled NO before, immediately following, and 3.5 and 21 h post-exposure. Blood markers focused on parameters related to acute response, blood coagulation, circulating leukocyte activation, including complete blood leukocyte counts and differentials, IL-6, fibrinogen, and clotting factor VII. Heart rate variability and repolarization phenomena were evaluated by continuous 24-h Holter monitoring. Preliminary findings indicated no particle-related symptoms.

In another study, Ghio et al. (2000a) also showed that inhalation of concentrated PM in healthy nonsmokers causes increased levels of blood fibrinogen. They exposed 38 volunteers exercising intermittently at moderate levels of exertion for 2 h to either filtered air or particles concentrated from the air in Chapel Hill, NC (23 to 311 $\mu g/m^3$). Blood obtained 18 h after exposure contained significantly more fibrinogen than blood obtained before exposure. The observed effects in blood may be associated with the mild pulmonary inflammation also found 18 h after exposure to CAPs (see Section 7.2.3).

Gardner et al. (2000) examined whether the instillation of particles would alter blood coagulability factors in laboratory animals. Sprague-Dawley rats were instilled with 0.3, 1.7, or 8.3 mg/kg of ROFA or 8.3 mg/kg Mount Saint Helens volcanic ash. Because fibrinogen is a

known risk factor for ischemic heart disease and stroke, the authors suggested that this alteration in the coagulation pathway could take part in the triggering of cardiovascular events in susceptible individuals. Elevations in plasma fibrinogen, however, were observed in healthy rats only at the highest treatment dose (8.3 mg/kg); and no other changes in clotting function were noted. Because the lower treatment doses are known to cause pulmonary injury and inflammation, albeit to a lesser extent, the absence of plasma fibrinogen changes at the lower doses suggests that only high levels of pulmonary injury are able to produce an effect in healthy test animals.

To establish the temporal relationship between pulmonary injury, increased plasma fibrinogen, and changes in peripheral lymphocytes, Kodavanti et al. (2002) exposed spontaneously hypertensive (SH) and Wistar-Kyoto (WKY) rats to ROFA using both intratracheal instillation and inhalation exposure (acute and long-term) scenarios. Increases in plasma fibrinogen and decreases in circulating white blood cells were found for both strains in response to acute ROFA exposure (15 mg/m^3; 6 h/day; 1 wk) by inhalation and were temporally associated with acute (1 wk post exposure), but not longer-term (2-4 wk) lung injury. A bolus intratracheal instillation of ROFA increased plasma fibrinogen in both SH and WKY rats; whereas the increase was evident only in SH rats after acute (1 week) ROFA inhalation. The increased fibrinogen in SH rats was associated with greater pulmonary injury and inflammation than was found in the WKY rats. The authors concluded that acute PM exposure can provoke an acute thrombogenic response associated with pulmonary injury/inflammation and oxidative stress in cardiovascular-compromised rats.

Kodavanti et al. (2003) exposed male SD, WKY, and spontaneously hypertensive (SH) male rats to nose-only doses of oil combustion-derived emission PM (EPM), which contains bioavailable zinc at doses of 2, 5, or 10 mg/m^3 for 6h/day for 4 consecutive days. A second exposure paradigm consisted of a 10 mg/m^3 dose for 6 h/day, 1 day/week, for 4 or 16 consecutive weeks. Cardiovascular effects were not seen in SD and SH rats with the acute or chronic exposure, but WKY rats from the 16 week exposure group demonstrated cardiac lesions consisting of chronic-active inflammation, multifocal myocardial degeneration, fibrosis, and decreased numbers of granulated mast cells. These results suggest that myocardial injury in sensitive rats can be caused by long-term inhalation of environmentally-relevant PM.

Nemmar et al. (2002) studied effects of ultrafine (60 nm) polystyrene particles on thrombus formation in a hamster model after IT administration of unmodified, carboxylate-polystyrene, or amine-polystyrene particles. Unmodified particles did not affect thrombosis at concentrations up to 5 mg/kg; whereas carboxylate-polystyrene particles significantly inhibited thrombus formation at 100 and 500 mg/kg, but not at 50 mg/kg body weight. Thrombosis was significantly enhanced by amine-polystyrene particles at 50 and 500 mg/kg, but not at 5 mg/kg body weight. Intratracheal instillation of 5 mg of amine-polystyrene particles also increased thrombosis formation. Thus, only positively charged ultrafine particles resulted in thrombus formation. The authors concluded that (a) the presence of ultrafine particles in the circulation may affect hemostasis and (b) this is dependent on the surface properties of the particles.

Suwa et al. (2002) studied the effect of PM_{10} on the progression of atherosclerosis in rabbits. They exposed Watanabe heritable hyperlipidemic rabbits (with naturally increased susceptibility to atherosclerosis) to 5 mg PM_{10} in 1 mL saline administered by intrapharyngeal instillation (2 × per wk for 4 wks) or to vehicle for four weeks, and then both (a) measured bone marrow stimulation and (b) used quantitative histologic methods to determine the morphologic features of the atherosclerotic lesions. Exposure to PM_{10} (99% < 3.0 μm) from Ottowa, CN air caused an increase in circulating polymorphonuclear leukocytes (PMN) band cell counts and an increase in the size of the bone marrow mitotic pool of PMNs. Exposure to PM_{10} also caused progression of atherosclerotic lesions toward a more advanced phenotype. The volume fraction (vol/vol) of the coronary atherosclerotic lesions was increased by PM_{10} exposure. The vol/vol of atherosclerotic lesions correlated with the number of alveolar macrophages that phagocytosed PM_{10}. Exposure to PM_{10} also caused an increase in plaque cell turnover and extracellular lipid pools in coronary and aortic lesions, as well as in the total amount of lipids in aortic lesions.

In summary, laboratory animal studies, to date, have provided interesting evidence indicating that high concentrations of inhaled or instilled particles can have systemic effects, but some of the studies have provided conflicting evidence. The controlled human exposure study by Ghio et al. (2002) also has shown that ambient levels (ranging to ~300 $\mu g/m^3$) of inhaled PM can produce some biochemical changes (increased fibrinogen) in blood. Although some of these changes have been used as clinical "markers" for cardiovascular diseases, the causal relationship between these changes and potential life-threatening diseases remains to be better established.

Among the hypotheses that have been proposed to account for the nonpulmonary, systemic effects of PM are activation of neural reflexes (Veronesi and Oortgiesen, 2001); cytokine effects on heart tissue (Killingsworth et al., 1997); alterations in coagulability (Seaton et al., 1995; Sjögren, 1997); perturbations in both conductive and hypoxemic arrythmogenic mechanisms (Watkinson et al., 1998; Campen et al., 2000); and altered endothelin levels (Vincent et al., 2001). Much progress has been made in obtaining evidence bearing on such hypotheses. More research using controlled exposures to PM of laboratory animals and human subjects will, however, be necessary to test further such mechanistic hypotheses generated to date (as well as those likely to be proposed in the future) in order to more fully understand pathways by which relatively low concentrations of inhaled ambient PM can produce systemic, life-threatening changes.

7.3 RESPIRATORY EFFECTS OF CONTROLLED PM EXPOSURES OF HUMANS AND LABORATORY ANIMALS IN VIVO

This section assesses the respiratory effects of controlled in vivo exposures of laboratory animals and humans to various types of PM; in vitro studies using animal or human respiratory cells are discussed in Section 7.4.

Biological responses occurring in the respiratory tract following controlled PM inhalation include changes in pulmonary inflammation and systemic effects that result from direct effects on lung tissue. The observed responses are dependent on the physicochemical characteristics of the PM, exposure parameters (duration, concentrations, etc.), and health status of the host.

As noted earlier, data available in the 1996 PM AQCD were from studies that evaluated respiratory effects of specific components of ambient PM or surrogate particles, e.g., pure sulfuric acid droplets. Pulmonary effects of controlled exposures to ambient PM have been investigated by the use of particles collected from emission source bag filters or ambient samplers (e.g., impactors; diffusion denuders, etc.) and, more recently, by the use of aerosol concentrators (e.g., Sioutas et al., 1995a,b, 2000; Gordon et al., 1998; Chang et al., 2000, Kim et al., 2000a,b). Particles from ambient air samplers are collected on filters or other media, stored, and resuspended in an aqueous medium for use in inhalation, intratracheal installation, or in vitro studies. Both ambient PM and concentrated ambient particles (CAPs) have been used to evaluate effects in normal and compromised laboratory animals and humans. Some ambient PM has been standardized as a reference material and compared to existing dust and soot standards, e.g., standard materials from the National Institutes of Standards and Technology (NIST).

Particle concentrators provide a technique for exposing animals or humans by inhalation to concentrated ambient particles (CAPs) at levels higher than typical ambient PM concentrations. The development of particle concentrators has permitted the study of ambient real-world particles under controlled conditions. This strength is somewhat offset by the inability of CAPs studies to precisely control the mass concentration and day-to-day variability in ambient particle composition. Nonetheless, these studies are invaluable in the attempt to understand the biological mechanisms responsible for cardiopulmonary responses to inhaled PM. Because the composition of concentrated ambient PM varies across both time and location, a thorough physical-chemical characterization is necessary to compare results among studies or even among exposures within studies or to link particle composition to effects.

The in vivo studies discussed here and in vitro studies discussed later have almost exclusively used PM_{10} or $PM_{2.5}$ as particle size cutoffs for studying the adverse effects of ambient PM. Studying particles in such size ranges is justified based in part on interests in evaluating the bases for existing PM_{10} and $PM_{2.5}$ standards. In addition, collection of these size fractions has been made easier by widespread availability of ambient sampling equipment for PM_{10} and $PM_{2.5}$. Unfortunately, the study of other important size fractions, such as the coarse fraction ($PM_{10-2.5}$) and $PM_{1.0}$ has been largely ignored, and only limited toxicology data are available to specifically address these potentially important particle sizes. Similarly, although organic compounds often comprise 20 to 60% of the dry fine particle mass of ambient PM (Chapter 3), little research has addressed mechanisms by which this organic fraction contributes to adverse effects associated with ambient PM exposures. The potential contribution of organics in mutagenesis and carcinogenesis has been studied, but these extensive findings are only briefly discussed in this chapter (Section 7.4.3.2), which mainly focuses on studies aimed at evaluating the biological plausibility of epidemiologic evidence for increased cardiopulmonary morbidity and mortality being associated with exposure to ambient PM.

7.3.1 Ambient, Complex Combustion-Related, and Surrogate Particulate Matter

Some new in vivo toxicology studies utilizing inhalation exposure as a technique for evaluating the respiratory effects of ambient particles in humans and laboratory animals have been conducted with CAPs and with DPM. However, many of the new in vivo exposure studies have utilized intratracheal instillation techniques. The pros and cons of this technique in comparison to inhalation are covered in Chapter 6 (Section 6.5), and these issues have also been reviewed elsewhere (Driscoll et al., 2000; Oberdörster et al., 1997; Osier and Oberdörster, 1997). In most of the studies, PM samples were collected on filters, resuspended in a vehicle (usually saline), and a small volume of the suspension was instilled intratracheally into the animals. The physiochemical characteristics of the collected PM may be altered by deposition and storage on a filter and resuspension in an aqueous medium. Therefore, in terms of direct extrapolation to humans in ambient exposure scenarios, greater importance should be placed on inhalation studies. However, delivery of PM by instillation has the advantages that much less material is needed and that the dose is better defined, even though the particle deposition and distribution

patterns differ somewhat from inhalation. Instillation studies have proven valuable in comparing the effects of different types of PM and for investigating some of the mechanisms by which particles may cause inflammation and lung injury. Tables 7-2a,b, 7-3a,b, and 7-4 summarize newly available studies in which various biological endpoints were measured following exposures to CAPs, ambient PM extracts, complex combustion-related PM, or laboratory-derived surrogate PM, respectively.

At the time of the 1996 PM AQCD, there were only limited data available from human studies or laboratory animal studies on ultrafine particles and even less on coarse particles. In vitro studies had shown that ultrafine particles have the capacity to cause injury to cells of the respiratory tract. High mass concentrations 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. At exposure concentrations less than 50 $\mu g/m^3$, freshly generated insoluble ultrafine PTFE fume particles can be severely toxic to the lung. However, it is not clear as to what roles in the observed effects may have been played by fume gases which adhered to the particles. Newer data from controlled exposures have demonstrated that particle composition, in addition to particle size, may be responsible for the adverse health effects associated with ambient PM exposures.

Toxicological studies of other types of PM species were also discussed in the previous criteria document (U.S. Environmental Protection Agency, 1996a). These studies included exposures to fly ash, volcanic ash, coal dust, carbon black, and miscellaneous other particles, either alone or in mixture. Some of the particles discussed were considered to be models of "respirable low toxicity particles" and were used in instillation studies to delineate nonspecific particle effects from effects of known toxicants. A number of studies on "other PM" examined effects of up to 50,000 $\mu g/m^3$ of respirable particles with inherently low toxicity. Although there was no mortality, some mild pulmonary function changes after exposure to 5,000 to 10,000 $\mu g/m^3$ (5 to 10 mg/m^3) of relatively inert particles were observed in rats and guinea pigs. Lung morphology studies revealed focal inflammatory responses, some epithelial hyperplasia, and fibrotic responses after exposure to > 5,000 $\mu g/m^3$. Changes in macrophage clearance after exposure to > 10,000 $\mu g/m^3$ were equivocal (no host defense effects). In studies of mixtures of particles and other pollutants, effects varied depending on the toxicity of the associated pollutant. In humans, co-exposure to carbon particles appeared to increase responses to formaldehyde but

TABLE 7-2a. RESPIRATORY EFFECTS OF INHALED CONCENTRATED AMBIENT PARTICULATE MATTER (CAPs) IN CONTROLLED EXPOSURE STUDIES OF HUMAN SUBJECTS AND LABORATORY ANIMALS[2]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Exposure Concentration*	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Humans, healthy nonsmokers; 18 to 40 yr old n = 38	CAPs (Chapel Hill)	Inhalation	23.1 to 311.1 µg/m^3	0.65 µm σg = 2.35	2 h; analysis at 18 h	Dose-dependent increase in BAL neutrophils in both bronchial and alveolar fractions. Increase noted at all exposure levels. Particles were concentrated 6- to 10-fold at the inlet of the chamber.	Ghio et al. (2000a)
Humans, healthy; 19-41 yr old n = 4	CAPs (LA)	Inhalation	148 to 246 µg/m^3	$PM_{2.5}$	2 h	No significant changes in lung function, symptoms, S_aO_2, or Holter ECGs observed. The maximum steady state fine particle concentration in the breathing zone was typically seven times the ambient concentration.	Gong et al. (2000)
Mongrel dogs, some with balloon occluded LAD coronary artery n = 14	CAPs (Boston)	Inhalation via tracheostomy	50 to 1055.8 µg/m^3 (variable from day-to-day)	0.23 to 0.34 µm σg = 0.2 to 2.9	6 h/day × 3 days	Decreased respiratory rate over time and modest increase in lavage fluid neutrophils in normal dogs. Study utilized Harvard ambient particle concentrator. Ambient particles concentrated by approximately 30-fold.	Godleski et al. (2000)
Rats, male S-D 200-225 g, control-avi, control-SO_2, CAPs, SO_2-CAPs n = 48	CAPs (Boston)	Inhalation; Harvard/EPA fine particle concentrator; animals restrained in chamber	206, 733, and 607 µg/m^3 for Days 1-3, respectively; 29 °C, 59% RH	0.18 µm σg = 2.9	5 h/day for 3 days	PEF and TV significantly increased in SO_2/CAPs exposed animals. CAPs exposed rats had significant increase in TV. Increased protein and percent neutrophils and lymphocytes in lavage fluid after CAPs exposure. Responses were greater in SO_2-bronchitis animals. No changes in LDH. No deaths occurred. Exposures were to 30-40 times greater PM concentrations than found in ambient air.	Clarke et al. (1999) Saldiva et al. (2002)
Rats, male F344 Hamsters, male, 8-mo-old Bi TO-2	CAPs (NY)	Inhalation	132 to 919 µg/m^3	0.2 to 1.2 µm σg = 0.2 to 3.9	1 × 3 h or 3 × 6 h	No inflammatory responses, no cell damage responses, no PFT changes. The PM mean concentration factor (gravimetric) was 19.5 ± 18.6.	Gordon et al. (2000)
Rats, male, 90 to 100-day-old S-D, with or without SO_2-induced bronchitis	CAPs (RTP)	Inhalation	650 µg/m^3		6 h/day × 2-3 days	No significant changes in healthy rats. Increased BAL protein and neutrophil influx in bronchitic rats sacrificed immediately afer last CAPs exposure; responses variable between exposure regimens. No CAPs effects seen at 18 h postexposure.	Kodavanti et al. (2000a)
Rats, male F344 7-8 mo	CAPs (NY)	Inhalation	100 to 350 µg/m^3 (mean 225 µg/m^3)	0.4 µm σg = 2.5	3 h	Basal levels of superoxide ($\bullet O_2^-$) reduced by 90% 72 h postexposure; zymosan-stimulated O_2^- formation increased by > 150% after 24 h; basal level H_2O_2 production by PAM depressed 90% 3 h following exposure and remained 60% below levels at least 24 h; zymosan-stimulated H_2O_2 unaffected. Concentration tested represents a range over the 3 h exposure period.	Zelikoff et al. (2003)

[a]PEF = Peak expiratory flow
TV = tidal volume
LDH = lactic dehydrogenase
S_aO_2 = arterial oxygen saturation
*Concentration =- range of CAP concentrations at inlet of exposure chamber or in breathing zone of exposed subjects.

TABLE 7-2b. RESPIRATORY EFFECTS OF INSTILLED AMBIENT PARTICULATE MATTER IN LABORATORY ANIMALS AND HUMAN SUBJECTS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Humans, healthy nonsmokers; 21 M, 3 F; 26.4 ± 2.2 yr old	Provo, UT, PM_{10} filters (10 years old)	Intrabronchial instillation	500 µg of PM extract in 10 mL saline	N/A	24 h BAL	Inflammation (PMN) and pulmonary injury produced by particles collected during steel mill operation was greater than for during period of mill closure.	Ghio and Devlin (2001)
Rats, male S-D 60-day-old	Provo, UT, TSP filters (10 years old)	Intratracheal instillation	0.25, 1.0, 2.5, 5.0 mg of PM extract in 0.3 mL saline	N/A	24 h	Dose-dependent increase in inflammation (PMN) and pulmonary injury produced by particles collected during steel mill operation was greater than for during period of mill closure for all exposed groups.	Dye et al. (2001)
Rats, S-D 60-day-old n = 8/fraction	Provo, UT, TSP filters (10 years old), soluble and insoluble extracts	Intratracheal instillation	100, 150, 500, and 1,000 µg of PM extract in 0.5 mL saline	N/A	24 h	Dose-dependent increase in inflammation (PMN) and lavage fluid protein. Effect was greater with the soluble fraction containing more metal (Zn, Fe, Cu) except for the 100 µg exposed group.	Ghio et al. (1999a)
Rats, Wistar (HAN strain)	Edinburgh PM_{10} filters Carbon black (CB) Ultrafine CB	Intratracheal instillation	Range of 50 to 125 µg in 0.2 mL phosphate buffered saline	PM_{10} CB = (200-500 nm) UCB = 20 nm	Sacrificed at 6 h	Increased PMN, protein, and LDH following 50-125 µg PM_{10}; greater response with ultrafine CB but not CB; decreased GSH level in BAL; free radical activity (deplete supercoil DNA); leukocytes from treated animals produced greater NO and TNF.	Li et al. (1996, 1997)
Rats, S-D	DEP	Intratracheal instillation	500 µg in 0.5 mL saline	N/A	3 times/wk, 2 wk	Decreased concentration of lavage ascorbate. Urate and glutathione concentrations unchanged; elevated MIP-2 and TNF; total cell count increased; lavage protein and LDH increased; increased total lavage iron concentration.	Ghio et al. (2000b)

[a]PEF = Peak expiratory flow
TV = tidal volume
LDH = lactic dehydrogenase
S_aO_2 = arterial oxygen saturation
DMTU = dimethylthiourea

TABLE 7-3a. RESPIRATORY EFFECTS OF INSTILLED COMPLEX COMBUSTION-RELATED PARTICULATE MATTER IN LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Hamsters, Syrian golden, male, 90-125 g	Kuwaiti oil fire particles; urban particles from St. Louis, MO	Intratracheal instillation	0.15, 0.75, and 3.75 mg/100 g	Oil fire particles: < 3.5 µm, 10 days of 24-h samples	Sacrificed 1 and 7 days postinstillation	Dose-dependent increases in PMN, albumin, LDH, myeloperoxidase, and β-N-acetylglucosaminidase; decrease in AM. Acute toxicity of the particles found in smoke from Kuwaiti oil fires roughly similar to that of urban particles.	Brain et al. (1998)
Mice, female, NMRI, 28-32 g	Coal fly ash (CFA) Copper smelter dust (CMP) Tungsten carbide (TC)	Intratracheal instillation	CMP: 20 µg arsenic/kg, or CMP: 100 mg particles/kg, TC alone (100 mg/kg), CFA alone (100 mg/kg [i.e., 20 µg arsenic/kg]), CMP mixed with TC (CMP, 13.6 mg/kg [i.e., 20 µg arsenic/kg; TC, 86.4 mg/kg]) and $Ca_3(AsO_4)_2$ mixed with TC (20 µg arsenic/kg; TC 100 mg/kg)	N/A	1, 6, 30 days post-treatment, lavage for total protein content, inflammatory cell number and type, and TNF-α production particle retention	Mild inflammation for TC; $Ca_3(AsO_4)_2$ caused significant inflammation; CMP caused severe but transient inflammation; CFA caused persistent alveolitis. Cytokine production was upregulated in TC-and $Ca_3(AsO_4)$ treated animals after 6 and 30 days, respectively; a 90% inhibition of TNF-α production was still observed at day 30 after CMP administration and CFA; a significant fraction persisted (10-15% of the arsenic administered) in the lung of CMP- and CFA-treated mice at day 30. Suppression of TNF-α production is dependent on the slow elimination of the particles and their metal content from the lung	Broeckaert et al. (1997)
Rats, male, S-D, 60 days old	Emission source PM (ROFA, DOFA, CFA) Ambient airshed PM ROFA	Intratracheal instillation	Total mass: 2.5 mg/rat or Total transition metal: 46 µg/rat	Emission PM: 1.78-4.17 µm Ambient PM: 3.27-4.09 µm	Analysis at 24 and 96 h following instillation	Increases in PMNs, albumin, LDH, PMN, and eosinophils following exposure to emission and ambient particles; induction of injury by emission and ambient PM samples is determined primarily by constituent metals and their bioavailability.	Costa and Dreher (1997)
Rats, male, S-D, 65 days old	ROFA	Intratracheal instillation	2.5 mg (8.3 mg/kg)	1.95 µm	Analysis at 24 and 96 h	Increased PMNs, protein, LDH at both time points; bioavailable metals were causal constituents of pulmonary injury.	Dreher et al. (1997)
Rats, S-D, 65-day-old	ROFA	Intratracheal Instillation	500 µg/rat ROFA 500 µg/rat ROFA plus DMTU	1.95 µm	24 h	ROFA-induced increased neutrophilic inflammation was inhibited by DMTU treatment, indicating role reactive oxygen species.	Dye et al. (1997)
Rats, male, S-D, 60-day-old	lo-S #6 ROFA,	Intratracheal Instillation	0.3, 1.7, 8.3 mg/kg BW in saline	1.95 µm σg = 2.19	24 h	Increased WBC count in ROFA-exposed rats; plasma fibrinogen increased 86% in ROFA rats at highest concentration.	Gardner et al. (2000)
	volcanic ash		8.3 mg/kg BW in saline	1.4 µm			

TABLE 7-3a (cont'd). RESPIRATORY EFFECTS OF INSTILLED COMPLEX COMBUSTION-RELATED PARTICULATE MATTER IN LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Rats, male, S-D, 60 days old	Two ROFA samples (R1 and R2)	Intratracheal instillation	2.5 mg (9.4 mg/kg)	R1: 1.88 µm R2: 2.03 µm	Analysis at 4 days	Four of the 24 animals treated with R2 or R2s (supernatant) died; none in R1s treated animals; more AM, PMN, eosinophils protein, and LDH in R2 and R2s animals; more focal alveolar lesions, thickened alveolar septae, hyperplasia of type II cells, alveolar fibrosis in R2 and R2s animals; baseline pulmonary function and airway hyperreactivity were worse in R2 and R2s groups. R1 had twice the saline-leachable sulfate, Ni, and V and 40 times Fe as R2; R2 had 31 times higher Zn.	Gavett et al. (1997)
Mice, female, Balb/cJ 7-15 weeks	#6 ROFA, lo-S	Intratracheal instillation	60 µg in saline (dose 3 mg/kg)	< 2.5	Analysis at 1, 3, 8, 15 days	ROFA caused increases in eosinophils, IL-4 and IL-5 and airway responsiveness in ovalbumin-sensitized and challenged mice. Increased BAL protein and LDH at 1 and 3 days but not at 15 days postexposure. Combined OVA and ROFA challenge increased all damage markers and enhanced allergen sensitization. Increased methacholine response after ROFA.	Gavett et al. (1999)
Rats, male, S-D	ROFA	Intratracheal instillation	500 µg	3.6 µm	Analyzed 4 and 96 h postexposure	Ferritin and transferrin were elevated; greatest increase in ferritin, lactoferrin, transferrin occurred 24 h postexposure.	Ghio et al. (1998a)
Mice, normal and Hp, 105 days old	ROFA	Intratracheal instillation	50 µg	1.95 µm	Analysis at 24 h	Diminished lung injury (e.g., decreased lavage fluid ascorbate, protein, lactate dehydrogenase, inflammatory cells, cytokines) in Hp mice lacking transferrin; associated with increased metal storage and transport proteins.	Ghio et al. (2000c)
Rats, male, S-D, 60 days old	ROFA	Intratracheal instillation	1.0 mg in 0.5 mL saline	1.95 µm	Analysis at 24 h	Increased PMNs, protein.	Kadiiska et al. (1997)
Rats, male, S-D and F-344 (60 days old)	ROFA	Intratracheal instillation	8.3 mg/kg	1.95 µm σg = 2.14	Sacrificed at 24 h	Increase in neutrophils in both S-D and F-344 rats; a time-dependent increase in eosinophils occurred in S-D rats but not in F-344 rats.	Kodavanti et al. (1996)

TABLE 7-3a (cont'd). RESPIRATORY EFFECTS OF INSTILLED COMPLEX COMBUSTION-RELATED PARTICULATE MATTER IN LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Rats, male, S-D, WISTAR, and F-344 (60 days old)	ROFA	Intratracheal instillation	8.3 mg/kg	1.95 µm σg = 2.14	Sacrificed at 6, 24, 48, and 72 h; 1, 3, and 12 weeks	Inflammatory cell infiltration, as well as alveolar, airway, and interstitial thickening in all three rat strains; a sporadic incidence of focal alveolar fibrosis in S-D rats, but not in WISTAR and F-344 rats; cellular fibronectin (cF_n) mRNA isoforms EIIIA(+) were up-regulated in S-D and WIS rats but not in F-344 rats. Fn mRNA expression by macrophage, alveolar and airway epithelium, and within fibrotic areas in S-D rats; increased presence of Fn EIIIA(+) protein in the areas of fibrotic injury and basally to the airway epithelium.	Kodavanti et al. (1997a)
Rats, male, S-D, 60 days old	ROFA $Fe_2(SO_4)_3$, VSO_4, $NiSO_4$	Intratracheal instillation	8.33 mg/kg ROFA-equivalent dose of metals	1.95 µm σg = 2.14	Analysis at 3, 24, and 96 h, postinstillation	ROFA-induced pathology lesions were as severe as those caused by Ni. Metal mixture caused less injury than ROFA or Ni alone; Fe was less pathogenic. Cytokine and adhesion molecule gene expression occurred as early as 3 h after exposure. V-induced gene expression was transient, but Ni caused persistent expression and injury.	Kodavanti et al. (1997b)
Rats, male, S-D, 60 days old	10 compositionally different ROFA particles from a Boston power plant	Intratracheal instillation	0.833, 3.33, 8.3 mg/kg	1.99-2.67 µm	Sacrificed at 24 h	ROFA-induced increases in BAL protein and LDH, but not PMN, associated with water-leachable total metal, Ni, Fe, and S; BALF neutrophilic inflammation was correlated with V but not Ni or S. Chemiluminescence signals in vitro (AM) were greatest with ROFA containing soluble V and less with Ni + V. Only data for the 8.3 mg/kg dosed group were reported.	Kodavanti et al. (1998a)
Rats, male, S-D 60-day-old treated with MCT (60 mg/kg)	ROFA	Intratracheal instillation	0, 0.83, 3.3 mg/kg	1.95 µm σg = 2.19	24-96 h	Dose-dependent increase in BALF protein and LDH activity and neutrophilic inflammation. Effects were variable due to high mortality. 58% of rats exposed to ROFA died within 96 h.	Kodavanti et al. (1999)
Rats, male, WKY and SH, 11-13 weeks old	ROFA VSO_4, $NiSO_4$, or saline	Intratracheal instillation	3.33 mg/mL/kg 1.5 µmol/kg	1.95 µm σg = 2.14	1 and 4 days; postinstillation analysis at 6 or 24 h	Increased BALF protein and LDH alveolitis with macrophage accumulation in alveoli; increased neutrophils in BAL. Increased pulmonary protein leakage and inflammation in SH rats. Effects of metal constituents of ROFA were strain specific; vanadium caused pulmonary injury only in WKY rats; nickel was toxic in both SH and WKY rats.	Kodavanti et al. (2001)

TABLE 7-3a (cont'd). RESPIRATORY EFFECTS OF INSTILLED COMPLEX COMBUSTION-RELATED PARTICULATE MATTER IN LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Rats, female, Brown Norway 8-10 wks old	ROFA and HDM	Intratracheal instillation	200 µg or 1000 µg	1.95	N/A	ROFA enhanced the response to house dust mite (HDM) antigen challenge. Eosinophil numbers and LDH were increased in highest exposed groups. BAL protein and IL-10 were increased in both ROFA groups + HDM versus HDM alone.	Lambert et al. (1999)
Rats, male, S-D, 60-day-old	#6 ROFA from Florida	Intratracheal instillation	1000 µg in 0.5 mL saline	1.95 ± 0.18 µm	15 min to 24 h	Production of acetaldehyde increased at 2 h postinstillation.	Madden et al. (1999)
	NC ROFA; Domestic oil fly ash	Intratracheal instillation	1000 µg in 0.5 mL saline		15 min to 24 h	ROFA induced production of acetaldehyde with a peak at about 2 h. No acetaldehyde was seen in plasma at any time. DOFA increased acetaldehyde, as did V and Fe.	
Rats, male, S-D; 60 days old	#6 ROFA (Florida) $NiSO_4$ VSO_4	Intratracheal instillation	3.3 mg/mL/kg; ROFA equivalent dose of metals	1.9 µm $\sigma_g = 2.14$	3 or 24 h	Inflammatory and stress responses were upregulated; the numbers of genes upregulated were correlated with metal type and ROFA	Nadadur et al. (2000); Nadadur and Kodavanti (2002)
Rats, male, S-D, 60-day-old	ROFA	Intratracheal instillation	400 and 1000 µg/mL (200 and 500 µg ROFA in 0.5 mL saline)	N/A	12 h post-IT	ROFA increased PGE_2 via cycloxygenase expression in the 400 µg/mL group. PGE_2 depressed in 1000 µg/mL group by COX2 inhibitor.	Samet et al. (2000)
Rats, male, S-D, 60-day-old	LoS, #6 ROFA	Intratracheal instillation	500 µg in 0.5 mL saline	3.6 µm	1, 4, or 24 h	Mild and variable inflammation at 4 h; no pronounced inflammation until 24 h when there were marked increases in P-Tyr and P-MARKS.	Silbajoris et al. (2000)
Rats, male, S-D; 60-day-old; WKY and SH; cold-stressed SH, ozone-exposed SH, and MCT-treated SH	Ottawa dust, ROFA, and volcanic ash	Intratracheal instillation	0, 0.25, 1.0, and 2.5 mg/rat	1.95 µm	96 h post-IT	IT ROFA caused acute and dose-related increase in pulmonary inflammation. Data on Ottawa dust and volcanic ash not reported.	Watkinson et al. (2000a,b)

[a]CFA = Coal fly ash
CMP = Copper smelter dust
TC = Tungsten carbide
MCT = Monocrotaline
DOFA = Fly ash from a domestic oil-burning furnace
ROFA = Residual oil fly ash
$Fe_2(SO_4)$ = Iron sulfate
VSO_4 = Vanadium sulfate
$NiSO_4$ = Nickel sulfate
LoS = low sulfur
OVA = Ovalbumin
HDM = House dust mite antigen
COX = Cyclooxygenase

TABLE 7-3b. RESPIRATORY EFFECTS OF INHALED COMPLEX COMBUSTION-RELATED PARTICULATE MATTER IN COMPROMISED LABORATORY ANIMAL MODELS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Rats, male, WISTAR Bor:WISW strain n = 20	Coal oil fly ash	Inhalation (chamber)	0, 11, 32, and 103 mg/m^3	1.9-2.6 µm σg = 1.6-1.8	6 h/day, 5days/week, 4 weeks	At 103 mg/m^3, type II cell proliferation, mild fibrosis and increased perivascular lymphocytes seen. At lowest concentration, main changes seen were particle accumulation in AM and mediastinal lymph nodes. Lymphoid hyperplasia observed at all concentrations. Effects increased with exposure duration.	Dormans et al. (1999)
Mice, BALB/C, 2-day-old, sensitized to ovalbumin (OVA)	Aerosolized ROFA leachate	Nose-only inhalation	50 mg/mL	N/A	30 min	Increased airway response to methylcholine and to OVA in ROFA exposed mice; increased airway inflammation also.	Hamada et al. (1999)
Rats, S-D, 250 g MCT	ROFA	Inhalation	580 ± 110 µg/m^3	2.06 µm σg = 1.57	6 h/day for 3 days	Death occurred only in MCT rats exposed to ROFA. Neutrophils in lavage fluid were increased significantly in MCT rats exposed to ROFA versus filtered air. MIP-2 mRNA expression in lavage cells was induced in normal animals exposed to fly ash.	Killingsworth et al. (1997)
Rats, male, S-D 60-day-old treated with MCT (60 mg/kg)	ROFA	Nose-only inhalation	15 mg/m^3	1.95 µm σg = 2.14	6 h/day for 3 days analysis at 0 or 18 h	No mortality occurred by inhalation. ROFA exacerbated lung lesions (edema, inflammation, alveolar thickening) and gene expression in MCT rats. Rats showed inflammatory responses (IL-6, MIP-2 genes upregulated).	Kodavanti et al. (1999)
Rats, male, WKY and SH, 11-13 weeks old	ROFA	Nose-only Inhalation	15 mg/m^3	1.95 µm σg = 2.14	6 h/day × 3 day, analysis at 0 or 18 h	More pulmonary injury in SH rats. Increased RBCs in BAL of SH rats. ROFA increased airway reactivity to Acctylcholine in both SH and WKY rats. Increased protein, albumin, and LDH in BAL after ROFA exposure (SH > WKY). Increased oxidative stress in SH rats. SH rats failed to increase glutathione. Inflammatory cytokine gene expression increased in both SH and WKY rats.	Kodavanti et al. (2000b)

[a]CFA = Coal fly ash
CMP = Copper smelter dust
TC = Tungsten carbide
MCT = Monocrotaline
DOFA = Fly ash from a domestic oil-burning furnace
ROFA = Residual oil fly ash
$Fe_2(SO_4)$ = Iron sulfate
VSO_4 = Vanadium sulfate
$NiSO_4$ = Nickel sulfate
LoS = low sulfur
OVA = Ovalbumin

TABLE 7-4. RESPIRATORY EFFECTS OF SURROGATE PARTICULATE MATTER IN LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Inhalation							
Hamsters, Syrian golden 900 male, 900 female, 4-wks-old	Toner (carbon) TiO_2 Silica	Nose-only inhalation	1.5, 6.0, or 24 mg/m^3 (toner)[b] 40 mg/m^3 (TiO_2)[b] 3 mg/m^3 (SiO_2)[b]	4.0 µm 1.1 µm 1.4 µm	3, 9, 15 mo 6 h/day 5 days/week	Retention increased with increased concentration and exposure duration. Retention half-times retarded (males) for all exposed groups.	Creutzenberg et al. (1998)
Mice, C57Bl/6J	PTFE TiO_2	Inhalation	PTFE: 1.25, 2.5, or 5×10^5 particles/cm^3 TiO_2-F: 10 mg/m^3 NiO: 5 mg/m^3 Ni_3S_2: 0.5 mg/m^3	PTFE: 18 nm TiO_2-F: 200 nm TiO_2-D: 10 nm	30 min or 6 h/day, 5 days/week, 13 wks	Effects on the epithelium caused by direct interactions with particles, not a result of macrophage-derived mediators, and suggest a more significant role in the overall pulmonary response than previously suspected; type II cell growth factor production may be significant in the pathogenesis of pulmonary fibrosis.	Finkelstein et al. (1997)
Rats, male, F-344 200-230 g	PTFE Fumes	Whole body inhalation	5×10^5 particles/cm^3 (~50 µg/m^3)	18 nm	15 min; analysis 4 h postexposure	Increased PMN, mRNA of MnSOD and MT, IL-1α, IL-1β, IL-6, MIP-2, TNF-α mRNA of MT and IL-6 expressed around all airways and interstitial regions; PMN expressed IL-6, MT, and TNF-α; AM and epithelial cells were actively involved.	Johnston et al. (1996)
Mice, male, C57BL/6J, 8 weeks and 18-mo-old	PTFE Fumes	Whole body inhalation	1, 2.5, or 5×10^5 particles/cm^3	18 nm	30-min exposure, analysis 6 h following exposure	Increased PMN, lymphocytes, and protein levels in old mice over young mice; increased TNF-α mRNA in old mice over young mice; increased LDH and β-Glucuronidase in young mice over old mice. Effects not seen at lowest exposure level.	Johnston et al. (1998)
Rats, male, S-D, MCT-treated	Fluorescent microspheres	Inhalation	3.85 ± 0.81 mg/m^3	1.38 ± 0.10 µm σ_g = 1.8 ± 0.28	3 h/day × 3 days	Monocrotaline-treated animals contained fewer microspheres in their macrophages, probably because of impaired chemotaxis.	Madl et al. (1998)

TABLE 7-4 (cont'd). RESPIRATORY EFFECTS OF SURROGATE PARTICULATE MATTER IN LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Inhalation (cont'd)							
Mice, male, Swiss-Webster, 6-8 weeks old (A/J, AKR/J, B6C3F1/J, BALB/cJ, C3H/HeJ-C3, C3HeOuJ, CSTBL/6J-B6, SJL/J, SWR/J, 129/J) strains raised in a pathogen free laboratory	Carbon black Regal 660 Carbon-associated $SO_4^=$	Nose only inhalation	10 mg/m^3 (carbon) 10 ppm SO_2 285 µg/m^3 (average concentration of particle-associated sulfates)	0.29 µm ± 2.7 µm	4 h	Differences in inflammatory responses (PMN) across strains. Appears to be genetic component to the susceptibility.	Ohtsuka et al. 2000a,b
Rats, F-344 8-wks, 20-mo-old Mice, TSK 14-17 mo old	Carbon	Inhalation	100 µg/m^3 and/or 1.0 ppm O_3 following exposure to endotoxin (12 min to 70 EU)	UF	6 h	Small effect on lung inflammation and inflammatory cell activation. Effects enhanced in compromised lung and in older animals. Greatest effect in compromised lung exposed to UF carbon and O_3.	Elder et al. (2000a,b)
Instillation							
Rats, male, S-D (200 g)	Diesel, SiO_2, carbon black	Intratracheal instillation	1 mg in 0.4 mL.	DEP collected as TSP-disaggregated in solution by sonication (20 nm); SiO_2 (7 nm); carbon black	Necropsy at 2, 7, 21, 42, and 84 days postinstillation	Amorphous SiO_2 increased permeability and neutrophilic inflammation. Carbon black and DEP translocated to interstitum and lymph nodes by 12 weeks.	Murphy et al. (1998)

[a]PTFE = polytetrafluoroethylene
TiO_2 = titanium oxide
SiO_2 = silicon dioxide
[b]Concentrations changed after 5 mo to 4, 16, and 64 mg/m^3 for toner; 0 mg/m^3 for TiO_2.

not to acid aerosol. None of the "other" particles mentioned above are present in ambient air in more than trace quantities. Thus, it was concluded that the relevance of any of these studies to standard setting for ambient PM may be extremely limited.

Newer studies, on the other hand, appear to provide evidence of likely greater relevance to understanding ambient PM exposure effects and underlying mechanisms, as discussed below.

7.3.1.1 Ambient Particulate Matter

New studies that examined the acute effects of intratracheal instillation of ambient PM obtained from specific ambient locations have most clearly shown that ambient PM can cause lung inflammation and injury.

Costa and Dreher (1997) showed that instillation of relatively high concentrations of PM samples from three emission sources (two oil and one coal fly ash) and four ambient airsheds (St. Louis, MO; Washington, DC; Dusseldorf, Germany; and Ottawa, Canada) resulted in increases in lung polymorphonuclear leukocytes (PMNs) and eosinophils in rats 24 h after instillation. Biomarkers of permeability (total protein and albumin) and cellular injury, lactic dehydrogenase (LDH), also were increased. Animals were dosed with (1) an equal dose by mass (nominal 2.5 mg/rat) of each PM mixture or (2) normalization of each PM mass to a metal content of 46 µg/dose and 35.5 µg of total metals (Cu, Fe, V, Zn) for the ambient PM and ROFA comparison. This study demonstrated that the lung dose of bioavailable transition metal, not instilled PM mass, was the primary determinant of the acute inflammatory response.

Kennedy et al. (1998) reported a similar dose-dependent inflammation (i.e., increase in protein and PMN in lavage fluid, proliferation of bronchiolar epithelium, and intraalveolar hemorrhage) in rats instilled with water-extracted particles (TSP) collected in Provo, UT. The particulate mixture was composed of 1.0 mg/g Zn, 0.04 mg/g Ni, 2.2 mg/g Fe, 0.01 mg/g Vn, 1.4 mg/g Cu, 1.7 mg/g Pb, and 78 mg/g $SO_4^=$ in 500 mL saline solution. This study also indicated that the metal constituent, in this case PM-associated Cu, was a plausible cause of the outcome based on IL-8 secretion and enhanced activation of the transcription factor NF-kB in cultured epithelium.

Further toxicological studies of ambient PM collected around Provo, UT (Utah Valley) in the late 1980s are particularly interesting (Ghio and Devlin, 2001; Dye et al., 2001; Wu et al., 2001; Soukup et al., 2000; Frampton et al., 1999). Epidemiologic studies by Pope (1989, 1991)

had shown that exposures to PM_{10} during closure of an open-hearth steel mill over a 13-mo period beginning in 1987 were associated with reductions in several health endpoints, e.g., hospital admissions for respiratory diseases, as discussed in the 1996 PM AQCD (U.S. Environmental Protection Agency, 1996a). Ambient PM was collected near the steel mill during the winter of 1986 (before closure), 1987 (during closure), and again in 1988 (after plant reopening). The fibrous glass hi-vol filters were stored, folded PM-side inward, in plastic sleeves at room temperature and humidity (Dye et al., 2001). A description of the in vivo toxicological studies follows; the in vitro studies are discussed in Section 7.4.2.1.

Ghio and Devlin (2001) investigated biologic effects of PM from the Utah Valley to determine if the biological responses mirrored the epidemiologic findings, with greater injury occurring after exposure to an equal mass of particles from those years when the mill was in operation. Aqueous extracts of the filters collected prior to temporary closure of the steel mill, during the closure, and after its reopening were instilled through a bronchoscope into the lungs of nonsmoking human volunteers. Twenty-four hours later, the same subsegment was lavaged. Exposure to aqueous extracts of PM collected before closure and after reopening of the steel mill provoked a greater inflammatory response than PM extracts from filters taken during the plant shutdown. These results indicate that pulmonary effects of experimental exposure of humans to the Utah Valley PM parallel health outcomes observed in epidemiologic studies of the human population exposed under ambient conditions.

Dye et al. (2001) also examined effects of Utah Valley ambient PM on respiratory health but in laboratory animals. Sprague-Dawley rats were intratracheally instilled with equivalent masses of aqueous extracts from filters originally collected during the winter before, during, and after closure of the steel mill. Twenty-four hours after instillation, rats exposed to extracts of particles collected when the plant was open developed significant pulmonary injury and neutrophilic inflammation. Additionally, 50% of rats exposed to these extracts had increased airway responsiveness to acetylcholine, compared to 17 and 25% of rats exposed to saline or the extracts of particles collected when the plant was closed. By 96 hr, these effects were largely resolved except for increases in lung lavage fluid neutrophils and lymphocytes in rats exposed to PM extracts from prior to the plant closing. Analogous effects were observed with lung histologic assessment. Extract analysis demonstrated that nearly 70% of the mass in all three extracts appeared to be sodium-based salts derived from the glass filter matrix. Extracts of

particles collected when the plant was open contained more sulfate, cationic salts (i.e., calcium, potassium, magnesium), and certain metals (i.e., copper, zinc, iron, lead, strontium, arsenic, manganese, nickel). Although total metal content was ≈1% of the extracts by mass, the greater quantity detected in the extracts of particles collected when the plant was open suggests that metals may be important determinants of the observed pulmonary toxicity. The authors concluded that the pulmonary effects induced in rats by exposure to aqueous extracts of local ambient PM filters were in good accord with the epidemiologic reports of adverse respiratory health effects in Utah Valley residents and with results from the Molinelli et al. (2002) in vitro study of Utah Valley PM filter extract effects on human epithelial cells (discussed below in Section 7.4).

In parallel work on potential importance of metals in mediating ambient PM effects, Kodavanti et al. (2002) examined the role of zinc in PM-induced health effects in several different animal models. Male Sprague-Dawley (SD) rats were instilled IT with an oil combustion emission PM (EPM) in saline (0.0, 0.8, 3.3, or 8.3 mg/kg); and, in order to examine the potential role of EPM leachable zinc, additional rats were instilled with either saline, whole EPM suspension, the saline leachable fraction of EPM, the particulate fraction of EPM (8.3 mg/kg, soluble Zn = 14.5 µg/mg EPM), or $ZnSO_4$ (0.0, 33.0, or 66.0 µg/kg Zn). Three rat strains of differing PM susceptibility, i.e., male SD, normotensive Wistar-Kyoto (WKY), and spontaneously hypertensive (SH) rats, were exposed at 90 days of age nose-only to either filtered air or EPM (2, 5, or 10 mg/m^3 for 6 h/day × 4 days/week × 1 week; or 10 mg/m^3 for 6 h/day × 1 day/week for 1, 4, or 16 weeks) and assessed at 2 days postexposure. Intratracheal exposures to whole EPM suspensions were associated with a dose-dependent increase in protein/albumin permeability and neutrophilic inflammation. Pulmonary protein/albumin leakage and neutrophilic inflammation caused by the leachable fraction of EPM and $ZnSO_4$ were comparable to the effects of the whole suspension. However, protein/albumin leakage was not associated with the particulate fraction, although significant neutrophilic inflammation did occur following instillation. With EPM nose-only inhalation, acute exposures (10 mg/m^3 only) for 4 days resulted in small increases in bronchoalveolar lavage fluid (BAL) protein and n-acetyl glucosaminidase activities (approximately 50% above control). Unlike IT exposures, no neutrophilic influx was detectable in BAL from any of the inhalation groups. The only major effect of acute and long-term EPM inhalation was a dose- and time-dependent increase in

alveolar macrophages (AM) regardless of the rat strain. Histological evidence also showed dose- and time-dependent accumulations of particle-loaded AM. Particles were also evident in interstitial spaces, and in the lung-associated lymph nodes following the inhalation exposures (SH > WKY = SD). There were strain-related differences in peripheral white blood cell counts and plasma fibrinogen, but no major EPM inhalation effect. The authors attributed the critical differences in pulmonary responsiveness to EPM between IT and inhalation exposures to the dose of bioavailable zinc. EPM IT exposures, but not acute and long-term inhalation of up to 10 mg/m^3, caused neutrophilic inflammation.

Also of interest are some other new instillation study results. For example, Li et al. (1996, 1997) reported that instillation of ambient PM_{10} (50-125 μg in 0.2 ml buffered saline) collected in Edinburgh, Scotland, also caused pulmonary injury and inflammation in rats. In addition, Brain et al. (1998) examined the effects of instillation of particles (< 3.5 μm) that resulted from the Kuwaiti oil fires in 1991 compared to effects of urban PM collected in St. Louis (NIST SRM 1648, collected in a bag house in the early 1980s). They showed that, on an equal mass basis, the acute toxicity of the Kuwaiti oil fire particles was similar to that of urban particles collected in the United States. At all exposure levels (0.15, 0.75, and 3.75 mg/100 g body weight), both the Kuwaiti oil fire and St. Louis urban particles significantly increased BAL neutrophils, macrophages, and levels of albumin and other biomarkers (LDH, MPO, GLN) of lung inflammation.

The fact that instillation of ambient PM collected from different geographical areas and from a variety of emission sources consistently caused pulmonary inflammation and injury tends to corroborate epidemiologic studies that report increased PM-associated respiratory effects in populations living in many different geographical areas and climates. On the other hand, there is a potential that more "realistic" doses of metals may activate cells and signaling pathways in a manner that is not observed at doses that are much greater than present in ambient air, such that these mechanisms may be overwhelmed. Thus, high-dose instillation studies may produce different effects on the lung than inhalation exposures at more relevant concentrations.

With regard to inhalation studies more directly mimicking ambient exposures, Ghio et al. (2000a) exposed 38 healthy volunteers exercising intermittently at moderate levels of exertion for 2 h to either filtered air or particles concentrated (23 to 311 $\mu g/m^2$) from the air in Chapel Hill, NC . Analysis of cells and fluid obtained 18 h after exposure showed a mild increase in

neutrophils in the bronchial and alveolar fractions of bronchoalveolar lavage (BAL) in subjects exposed to the highest quartile concentration of concentrated PM (mean of 206.7 $\mu g/m^3$). Lavage protein did not increase, and there were no other indicators of pulmonary injury. No respiratory symptoms or decrements in pulmonary function were found after exposure to CAPs. The 38 human volunteers reported on by Ghio et al. (2000a) were also examined for changes in host defense and immune parameters in BAL and blood (Harder et al., 2001). There were no changes in the number of lymphocytes or macrophages, subcategories of lymphocytes (according to surface marker analysis by flow cytometry), cytokines IL-6 and IL-8, or macrophage phagocytosis. Similarly, there was no effect of concentrated ambient PM exposure on lymphocyte subsets in blood. Thus, a mild inflammatory response to concentrated ambient PM was not accompanied by an effect on immune defenses as determined by lymphocyte or macrophage effects. The increase in neutrophils may represent an adaptive response of the lung to particles, although the presence of activated neutrophils may release biochemical mediators which produce lung injury. Whether this mild inflammatory increase in neutrophils constitutes a biologically significant injury to the lung is an ongoing controversial issue.

Other human inhalation studies with CAPs are limited by the small numbers of subjects studied. Petrovic et al. (1999) exposed four healthy volunteers (aged 18 to 40) under resting conditions to filtered air and 3 concentrations of concentrated ambient PM (23 to 124 $\mu g/m^3$) for 2 hours using a face mask. The exposure was followed by 30 minutes of exercise. No cellular signs of inflammation were observed in induced sputum samples collected at 2 or 24 hours after exposure. There was a trend toward an increase in nasal lavage neutrophils although no statistical significance was presented. The only statistically significant change in pulmonary function was a 6.4% decrease in thoracic gas volume after exposure to 124 $\mu g/m^3$ PM versus a 5.6% increase after air. A similar, small pilot study has been reported (Gong et al., 2000) in which no changes in pulmonary function or symptoms were observed in four subjects aged 19 to 41 after a 2 hour exposure to air or mean concentrations of 148 to 246 $\mu g/m^3$ concentrated ambient PM in Los Angeles, CA. Both of these laboratories are currently expanding on these preliminary findings, but no additional data are available at this time.

Saldiva et al. (2002) studied the effects on rat lung of CAPs from Boston. The study was designed (1) to determine whether short-term exposures to CAPs cause pulmonary inflammation in normal rats and rats with chronic bronchitis (CB); (2) to identify the site within the lung

parenchyma where CAPs-induced inflammation occurs; and (3) to characterize the component(s) of CAPs significantly associated with development of the inflammatory reaction. Four groups of animals were studied: (1) air treated, filtered air exposed (air-sham); (2) sulfur dioxide treated (CB), filtered air exposed (CB-sham); (3) air treated, CAPs exposed (air-CAPs); and (4) sulfur dioxide treated, CAPs exposed (CB-CAPs). Chronic bronchitis and normal rats were exposed by inhalation either to filtered air or CAPs during 3 consecutive days (5 hours/day). CAPs (as a binary exposure term) and CAPs mass (in regression correlations) induced a significant increase in bronchoalveolar lavage (BAL) neutrophils and in normal and CB animals. Numerical density of neutrophils (Nn) in the lung tissue significantly increased with CAPs in normal animals only. Greater Nn was observed in central, compared with peripheral, regions of the lung. A significant dose-dependent association was found between CAPs components and BAL neutrophils or lymphocytes, but only vanadium and bromine concentrations had significant associations with both BAL neutrophils and Nn in CAPs-exposed groups analyzed together. The authors concluded that (a) short-term exposures to CAPs from Boston induce a significant inflammatory reaction in rat lungs and (b) the reaction is influenced by particle composition.

Zelikoff et al. (2003a) reported effects on pulmonary or systemic immune defense mechanisms in Fischer rats exposed to New York City CAPs at 0 or 90 to 600 $\mu g/m^3$ for 3 h prior to IT instillation of Streptococcus pneumoniae ($2 - 4 \times 10^7$ organisms delivered dose). The number of lavageable cells (PAM and PMN) increased in both control and experimental groups, but were elevated faster and were twice as high in the CAPs-exposed group, as well as staying elevated longer. Lymphocyte values and WBC were significantly increased 24 and 72 h postinfection in both groups. CAPs exposure slowed the decline of TNFα and IL-6 levels three days postinfection compared to bacteria-only exposed rats; but the differences were not significant. CAPs exposure significantly increased bacterial burdens at 24 h postinfection. Thereafter, CAPs-exposed animals exhibited significantly lower bacterial burdens. In another study, Zelikoff et al. (2003b) evaluated the effects of CAPs exposure in rats following a single 5 h exposure to IT instilled Streptococcus pneumoniae. CAPs exposure significantly reduced percentages of lavageable PMN 24 h following CAPs exposure and remained well below control levels for up to 3 days. Lavageable PAM was significantly increased in the CAPs exposed animals. CAPs exposure reduced the levels of TNFα, IL-1, and IL-6. The bacterial burden decreased in both exposed groups over time; however, CAPs exposed animals had a significantly

greater burden after 24 h than did control rats. Lymphocyte and monocyte levels were unaffected by CAPs exposure.

7.3.1.2 Diesel Particulate Matter

Other studies of controlled human exposures to ambient PM that may be relevant to this discussion are those previously examined in detail in earlier assessment documents (Health Effects Institute, 1995; U.S. Environmental Protection Agency, 2002). Briefly, the data from work shift studies suggest that the principle noncancer human hazard from exposure to diesel exhaust (DE) includes increased acute sensory and respiratory symptoms (e.g., cough, phlegm, chest tightness, wheezing) that are more sensitive indicators of possible health risks from exposure to DE than pulmonary function decrements. Immunological changes also have been demonstrated under short-term exposure scenarios to either DE or diesel particulate (DPM), and the evidence indicates that these immunological effects are caused by both the non-extractable carbon core and the adsorbed organic fraction of the diesel particle. While noncancer effects from long-term exposure to a high concentration of DE in several laboratory animal species include pulmonary histopathology and chronic inflammation, noncancer effects in humans from long-term chronic exposure to DE are not evident. The mode of action of DE is not completely understood; but the effects on the upper respiratory tract, observed in acute studies, suggest a non-inflammatory irritant response while the effects on the lung, observed in chronic studies, indicate an underlying inflammatory response. The noncancer lung effects occur in response to DE in several species and occur in rats at doses lower than those inducing particle overload.

Diesel particulate matter, therefore, can be relevant to the urban environment, particularly in urban micro-environments with heavy diesel engine traffic. The findings of controlled-exposure studies to DE are discussed both here and in Section 7.5.3 (Particulate Matter Effects on Allergic Hosts).

Pulmonary function and inflammatory markers (as assayed in induced sputum samples or BAL) have been studied in human subjects exposed to either resuspended or freshly generated and diluted DPM. In a controlled human study, Sandstrom and colleagues (Rudell et al., 1994) exposed eight healthy subjects in an exposure chamber to diluted exhaust from a diesel engine for 1 h with intermittent exercise. Dilution of the DE was controlled to provide a median NO_2 level of approximately 1.6 ppm. Median particle number was 4.3×10^6 /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 airway closing volume. Five of eight subjects experienced unpleasant smell, eye irritation, and nasal irritation during exposure. BAL 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 yet statistically significant reductions were seen in BAL mast cells, AM phagocytic function, and lymphocyte CD4 to CD8+ cell ratios. A small increase in neutrophils was also observed. These findings suggest that DE may induce mild airway inflammation in the absence of spirometric changes. Although this early study provided important information on the effect of DE exposure in humans, only one exposure level was used, the number of subjects was low, and a limited range of endpoints was reported. Several follow-up studies have been done by the same and other investigators.

Rudell et al. (1996) later exposed 12 healthy volunteers to DE for 1 h in an exposure chamber. Light work on a bicycle ergometer was performed during exposure. Random, double-blinded exposures included air, DE, or DE with particle numbers reduced 46% by a particle trap. The engine used was a new Volvo model 1990, a six-cylinder direct-injection turbocharged diesel with an intercooler, which was run at a steady speed of 900 rpm during the exposures. It is difficult to compare this study with others, because neither exhaust dilution ratios nor particle concentrations were reported. Concentrations of 27-30 ppm CO and of 2.6-2.7 ppm NO, however, suggested DPM concentrations may have equaled several mg/m^3. The most prominent symptoms during exposure were irritation of the eyes and nose, accompanied by an unpleasant smell. Both airway resistance and specific airway resistance increased significantly during the exposures. Despite the 46% reduction in particle numbers by the trap, effects on symptoms and lung function were not significantly reduced. A follow-up study on the usefulness of a particle trap confirmed the lack of effect of the filter on DE-induced symptoms (Rudell et al., 1999). In this study, 10 healthy volunteers also underwent BAL 24 hours after exposure. Exposure to DE produced inflammatory changes in BAL, as evidenced by increases in neutrophils and decreases in macrophage phagocytic function in vitro. A 50% reduction in the particle number concentration by the particle trap did not alter these BAL cellular changes.

As reported in the studies by Rudell and Sandstrom (Rudell et al., 1990, 1996, 1999; Blomberg et al., 1998; Salvi et al., 1999), significant increases in neutrophils and B lymphocytes, as well as histamine and fibronectin in airway lavage fluid, were not

accompanied by decrements in pulmonary function. Salvi et al. (1999) exposed healthy human subjects to diluted DE (DPM = 300 $\mu g/m^3$) for 1 h with intermittent exercise. Bronchial biopsies obtained 6 h after DE exposure showed a significant increase in neutrophils, mast cells, and CD4+ and CD8+ T lymphocytes, along with upregulation of the endothelial adhesion molecules ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) and increases in the number of leukocyte function-associated antigen-1 (LFA-1+) in the bronchial tissue. Importantly, extra-pulmonary effects were observed in these subjects. Significant increases in neutrophils and platelets were found in peripheral blood following exposure to DE.

Several DE toxicity studies cited in the EPA Assessment of Health Effects of Diesel Exhaust (U.S. Environmental Protection Agency, 2002) compared the effects of whole, unfiltered exhaust to those produced by the gaseous components of the exhaust. A comparison of the toxic responses in laboratory animals exposed to whole exhaust or filtered exhaust containing no particles demonstrates across studies that, when the exhaust is sufficiently diluted to limit the concentrations of gaseous irritants (NO_2 and SO_2), irritant vapors (aldehydes), CO, or other systemic toxicants, the diesel particles are the prime etiologic agents of noncancer health effects, although additivity or synergism with the gases cannot be ruled out. These toxic responses are both functional and pathological and represent a cascading sequelae of lung pathology based on concentration and species. The diesel particles plus gas exposures produced biochemical and cytological changes in the lung that are much more prominent than those evoked by the gas phase alone. Such marked differences between whole and filtered DE are also evident from general toxicological indices, such as decreases in body weight and increases in lung weights, pulmonary function measurements, and pulmonary histopathology (e.g., proliferative changes in Type II cells and respiratory bronchiolar epithelium fibrosis). Hamsters, under equivalent exposure regimens, have lower levels of retained DPM in their lungs than rats and mice and, consequently, less pulmonary function impairment and pulmonary pathology. These differences may result from lower DPM inspiration and deposition during exposure, greater DPM clearance, or lung tissue less susceptible to the cytotoxicity of deposited DPM.

The IL-6 increase seen here 6 hours after DE exposure in asthmatic subjects parallels similar significant IL-6 increases in sputum 6 hours after DE exposure of healthy subjects, suggesting that the IL-6 release represents an acute response of both healthy and asthmatic

persons to DE exposures. Other work by Steerenberg, et al. (1998) showed that DE particles are effective in inducing release of IL-6 from human bronchial epithelial cells.

In a follow-up investigation of potential mechanisms underlying the DE-induced airway leukocyte infiltration, Salvi et al. (2000) exposed healthy human volunteers to diluted DE on two separate occasions for 1 h each, in an exposure chamber. Fiber-optic bronchoscopy was performed 6 h after each exposure to obtain endobronchial biopsies and bronchial wash (BW) cells. These workers observed that diesel exhaust (DE) exposure enhanced gene transcription of interleukin-8 (IL-8) in the bronchial tissue and BW cells and increased growth-regulated oncogene-α protein expression and IL-8 in the bronchial epithelium; there was also a trend toward an increase in interleukin-5 (IL-5) mRNA gene transcripts in the bronchial tissue.

Nightingale et al. (2000) have reported inflammatory changes in healthy volunteers exposed to 200 $\mu g/m^3$ resuspended DPM under resting conditions in a double-blinded study. Small but statistically significant increases in neutrophils and myeloperoxidase (an index of neutrophil activation) were observed in sputum samples induced 4 hours after exposure to DPM in comparison to air. Exhaled carbon monoxide was measured as an index of oxidative stress and was found to increase maximally at 1 hour after exposure. These biochemical and cellular changes occurred in the absence of any decrements in pulmonary function, thus confirming that markers of inflammation are more sensitive than pulmonary function measurements.

Because of the considerable concern about inhalation of ambient particles by sensitive subpopulations, (Nordenhäll et al., 2001) also studied the effect of a 1 hour exposure to DE (containing 300 $\mu g/m^3$ DPM, 1.2 ppm NO_2, 3.4 ppm NO, 2.6 ppm HC, and 9.1 ppm CO) on 14 atopic asthmatics with stable disease and on inhaled corticosteroid treatment. At 6 hours after exposure, there was a significant increase in airway resistance ($-p < 0.004$) and in IL-6 in induced sputum ($p < 0.048$) following exposure to DE versus filtered air. At 24 hours after exposure, there was a significant increase in the nonspecific airway responsiveness to inhaled methacholine. Although the exposure level was high relative to ambient PM levels, these findings may be important, as noted by the authors, in terms of supporting epidemiologic evidence for increased asthma morbidity associated with episodic exposure to ambient PM.

The role of antioxidant defenses in protecting against acute diesel exhaust exposure has also been studied. Blomberg et al. (1998) investigated changes in the antioxidant defense network within the respiratory tract lining fluids of human subjects following diesel exhaust

exposure. Fifteen healthy, nonsmoking, asymptomatic subjects were exposed to filtered air or diesel exhaust (DPM 300 mg/m^3) for 1 h on two separate occasions at least 3 weeks apart. Nasal lavage fluid and blood samples were collected prior to, immediately after, and 5.5 h post-exposure. Bronchoscopy was performed 6 h after the end of diesel exhaust exposure. Nasal lavage ascorbic acid concentration increased tenfold during diesel exhaust exposure, but returned to basal levels 5.5 h post-exposure. Diesel exhaust had no significant effects on nasal lavage uric acid or GSH concentrations and did not affect plasma, bronchial wash, or bronchoalveolar lavage antioxidant concentrations or malondialdehyde or protein carbonyl concentrations. The authors concluded that the acute increase in ascorbic acid in the nasal cavity induced by diesel exhaust may help prevent further oxidant stress in the upper respiratory tract of healthy individuals.

7.3.1.3 Complex Combustion-Related Particles

Because combustion emission sources contribute to the overall ambient air particulate burden (Spengler and Thurston, 1983), many of the studies investigating the response of laboratory animals to particle exposures have used complex combustion-related particles (see Table 7-2). For example, the residual oil fly ash (ROFA) samples used in toxicological studies have been collected from a variety of sources, e.g., boilers, bag houses used to control emissions from power plants, and from particles emitted downstream of such collection devices. ROFA has a high content of water soluble sulfate and metals, accounting for 82 to 92% of water-soluble mass, while the water-soluble mass fraction in ambient air varies from low teens to more than 60% (Costa and Dreher, 1997; Prahalad et al., 1999). More than 90% of the metals in ROFA are transition metals; whereas these metals are only a small subfraction of the total ambient PM mass. Transition metals generate reactive oxygen species and are relevant to understanding the mechanisms of toxicity and the components contributing to the toxic responses. Thus, the dose of bioavailable metal that is delivered to the lung when ROFA is instilled into a laboratory animal can be orders of magnitude greater than an ambient PM dose, even under a worst-case scenario.

Intratracheal instillation of various doses of ROFA suspension has been shown to produce severe inflammation, an indicator of pulmonary injury that includes recruitment of neutrophils, eosinophils, and monocytes into the airway. The biological effects of ROFA in rats have been

shown to depend on aqueous leachable chemical constituents of the particles (Dreher et al., 1997; Kodavanti et al., 1997b). A leachate prepared from ROFA, containing predominantly Fe, Ni, V, Ca, Mg, and sulfate, produced similar lung injury to that induced by the complete ROFA suspension (Dreher et al., 1997). Depletion of Fe, Ni, and V from the ROFA leachate eliminated its pulmonary toxicity. Correspondingly, minimal lung injury was observed in animals exposed to saline-washed ROFA particles. A surrogate transition metal sulfate solution containing Fe, V, and Ni largely reproduced the lung injury induced by ROFA. Interestingly, ferric sulfate and vanadium sulfate antagonized the pulmonary toxicity of nickel sulfate. Interactions between different metals and the acidity of PM were found to influence the severity and kinetics of lung injury induced by ROFA and its soluble transition metals.

To further investigate the response to ROFA with differing metal and sulfate composition, male Sprague-Dawley rats (60 days old) were intratracheally instilled with ROFA (2.5 mg/rat) or metal sulfates (iron –0.54 µmole/rat, vanadium –1.7 µmole/rat, and nickel –1.0 µmole/rat, individually or in combination) (Kodavanti et al., 1997b). Transition metal sulfate mixtures caused less injury than ROFA or Ni alone, suggesting metal interactions. This study also showed that V-induced effects were less severe than that of Ni and were transient. Ferric sulfate was least pathogenic. Cytokine gene expression was induced prior to the pathology changes in the lung, and the kinetics of gene expression suggested persistent injury by nickel sulfate. Another study by the same investigators was performed using 10 different ROFA samples collected at various sites within a power plant burning residual oil (Kodavanti et al., 1998a). Animals received intratracheal instillations of either saline (control), or a saline suspension of whole ROFA (< 3.0 µm MMAD for all ground PM) at three doses (0.833, 3.33, or 8.33 mg/kg). This study showed that ROFA-induced PMN influx was associated with its water-leachable V content; but protein leakage was associated with water-leachable Ni content. ROFA-induced in vitro activation of alveolar macrophages (AMs) was highest with ROFA containing leachable V but not with Ni plus V, suggesting that the potency and the mechanism of pulmonary injury may differ between emissions containing bioavailable V and Ni.

Other studies have shown that soluble metal components play an important role in the toxicity of emission source particles. Gavett et al. (1997) investigated the effects of two ROFA samples of equivalent diameters, but having different metal and sulfate content, on pulmonary responses in Sprague-Dawley rats. ROFA sample 1 (R1) (the same emission particles used by

Dreher et al. [1997]) had approximately twice as much saline-leachable sulfate, nickel, and vanadium, and 40 times as much iron as ROFA sample 2 (R2); whereas R2 had a 31-fold higher zinc content. Rats were instilled with suspensions of 2.5 mg R2 in 0.3 mL saline, the supernatant of R2 (R2s), the supernatant of 2.5 mg R1 (R1s), or saline only. By 4 days after instillation, 4 of 24 rats treated with R2s or R2 had died. None treated with R1s or saline died. Pathological indices, such as alveolitis, early fibrotic changes, and perivascular edema, were greater in both R2 groups. In surviving rats, baseline pulmonary function parameters and airway hyperreactivity to acetylcholine were significantly worse in the R2 and R2s groups than in the R1s groups. Other than BAL neutrophils, which were significantly higher in the R2 and R2s groups, no other inflammatory cells (macrophages, eosinophils, or lymphocytes) or biochemical parameters of lung injury were significantly different between the R2 and R2s groups and the R1s group. Although (a) soluble forms of zinc had been found in guinea pigs to produce a greater pulmonary response than other sulfated metals (Amdur et al., 1978) and (b) the level of zinc was 30-fold greater in R2 than R1, the precise mechanisms by which zinc may induce such responses are unknown. Still, these results show that the composition of soluble metals and sulfate is critical in the development of airway hyperractivity and lung injury produced by ROFA, albeit at very high instilled doses.

Dye et al. (1997) pretreated rats with an intraperitoneal injection of 500 mg/kg dimethylthiourea (DMTU) or saline, followed 30 min later by intratracheal instillation of either acidic saline (Ph = 3.3) or an acidified suspension of ROFA (500 µg/rat). Dimethylthiourea reduces the activity of the reactive oxygen species. The systemic administration of DMTU impeded development of the cellular inflammatory response to ROFA but did not ameliorate biochemical alterations in BAL fluid. In a subsequent study, it was determined that oxidant generation, possibly induced by soluble vanadium compounds in ROFA, is responsible for the subsequent rat tracheal epithelial cells gene expression, inflammatory cytokine production (MIP-2 and IL-6), and cytotoxicity (Dye et al., 1999).

In addition to transition metals, other components in fly ash also may cause lung injury. The effects of arsenic compounds in coal fly ash or copper smelter dust on the lung integrity and on the ex vivo release of TNFα by alveolar phagocytes were investigated by Broeckaert et al. (1997). Female NMRI mice were instilled with different particles normalized for the arsenic content (20 µg/kg body weight [i.e., 600 ng arsenic/mouse]) and the particle load (100 mg/kg

body weight [i.e., 3 mg/mouse]). Mice received tungsten carbide (WC) alone, coal fly ash (CFA) alone, copper smelter dust (CMP) mixed with WC, and $Ca_3(AsO_4)_2$ mixed with WC (see Table 7-2 for concentration details). Copper smelter dust caused a severe but transient inflammatory reaction; whereas a persisting alveolitis (30 days postexposure) was observed after treatment with coal fly ash. In addition, TNFα production in response to lipopolysaccharide (LPS) by alveolar phagocytes were significantly inhibited at day 1 but was still observed at 30 days after administration of CMP and CFA. Although arsenic was cleared from the lung tissue 6 days after $Ca_3(AsO_4)_2$ administration, a significant fraction persisted (10 to 15% of the arsenic administered) in the lung of CMP- and CFA-treated mice at Day 30. It is possible that suppression of TNF-α production is dependent upon the slow elimination of the particles and their metal content from the lung.

Antonini et al. (2002) investigated the effect of preexposure to ROFA on lung defenses and injury after pulmonary challenge with *Listeria monocytogenes*, a bacterial pathogen. Male Sprague-Dawley rats were dosed IT at day 0 with saline (control) or ROFA (0.2 or 1 mg/100 g body weight). Three days later, both groups of rats were instilled IT with a low (5×10^3) or high (5×10^5) dose of *L. monocytogenes*. Chemiluminescence (CL) and nitric oxide (NO) production, two indices of alveolar macrophage (AM) function, were measured on cells recovered from the right lungs by bronchoalveolar lavage. The left lungs and spleens were homogenized, cultured, and colony-forming units were counted after overnight incubation. Exposure to ROFA and the high dose of *L. monocytogenes* led to marked lung injury and inflammation as well as to an increase in mortality, compared with rats treated with saline and the high dose of *L. monocytogenes*. Preexposure to ROFA significantly enhanced injury and delayed the pulmonary clearance of *L. monocytogenes* at both bacterial doses when compared to the saline-treated control rats. ROFA had no effect on AM CL but caused a significant suppression of AM NO production. The authors concluded that acute exposure to ROFA slowed pulmonary clearance of *L. monocytogenes* and altered AM function. They postulated that these changes could lead to increased susceptibility to lung infection in exposed populations.

In summary, intratracheally instilled high doses of ROFA produced acute lung injury and inflammation. Water soluble metals in ROFA appear to play a key role in the acute effects of instilled ROFA through the production of reactive oxygen species. These ROFA studies clearly show that combustion-generated particles with a high metal content can cause substantial lung

injury; but how well such effects can be extrapolated as likely to occur with ambient PM exposure remains to be more fully established. Still, the Appendix 7A dosimetric modeling results suggest much less difference between the PM doses deposited with the ROFA instillation and PM amounts expected to be regionally deposited with acute human exposures to ambient PM.

7.3.2 Acid Aerosols

Extensive earlier studies (conducted up to the early 1990's) on the effects of controlled exposures to aqueous acid aerosols on various aspects of lung function in humans and laboratory animals were reviewed in an EPA Acid Aerosol Issue Paper (U.S. Environmental Protection Agency, 1989 and in the 1996 PM AQCD (U.S. Environmental Protection Agency 1996a). Methodology and measurement methods for controlled human exposure studies were also reviewed elsewhere (Folinsbee et al., 1997).

The studies summarized in the 1996 PM AQCD illustrate that aqueous acidic aerosols have minimal effects on symptoms and mechanical lung function in young healthy adult volunteers at concentrations as high as 1000 $\mu g/m^3$. Asthmatic subjects appear to be more sensitive to the effects of acidic aerosols on mechanical lung function. Responses have been reported in adolescent asthmatics at concentrations as low as 68 $\mu g/m^3$, and modest bronchoconstriction has been seen in adult asthmatics exposed to concentrations $\geq$ 400 $\mu g/m^3$, but the available data are not consistent. However, at concentrations as low as 100 $\mu g/m^3$, acid aerosols can alter mucociliary clearance. Brief exposures ($\leq$ 1 h) to low concentrations ($\approx$ 100 $\mu g/m^3$) may accelerate clearance while longer (multihour) exposures to higher concentrations (> 100 $\mu g/m^3$) can depress clearance.

Some earlier acid aerosol studies not assessed in the 1996 PM AQCD or published more recently are summarized in Table 7-5. For example, Frampton et al. (1992) found that acid aerosol exposure in humans (1000 $\mu g/m^3$ H_2SO_4) did not result in airway inflammation and there was no evidence of altered macrophage host defenses. Also, Leduc et al. (1995) found no increase in bronchoconstriction or bronchial responsiveness among asthmatic human adults exposed via facemask to 500 $\mu g/m^3$ of simulated acid fog containing H_2SO_4 or ammonium sulfate aerosol.

TABLE 7-5. RESPIRATORY EFFECTS OF ACID AEROSOLS IN HUMANS AND LABORATORY ANIMALS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Humans, healthy nonsmokers; 10 M, 2 F; 20-39 years old	H_2SO_4 aerosol NaCl (control)	Inhalation	1,000 µg/m^3	0.8-0.9 µm MMAD	2 h; analysis 18 h	No inflammatory responses; slight increase in BAL protein and slight decrease in albumin in H_2SO_4 subjects compared to NaCl. No effect on bacterial killing by macrophages was found.	Frampton et al. (1992)
Humans, asthmatic; 13 M, 11 F	H_2SO_4 aerosol NH^+_4/SO^{-2}_4 aerosol	Inhalation by face mask	500 µg/m^3	9 µm MMAD 7 µm MMAD	1 h	Exposure to simulated natural acid fog did not induce bronchoconstriction nor change bronchial responsiveness in asthmatics.	Leduc et al. (1995)
Dogs, beagle, healthy; n = 16	Neutral sulfite aerosol	Inhalation	1.5 mg/m^3	1.0 µm MMAD σg = 2.2	16.5 h/day for 13 mo	Long-term exposure to particle-associated sulfur and hydrogen ions caused only subtle respiratory responses and no change in lung pathology.	Heyder et al. (1999)
	Acidic sulfate aerosol	Inhalation	5.7 mg/m^3	1.1 µm MMAD σg = 2.0	6 h/day for 13 mo		
Rats, female, F-344; Guinea Pigs, female, Hartley	H_2SO_4 aerosol	Inhalation	94 mg/m^3 43 mg/m^3	0.80 σg 1.89 0.93 σg 2.11	4 h	Acid aerosol increased surfactant film compressibility in guinea pigs.	Lee et al. (1999)
Rabbits, New Zealand white Humans, healthy nonsmokers; 10 M, 21-37 years old	H_2SO_4	Inhalation	1,000 µg/m^3	0.8 µm σg 1.6	2 h	No inflammatory response; LDH activity in BAL elevated in both species; effect on bacterial killing by humans was inconclusive.	Zelikoff et al. (1997)

H_2SO_4 = Sulfuric acid
BAL = Bronchoalveolar lavage
LDH = Lactate dehydrogenase
MMAD = Mass median aerodynamic diameter
MMD = Mass median diameter
σg = Geometric standard deviation

Zelikoff et al. (1997) compared the responses of rabbits and humans exposed to similar concentrations (i.e., 1000 μg/m^3) of H_2SO_4 aerosol. For both rabbits and humans, there was no evidence of PMN infiltration into the lung and no change in BAL fluid protein level, although there was an increase in LDH in rabbits but not in humans. Macrophages showed somewhat less antimicrobial activity in rabbits; but insufficient data were available for humans. Macrophage phagocytic activity was also slightly reduced in rabbits but not in humans. Superoxide production by macrophages was somewhat depressed in both species. Ohtsuka et al. (2000a,b) have also shown that a single 4 h exposure of mice to acid-coated carbon particles at a high mass concentration of 10,000 μg/m^3 carbon black causes decreased phagocytic activity of alveolar macrophages, even in the absence of lung injury.

In another study, Lee et al. (1999) found little effect on female rats or guinea pigs of inhalation exposure to very high concentrations (43 or 94 mg/m^3) of H_2SO_4 aerosol. Nor were any respiratory effects of long-term exposure to acid aerosol (1.5 mg/m^3; 16.5 h/day; for 13 months) found in dogs (Heyder et al., 1999). Thus, recent studies provide very little additional evidence demonstrating that relevant concentrations of aqueous acid aerosols contribute to acute respiratory effects of ambient PM.

Although pulmonary effects of acid aerosols have been the subject of extensive research in past decades, the cardiovascular effects of acid aerosols have received little attention. Zhang et al. (1997) reported that inhalation of acetic acid fumes caused reflex-mediated increases in blood pressure in normal and spontaneously hypertensive rats. Thus, acid components should not be ruled out as possible mediators of PM health effects. In particular, the cardiovascular effects of acid aerosols at realistic concentrations need further investigation.

7.3.3 Metal Particles, Fumes, and Smoke

Data from occupational and laboratory animal studies reviewed in the 1996 PM AQCD (U.S. Environmental Protection Agency, 1996a) indicated that acute exposures to very high levels (hundreds of μg/m^3 or more) or chronic exposures to lower levels (as low as 15 μg/m^3) of metallic particles could affect the respiratory tract. It was concluded, on the basis of data available at that time, that the metals at typical concentrations present in the ambient atmosphere (1 to 14 μg/m^3) were not likely to have a significant acute effect in healthy individuals. This included metals such as arsenic, cadmium, copper, nickel, vanadium, iron, and zinc. Other

metals found at concentrations less than 0.5 μg/m^3 were not reviewed in the 1996 criteria document. More recently published data from high-dose laboratory and other types of studies, however, are suggestive of particle-associated metals likely being among PM components contributing to health effects attributed to ambient PM. Such studies are summarized in Table 7-6a, b.

Controlled human exposure studies have been performed with metal containing fumes or particles. Controlled inhalation exposure studies to high concentrations of two different metal fume particles, MgO and ZnO, demonstrate differences in response depending on particle metal composition (Kuschner et al., 1997; Kuschner et al., 1995). Up to 6400 mg/m^3/min cumulative dose of MgO had no effect on lung function (spirometry, $D_{L_{CO}}$), symptoms of metal fume fever, or changes in inflammatory mediators or cells recovered by BAL. However, lower concentrations of ZnO fume (166 to 1110 mg/m^3/min) induced a neutrophilic inflammatory response in the airways 20 h postexposure. Lavage fluid PMNs, TNF-α, and IL-8 were increased by ZnO exposure. Although the concentrations used in these exposure studies exceed ambient levels by more than 1000-fold, the absence of a response to an almost 10-fold higher concentration of MgO compared with ZnO indicates that differential metal composition, in addition to particle size (ultrafine/fine), is likely an important determinant of observed health responses to inhaled ambient PM.

Several metals (e.g., zinc, chromium, cobalt, copper, and vanadium) have been shown to stimulate cytokine release in cultured human pulmonary cells. Boiler makers, exposed occupationally to ˜400 to 500 μg/m^3 of fuel oil ash, containing high levels of soluble metals, showed acute nasal inflammatory responses characterized by increased myeloperoxidase (MPO) and IL-8 levels; these changes were associated with increased vanadium levels in the upper airway (Woodin et al., 1998). Also, Irsigler et al. (1999) reported that V_2O_5 can induce asthma and bronchial hyperreactivity in exposed workers.

Iron is the most abundant of the elements capable of catalyzing oxidant generation and is also present in ambient urban particles. Lay et al. (1998) and Ghio et al. (1998b) tested the hypothesis that the human respiratory tract will attempt to diminish the added, iron-generated oxidative stress. They examined cellular and biochemical responses of human subjects instilled, via the intrapulmonary route, with a combination of iron oxyhydroxides that introduced an oxidative stress to the lungs. Saline alone and iron-containing particles suspended in saline were

TABLE 7-6a. RESPIRATORY EFFECTS OF INHALED METAL PARTICLES, FUMES, AND SMOKE IN HUMANS AND LABORATORY ANIMALS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Humans, boilermakers (18 M), 26-61 years old, utility worker controls (11 M), 30-55 years old	ROFA	Inhalation of fuel-oil ash	0.4-0.47 mg/m^3 0.1-0.13 mg/m^3	10 µm	6 weeks	Exposure to fuel-oil ash resulted in acute upper airway inflammation, possibly mediated by increased IL-8 and PMNs. Effects seen at the 0.47 mg/m^3 exposure level, representing exposures inside the boiler.	Woodin et al. (1998)
Humans, vanadium plant workers; 40 M; 19-60 years old	V_2O_5	Inhalation	< 0.05-1.53 mg/m^3	N/A	Variable	12/40 workers had bronchial hyperreactivity that persisted in some for up to 23 mo.	Irsigler et al. (1999)
Humans, healthy nonsmokers; 4 M, 2 F; 21-43 years old	MgO	Inhalation	5.8-230 mg/m^3	99% < 1.8 µm 29% < 0.1 µm	15-45 min	No significant differences in BAL inflammatory cell concentrations, BAL interleukins (IL-1, IL-6, IL-8), tumor necrosis factor, pulmonary function, or peripheral blood neutrophils.	Kuschner et al. (1997)
Humans, healthy nonsmokers; 8 M, 8 F; 18-34 years old	Fe_2O_3	Inhalation	12.7 mg/m^3	1.5 µm σg = 2.1	30 min	No significant difference in $^{98m}T_c$-DTPA clearance half-times, D_LCO, or spirometry	Lay et al. (2001)
Rats, SD; 60 days old	VSO_4 $NiSO_4$	Inhalation	0.3 - 1.7 mg/m^3 0.37 - 2.1 mg/m^3	N/A	6h/day x 4 days	V did not induce any significant changes in BAL or HR. Ni caused delayed bradycardia, hypothermia, and arrhythmogenesis at > 1.3 mg/m^3. Possible synergistic effects were found.	Campen et al. (2001)
Rats, WISTAR Furth; 7-week-old, Mice, C57BL6 and DBA3NCR	CdO Fume	Nose-only inhalation	1.04 mg/m^3 Rats dose = 18.72 µg Mouse dose = 4.59 µg	CMD = 0.008 µm σg = 1.1	1 × 3 h	Mice created more metallothionein than rats, which may be protective of tumor formation.	McKenna et al. (1998)

CdO = Cadmium oxide
Fe_2O_3 = Iron oxide
MgO = Magnesium oxide
MnO_2 = Manganese oxide
TiO_2 = Titanium oxide
$VOSO_4$ = Vanadyl sulfate
V_2O_5 = Vanadium oxide
ZnO = Zinc oxide

BAL = Bronchoalveolar lavage
CMD = Count median diameter
IL = Interleukin
LDH = Lactate dehydrogenase
MIP-2 = Macrophage inflammatory protein-2
mRNA = Messenger RNA (ribonucleic acid)
N/A = Data not available

TABLE 7-6b. RESPIRATORY EFFECTS OF INSTILLED METAL PARTICLES, FUMES, AND SMOKE IN HUMAN SUBJECTS AND LABORATORY ANIMALS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Humans, healthy nonsmokers; 12 M, 4 F; 18-35 years old	Colloidal iron oxide	Bronchial instillation	5 mg in 10 mL	2.6 µm	1, 2, and 4 days after instillation	L-ferritin increased after iron oxide particle exposure; transferrin was decreased. Both lactoferrin and transferrin receptors were increased.	Ghio et al. (1998b)
Humans, healthy nonsmokers; 27 M, 7 F; 20-36 years old	Fe_2O_3	Intrapulmonary instillation	3×10^8 microspheres in 10 mL saline.	2.6 µm	N/A	Initially-induced transient inflammation (neutrophils, protein, LDH, IL-8) resolved by 4 days postinstillation.	Lay et al. (1998)
Mice, Swiss	EHC-93 soluble metal salts	Intratracheal instillation	1 mg in 0.1 mL H_2O	0.8 ± 0.4 µm	3 days	Solution containing all metal salts (Al, Cu, Fe, Pb, Mg, Ni, Zn) or ZnCl alone increased BAL inflammatory cells and protein.	Adamson et al. (2000)
Rats, Fischer 344. (250 g)	Fe_2O_3	Intratracheal instillation	7.7×10^7 microspheres in 5 mL saline	2.6 µm	N/A	Transient inflammation at 1 day postinstillation.	Lay et al. (1998)
Mice, NMRI; Mouse peritoneal macrophage	MnO_2	Intratracheal instillation; in vitro	0.037, 0.12, 0.75, 2.5 mg/animal	surface area of 0.16, 0.5, 17, 62 m^2/g	Sacrificed at 5 days	LDH, protein and cellular recruitment increased in a dose-related manner with increasing surface area for particles with surface areas of 17 and 62 m^2/g; freshly ground particles with surface areas of 0.5 m^2/g had enhanced cytotoxicity.	Lison et al. (1997)
Rats, M, F344, 175-225 g	TiO_2	Intratracheal inhalation and Intratracheal instillation	Inhalation at 125 mg/m^3 for 2 h; Instillation at 500 µg for fine, 750 µg for ultrafine	Fine: 250 nm Ultrafine: 21 nm	Inhalation exposure, 2 h; sacrificed at 0, 1, 3, and 7 days postexposure for both techniques	Inflammation produced by intratracheal inhalation (both severity and persistence) was less than that produced by instillation; ultrafine particles produced greater inflammatory response than fine particles for both dosing methods.	Osier and Oberdörster (1997)
Rats, M. F344, 175-225 g	TiO_2	Intratracheal inhalation and Intratracheal instillation	Inhalation at 125 mg/m^3 for 2 h; Instillation at 500 µg for fine, 750 µg for ultrafine	Fine: 250 nm Ultrafine: 21 nm	Inhalation exposure, 2 h; sacrificed at 0, 1, 3, and 7 days postexposure for both techniques	MIP-2 increased in lavage cells but not in supernatant in those groups with increased PMN (more in instillation than in inhalation; more in ultrafine than in fine); TNF-α levels had no correlation with either particle size or dosing methods.	Osier et al. (1997)
Rats, Female, CD	$NaVO_3$ $VOSO_4$ V_2O_5	Intratracheal instillation	21 or 210 µg V/kg ($NaVO_3$, $VOSO_4$ soluble) 42 or 420 µg V/kg (V_2O_s) less soluble	N/A	1 h or 10 days following instillation	PMN influx was greatest following $VOSO_4$, lowest for V_2O_5 (no effect at lowest concentration); $VOSO_4$ induced inflammation persisted longest; MIP-2 and KC (CXC chemokines) were rapidly induced as early as 1 h postinstillation and persisted for 48 h; Soluble V induced greater chemokine mRNA expression than insoluble V; AMs have the highest expression level.	Pierce et al. (1996)

CdO = Cadmium oxide
Fe_2O_3 = Iron oxide
MgO = Magnesium oxide
MnO_2 = Manganese oxide
$NaVO_3$ =
TiO_2 = Titanium oxide
$VOSO_4$ = Vanadyl sulfate
V_2O_5 = Vanadium oxide
ZnO = Zinc oxide
BAL = Bronchoalveolar lavage
CMD = Count median diameter
IL = Interleukin
LDH = Lactate dehydrogenase
MIP-2 = Macrophage inflammatory protein-2
mRNA = Messenger RNA (ribonucleic acid)
N/A = Data not available

instilled into separate lung segments of human subjects. Subjects underwent bronchoalveolar lavage at 1 to 91 days after instillation of 2.6-μm diameter iron oxide (approximately 5 mg or 2.1×10^8 particles) agglomerates. Lay and colleagues found iron-oxide-induced inflammatory responses in both the alveolar fraction and the bronchial fraction of the lavage fluid at 1 day postinstillation. Lung lavage 24 h after instillation revealed decreased transferrin concentrations and increased ferritin and lactoferrin concentrations, consistent with a host-generated response to decrease the availability of catalytically reactive iron (Ghio et al., 1998b). Normal iron homeostasis returned within 4 days of the iron particle instillation. The same iron oxide preparation, which contained a small amount of soluble iron, produced similar pulmonary inflammation in rats. In contrast, instillation of rats with two iron oxide preparations that contained no soluble iron failed to produce injury or inflammation, thus suggesting that soluble iron was responsible for the observed intrapulmonary changes.

In a subsequent inhalation study, Lay et al. (2001) studied the effect of iron oxide particles on lung epithelial cell permeability. Healthy, nonsmoking human subjects inhaled 12.7 mg/m^3 low- and high-solubility iron oxide particles (MMAD = 1.5 μm and σg = 2.1) for 30 minutes. Neither pulmonary function nor alveolar epithelial permeability, as assessed by pulmonary clearance of technetium-labeled DPTA, was changed at 0.5 or 24 hours after exposure to either type of iron oxide particle. Because the exposure concentration was so high, the data suggest that iron may play little role in the adverse effects of ambient, urban PM. Ghio et al. (2001) reported a case study, however, in which acute exposure to oil fly ash from a domestic oil-fired stove produced diffuse alveolar damage, difficulty in breathing, and symptoms of angina. While steroid treatment led to rapid improvement in symptoms and objective measurements, this report suggests that the high metal content of oil fly ash can alter the epithelial cell barrier in the alveolar region.

In addition to the above experimental studies, autopsy data suggest that chronic exposure to urban air pollution leads to an increased retention of metals in human tissues. A comparison of autopsy cases in Mexico City from the 1950s with those from the 1980s indicated substantially higher (5- to 20-fold) levels of Cd, Co, Cu, Ni, and Pb in lung tissue from the 1980s (Fortoul et al., 1996). Similar studies have examined metal content in human blood and lung tissue (Tsuchiyama et al., 1997; Osman et al., 1998), with similar results.

7.3.4 Ambient Bioaerosols

The American Conference of Industrial Hygienists defines bioaerosols as airborne particles, large molecules or volatile compounds that are living, contain living organisms, or have been released from living organisms. Such particles may be suspended in the air adhered to dust particles or tiny droplets of water. Bioaerosols include fungal materials, pollen, bacteria, viruses, endotoxins, and plant and animal debris, and range in size from 0.01 μm (viruses) to well over 20 μm (pollen). The smallest of the bioaerosol particles, < 5 μm, can penetrate into the deep lung. They are naturally present in the environment and generally pose little threat to human health. However, for some sensitive individuals, some bioaerosols when inhaled may cause diseases such as asthma, allergic rhinitis, and respiratory infections.

As discussed in the 1996 PM AQCD (U.S. Environmental Protection Agency, 1996a), biologically-derived particles are frequently ignored components of both ambient and indoor aerosols, due in part to the bioaerosols being considered "natural" and not readily amenable to control. The 1996 PM AQCD highlighted several examples of common bioaerosol sources, particles, and agents, as listed in Table 7-6 and discussed in several earlier bioaerosols reviews, e.g., Cox (1987), Pope et al. (1993), Lighthart and Mohr (1994), and Cox and Wathes (1995).

The terms reservoir, source, particle, and agent are often encountered in discussion of bioaerosols. As employed in the 1996 PM AQCD and here, the following definitions apply:

- *Reservoir:* the environmental niche in which source organisms are living
- *Source:* the organism that produced the bioaerosol particle
- *Particle:* the particle shed from the organism
- *Agent:* the part(s) of the particle that actually mediate the disease process.

Information from the 1996 PM AQCD on different types of bioaerosol components noted in Table 7-6 still largely applies and is first summarized below. Then, updating is provided in light of important newly available findings for some bioaerosol components.

TABLE 7-6. EXAMPLES OF MAJOR SOURCES, TYPES OF PARTICLES, AND DISEASE AGENTS ASSOCIATED WITH BIOAEROSOLS

Sources	Aerosol Particles	Disease Agents
Plants	Pollen and pollen fragments, fragments of other plant parts, spores (ferns, mosses), algal cells	Glycoprotein allergens
Animals	Skin scales, secretions (saliva, skin secretions), excreta, body parts (arthropods)	Glycoprotein allergens
Fungi	Spores, hyphae, yeast cells, metabolites (toxins, digested substrate material)	Glycoprotein allergens, infectious units, glucans, mycotoxins
Bacteria	Cells, fragments, metabolites (toxins, digested substrate material)	Infectious units, allergens, endotoxin, exotoxins
Viruses	Viral particles	Infectious units

Source: Modified from 1996 PM AQCD (U.S. EPA, 1996a).

7.3.4.1 Plant Aerosols

Pollen

Among the best known plant aerosols are various types of pollen produced by flowering plants including trees (e.g., pines, cedars, birch, elm, maple, oak, hickory, walnut, etc.), weeds (e.g., ragweed, sage, etc.), and grasses (e.g., rye grass, Bermuda grass, etc.). Within these groupings, specific types are regionally more common, e.g., ragweed more so in the eastern United States, Birch pollen during the spring pollen season in New England, mountain cedar pollen early in the year in the southwest, etc. (Lewis et al., 1983).

Outdoor pollen levels are determined by numbers of plants available for pollen release, the amount of pollen produced by each plant, factors that control pollen release and dispersion from the plant, and factors that directly affect the aerosols (Edmonds, 1979). The number of plants available depends on many environmental factors (some human) that control plant prevalence. For example, the abundance of the ragweed plant in a given year depends on numbers of plants that produced seed in the previous year, disturbed ground available for seed germination and growth, and meteorological factors during the growing season. Once a crop of ragweed matures, pollen production depends on temperature, rainfall, and day length.

Pollen grains are relatively large complex particles that consist of cellular material surrounded by a cell membrane and a complex wall. Pollen grain structure has been well

studied. Pollen shed is controlled by temperature, humidity, wind, and rain. Pollen levels in air depend on all of these factors as well as wind and rain conditions after release, and on surfaces available for impaction. Pollen allergens are thought to be water-soluble glycoproteins that rapidly diffuse from the grain when it contacts a wet surface. The glycoproteins are generally specific to the type of pollen, although large groups include a single allergen. For example, many different kinds of grasses carry similar allergens in their pollen grains. Several pollen allergens have been characterized: Amb a I (ragweed), Bet v I (birch), Par j I (parietaria), etc.

Other Natural Plant Aerosols

Other plant-derived particles naturally occurring in outdoor air include algal cells; spores of mosses, liverworts, club mosses, and ferns; and fragments of all kinds of plants. Very little has been reported about the prevalence or human impact of any of these aerosol particles, but they are presumed to carry allergens.

Plant-Related Bioaerosols Generated by Human Activities (Grain Dust, Latex, etc.)

Human accumulations of plant materials that are subsequently handled by humans inevitably produce bioaerosols. The most common examples of such accumulations are storage, handling, and transport of farm products (hay, straw, grain), composting, and other anthropogenic processes that involve the use of plant material. Of particular interest are grain dusts that include respirable-size particles (< 10 μm). For example, soybean dust aerosols released from freighters unloading the beans in port have been blamed for epidemics of asthma. Also, human uses of some plant products can result in disease-causing aerosols (Alberts and Brooks, 1992). One example is wood trimmer's disease (from inhalation of wood dust particles released during high-speed wood cutting); and sewage composting involves the use of wood chips, which can release allergenic aerosols. Also, latex particles from automobile tires can contaminate reentrained roadway dust.

7.3.4.2 Animal Aerosols

Mammalian Aerosols

All mammals produce aerosols, from the smallest mouse to humans . Human aerosols (skin scales, respiratory secretions) generally do not cause disease except, of course, for agents of infection (see below). Other mammals release aerosols that cause hypersensitivity diseases, the most common sources being cats, dogs, farm animals, laboratory animals, and house mice — although all animals release aerosols that could be sensitizing under appropriate conditions (Burge, 1995). Mammals only cause human disease when appropriate exposure conditions occur. For example, simply having a cat in a house can create such conditions, as can handling of any animal. Cat allergens apparently become aerosolized on very small particles (< 1 μm) shed from skin and saliva. There is some indication that dog, mouse, and other rodent allergens are borne on dried urine particles, with particle sizes similar to those of cat allergen. Little is known about other mammalian aerosols. Cat and dog allergens (Fel d I, Can f I) have been characterized, and other mammalian allergens are under active study.

Avian Aerosols

Examples of wild and domesticated birds associated with disease-causing aerosols include: starlings (histoplasmosis); pigeons (histoplasmosis, pigeon-breeders disease); parrots (psittacosis); poultry (poultry-handlers disease); etc. Only the hypersensitivity diseases (e.g., pigeon breeders and poultry handlers disease) are caused by "bird" aerosols per se. The others are infectious diseases caused by agents inhabiting the birds (see below). The avian aerosol-hypersensitivity diseases are almost exclusively confined to sites where birds are bred and handled extensively, especially in indoor environments; and birds that release antigens observed to cause human disease are those that congregate or are typically confined close to people. Relatively little is known about avian aerosols. Probably skin scales, feather particles, and fecal material are all released as antigen-containing aerosols. The antigens (allergens) responsible for avian-related hypersensitivity diseases have yet to be well characterized.

Insect Aerosols

Dust Mites. Dust mites are arthropods belonging to the family Pyrogliphidae. There are two common species in temperate climates: *Dermatophagoides farinae*, which proliferates under relatively dry conditions; and *D. pteronyssinus*, which dominates in more humid environments (Arlian, 1989). Dust mites thrive in environments where relative humidity consistently exceeds 60 % and where skin scales and fungal spores are available as food. Bedding and carpet dust are primary reservoirs for exposure. The mite itself is about 100 µm long, but excretes 20 µm membrane-bound fecal particles that contain allergens. Exposure to dust mite allergens apparently occurs only when reservoirs are disturbed. Dust mites produce allergens that are a major cause of sensitization in children. The allergens are digestive enzymes that gradually diffuse from fecal particles after deposition on mucous membranes. Several dust mite allergens have been characterized and monoclonal antibodies against each raised and cloned, including Der f I and II; and Der p I and II (Platts-Mills and Chapman, 1987).

Cockroaches. Cockroaches are insects belonging to the Orthoptera (Mathews, 1989). The cockroach most commonly infesting buildings in temperate climates is *Blatella germanica* (the German cockroach). Cockroaches are nocturnal, and inhabit dark environments where food and water are available. Common food sources include stored animal or human food, and discarded food (garbage). Cockroaches are extremely prolific, given favorable environmental conditions. Population pressure will eventually drive the roaches into the daylight in search of food. Cockroaches shed body parts, egg cases, and fecal particles, all of which probably carry allergens. Little is known about the particles that actually carry the allergens. Two German cockroach allergens have been characterized: Bla g I, and Bla g II. Cockroach allergens are likely a major cause of asthma for some populations of children.

Other Insects. Fragments of gypsy moths and other insects that undergo massive migrations can become abundant in ambient air. Sizes, nature, and allergen content of such particles have not been studied, but cases of occupational asthma from exposure to insects (e.g., sewer flies) have been reported.

Other Animal Allergens

It is likely that proteinaceous particles shed from any animal could cause sensitization if exposure conditions are appropriate. For example, exposure to proteins aerosolized during seafood processing have caused epidemics of asthma.

7.3.4.3 Fungal Aerosols

Fungi are primarily filamentous microorganisms that reproduce and colonize new areas by means of airborne spores. Most use complex non-living organic material for food, require oxygen, and have temperature optima within the human comfort range. The major structural component of the cell wall is acetyl-glucosamine polymers (chitin). Cell walls also may contain B-glucans, waxes, mucopolysaccharides, and many other substances. In the process of degrading organic material, the fungi produce CO_2, ethanol, many other volatile organic compounds, water, organic acids, ergosterol, and a broad spectrum of secondary metabolites that include many antibiotics and mycotoxins.

Fungi colonize dead organic materials in both outdoor and indoor environments. Some invade living plant tissue and cause many important plant diseases; a few invade living animal hosts, including people. Fungi are universally present in indoor environments unless specific efforts are made for their exclusion (i.e., as in clean rooms). Kinds of fungi able to colonize indoor materials are generally those with broad nutritional requirements (e.g., *Cladosporium sphaerospermum*), those that can colonize dry environments (e.g., members of the *Aspergillus glaucus* group), or organisms that readily degrade cellulose and lignin present in many indoor materials (e.g., *Chaetomium globosum*, *Stachybotrys atra*, *Merulius lacrymans*). Yeasts (which are unicellular fungi) and other hydrophilic taxa (e.g., *Fusarium*, *Phialophora*) are able to colonize air/water interfaces. Moisture, in fact, is the most important factor determining indoor fungal growth, since food sources are ubiquitous (Kendrick, 1992).

Particles that become airborne from fungal growth include spores (the unit of most fungal exposure); fragments of the filamentous body of the fungus; and fragments of decomposed substrate material. Fungal spores range from about 1.5 µm to >100 µm in size and come in many different shapes, the simplest being smooth spheres and the most complex large multicellular branching structures. Most fungal spores are near unit density or less. Some include large air-filled vacuoles. Fungal spores form the largest and most consistently present

component of outdoor bioaerosols. Levels vary seasonally, with lowest levels occurring during periods of snow. While rain may initially wash large dry spores from the air, these are immediately replaced by wet (hydrophilic) spores that are released in response to the rain.

Some kinds of spores are widespread in outdoor air (e.g., *Cladosporium herbarum*, *Alternaria tenuissima*). Others produced by fungi with more fastidious nutritional requirements are only locally abundant. Typical indoor fungal aerosols are composed of particles penetrating from outdoors, particles released from active growth on indoor substrates, and reaerosolized particles that had settled into dust reservoirs. Indoor fungal aerosols are produced by active forcible discharge of spores; by mechanisms intrinsic to the fungus that "shake" spores from the growth surface; and, most commonly, by mechanical disturbance (e.g., air movement, vibration).

Allergic rhinitis and asthma are the only commonly reported diseases resulting from fungal exposures outdoors, and which also commonly occur indoors. The allergens of fungi are probably digestive enzymes that are released as the spore germinates. Other spore components (of unknown function) may also be allergenic. Only very few fungal allergens (e.g., *Alt a* I, *Cla h* I, and *Asp f* I), out of possibly hundreds of thousands, have been characterized.

Allergic fungal sinusitis and allergic bronchopulmonary mycoses occur when fungi colonize thick mucous in the sinuses or lungs of allergic people. The patterns of incidence of allergic fungal sinusitis may be explained in part by geographic variability in ambient fungal exposures. This disease is most commonly caused by *Bispora*, *Curvularia*, and other dark-spored fungi. Exposure patterns required for allergic bronchopulmonary mycoses are unknown. This disease is usually caused by *Aspergillus fumigatus*. Coccidioidomycoses and Histoplasmosis are infectious fungal diseases that result from outdoor exposures to *Histoplasma capsulatum* (a fungus that contaminates damp soil enriched with bird droppings) and *Coccidioides inmitis* (a fungus that grows in desert soils). Indoor aerosol-acquired fungal infections are rare and mostly restricted to immunocompromised people (Rippon, 1988).

Toxic agents produced by fungi include antibiotics, mycotoxins, and some cell-wall components that have irritant or toxic properties. The antibiotics and mycotoxins are secondary metabolites produced during fungal digestion of substrate materials, and their presence depends, in part, on the nature of the substrate. The locations of the toxins in spores or other mycelial fragments are unknown, as are the dynamics of release in the respiratory tract. Aerosol exposure to fungal antibiotics in levels sufficient to cause disease is unlikely. Mycotoxicoses have been

reported as case studies from exposure to spores of *Stachybotrys atra* (Croft et al., 1986), and epidemiologically for *Aspergillus flavus* (Baxter et al., 1981).

7.3.4.4 Bacterial Aerosols

Most bacteria are unicellular, although some form "pseudo" filaments when cells remain attached following cell division. The actinomycetes are bacteria that do form filaments and, in some cases, dry spores designed for aerosol dispersal. Bacteria can be broadly categorized into two groups based on a response to the Gram stain procedure. The cell walls of Gram positive (Gram+) bacteria are able to absorb a purple stain; the cell walls of Gram negative (Gram-) bacteria resist staining and contain endotoxin consisting of proteins, lipids, and polysaccharides.

Most infectious agents are maintained in diseased hosts. A few, including *Legionella pneumophila*, reside in water-filled environmental reservoirs such as water delivery systems, cooling towers, air conditioners, and lakes, streams, oceans, etc. Infectious agents are often released from hosts in droplets exhaled from the respiratory tract. Each droplet contains one or more of the infectious agent, possibly other organisms, and respiratory secretions. Most droplets are very large and fall quickly. Smaller droplets dry quickly to droplet nuclei, which range from the size of the individual organism (< 1 μm for the smallest bacteria) to clumps of larger organisms (> 10 μm for larger bacteria).

Environmental-source aerosols are produced by mechanical disturbances that include wind, rain splash, wave action, and as occurs in air recirculation, in sprays of washes and coolants, and in humidifiers. Particle sizes from all of these activity cover a wide range from well below 1 μm to > 50 μm. The thermophilic actinomycetes produce dry aerial spores that require only slight air movements to stimulate release. Each spore is about 1 μm in diameter.

Whole living bacteria are agents of infectious disease (e.g., tuberculosis, Legionnaires' disease, etc.). For tuberculosis, a single virulent bacterial cell deposited in the appropriate part of the lung can cause disease in a host without specific immunity. For Legionnaires' disease, the number of organisms needed for disease development likely depends on how well the host's general protective immune system is operating. Some bacteria release antigens that cause hypersensitivity pneumonitis. The antigens may be enzymes (e.g., *Bacillus subtilis* enzymes used in the detergent industry) or may be cell wall components (e.g., endotoxin or glucans).

7.3.4.5 Viral Aerosols

Viruses are either RNA or DNA units surrounded by a protein coat that have no intrinsic mechanism for reproduction, but rather require living cells (whose enzyme systems they utilize to make new viral particles). Viruses can be crystallized and yet remain able to reproduce, and they are often considered intermediates between non-life and life. Because viruses require living cells to reproduce, reservoirs for them are almost exclusively living organisms. Viruses, in rare instances, even survive (but do not reproduce) in environmental reservoirs from which they are re-aerosolized to cause disease. Hanta virus that causes severe respiratory disease in people exposed to intense aerosols of infected mouse urine is an example of this. Viral aerosols are produced when the infected organism coughs, sneezes, or otherwise forces respiratory or other secretions into the air. The viral particles are coated with secretions from the host and, as in the case for bacteria, there may be one to many in a single droplet. The size of a single viral particle is very small (a small fraction of a μm). However, infectious droplets more usually occur within a larger size range (1 to 10 μm). Each kind of virus produces a specific disease, although some of the diseases present with similar symptoms. Thus, the measles virus produces measles, the chicken pox viruses produces chicken pox and shingles, etc. Influenza and common colds are produced by a variety of viruses, all of which produce similar (but not necessarily identical) symptoms.

7.3.4.6 Ambient and Indoor Air Concentrations of Bioaerosols

Biological aerosols such as those discussed above can produce various health effects, e.g., irritation, infection, hypersensitivity, and other toxic responses. Bioaerosols present in the ambient environment have the potential to cause disease in humans under certain conditions. However, the 1996 PM AQCD concluded in that bioaerosols, at typically low levels present in the ambient environment, appear unlikely to account for the observed effects of PM on human mortality and morbidity reported in PM epidemiologic studies, given that bioaerosols generally represent a rather small fraction of measured urban ambient PM mass and are typically present at lower levels during the winter months when notable ambient PM effects have been found.

The 1996 PM AQCD further noted that a general rough estimate of the contribution of bioaerosols to collected PM mass can be made as follows: for an “average” 3 μm spherical spore of 0.9 density, each spore would weigh $\approx 13 \times 10^{-6}$ μg; for a clean indoor environment

with $\approx 10^3$ spores/m^3, the mass would be on the order of 0.01 µg/m^3; for a typical outdoor condition, with $\approx 50 \times 10^3$ spores/m^3, the contribution would be on the order of 0.5 µg/m^3. In contaminated indoor environments, where spore levels above 10^6 spores/m^3 are possible, the airborne spore concentration could be on the order of 10 µg/m^3 or more. In summary, it was estimated that the minor mass concentrations of bioaerosols in ambient air generally appear to be independent of the concentrations of non-bioaerosol constituents in ambient air and are unlikely to account for health effects attributed to ambient PM. However, the deposition of bioaerosols at the same respiratory tract loci as the other PM could possibly cause irritation and provide infection foci that may make the affected host more susceptible to the effects of other deposited PM.

7.3.4.7 Newly Available Bioaerosols Research

Since the 1996 PM AQCD, numerous newly available studies have yielded interesting new information pertinent to evaluating potential involvement of certain types of bioaerosols in contributing to health effects associated with exposures to ambient PM components. Of particular interest for present purposes are newly published findings which (a) indicate greater contributions (than previously thought) of bioaerosols to airborne ambient PM concentrations; (b) improve our understanding of factors and mechanisms affecting release of some bioaerosol materials into ambient air; and (c) provide evidence indicative of bioaerosols contributing to ambient PM-related health effects, including contributions made in combination with other, non-biological, PM components.

The fate of bioaerosols is dependent on a number of variables: geography, time of day, moisture levels, air temperature/humidity, wind speed and direction, and seasonal variations in the latter variables. Once airborne, depending on the particle size, bioaerosols may travel great distances. As discussed in more detail below, bioaerosols generally represent a rather small fraction of the measured urban ambient PM mass and are typically present at even lower concentrations outdoors during cold seasons, when notable ambient PM effects have been demonstrated (Ren et al., 1999; Kuhn and Ghannoum, 2003). Bioaerosols tend to be in the coarser fraction of PM; but some bioaerosols, including fungal spores, and fragmented pollens and nonagglomerated bacteria are found in the fine fraction (Meklin, 2002a; Schäppi, 1999) as

well, possibly due to reactions of the biological agents with ambient particles (Schäppi et al., 1999; Oikonen et al., 2003; Behrendt et al., 2001; Ormstad et al., 1998).

For the sake of bringing together information regarding bioaerosols, the following discussions include not only toxicology studies, but also some studies conducted in occupational settings or results from epidemiology studies assessing health responses to airborne allergens or biological material. To the extent that other aspects of air pollution evaluated in these epidemiology studies are deemed pertinent and important, the results are discussed in Chapter 8.

Atmospheric Levels of Cellulose/Other Plant Debris Markers

Puxbaum and Tenze-Kunit (2003) investigated seasonal variations in atmospheric cellulose levels (as a "microtracer" for airborne plant debris) in and around Vienna, Austria. The 9 mo average of "free" cellulose concentrations at the downtown site was 0.374 $\mu g/m^3$, (reflective of 0.75 $\mu g/m^3$ plant debris). Given an annual average for organic carbon (OC) at the downtown site of 5.7 $\mu g/m^3$, plant debris appears to be more than a minor contributor to ambient organic aerosol at that site. Unexpectedly, size distribution determinations via impactor measurements indicated that the "free cellulose" (on a mass basis) comprised approximately 0.7% of ambient fine PM (0.1 - 1.5 µm), forming a "wetable, but insoluble part of the accumulation mode aerosol," as noted by Puxbaum and Tenze-Kunit (2003). They further noted that the cellulose levels at the downtown site showed maximum concentration during the fall (probably due to increased biological activity involving seed production and entrainment of other plant cellulose materials into the air). Comparison of simultaneous measurements of cellulose at the downtown site to those from a suburban site indicated that the ambient PM cellulose did not originate in notable amounts from within the city.

The Puxbaum and Tenze-Kunit (2003) study adds further to a growing database which points toward plant debris being a significant contributor to organic aerosols present at continental sites. As discussed by Puxbaum and Tenze-Kunit, Rogge et al. (1993a) and Zappoli et al. (1999) have shown a considerable portion of the organic aerosols not to be soluble in water or organic solvents, suggesting larger molecular sizes of the insoluble compounds. Also, Matthias-Maser and Jaenike (1995) found up to 40% of the number of particles > 0.2 µm (AD) at a continental site to be of primary "biological origin". Puxbaum and Tenze-Kunit further noted that Bauer et al. (2002) found fungal spores in the 2.15 - 10 µm fraction of organic

background aerosol at a mountain site to comprise on average, about 6% of the OC in the coarse PM fraction. Also, they noted that the main constituents of the organic aerosol appear to be humic-like substances (HULIS) that are present in continental aerosol samples at concentrations (HULIS-carbon) ranging from 7 to 24% of the OC (Havers et al., 1998; Zappoli et al., 1999; Facchini et al., 1999). The macromolecular HULIS materials likely have many origins, e.g., from biomass fires (Facchini et al., 1999) or secondary atmospheric reactions (Gelenser et al., 2003). It was further noted by Puxbaum and Tenze-Kunit that cellulose is also contained in pollen at 3 - 7% dry mass (Standby and Linsken, 1985).

Other new studies evaluated atmospheric levels of levoglucosan (LVG) and other markers (e.g., palmitic acid, stearic acid) of biomass burning so as to investigate potential inputs of materials from that source category to ambient PM. One study (Fraser and Lakshamann, 2000), measuring effects in Texas of biomass fires in Mexico/Central America, found 0.2 - 1.2 $\mu g/m^3$ of LVG during episodes resulting from long-range transport of smoke haze. In another study, Poore (2002) reported on LVG concentrations in $PM_{2.5}$ samples taken at the Fresno, California supersite during the year 2000. Highest levels of LVG (up to 4.05 $\mu g/m^3$) were found during late fall/winter months (November - January), whereas LVG concentrations during spring/summer months were near or below the detection limit of 0.01 $\mu g/m^3$. Analogous seasonal patterns of variations in concentrations of palmitic and stearic acid were also seen for the Fresno supersite $PM_{2.5}$ samples. Given that agriculturally-related biomass burning in the Fresno area is typically completed by the end of October, the elevated LVG levels during fall/winter months were most likely derived from residential woodsmoke emissions. The same may also be true for fall/winter increases in palmitic and stearic acid levels, although as noted by Poore (2003), both of these acids are emitted from a variety of sources, including food production. In any case, these results appear to be indicative of episodic or more prolonged seasonal increases in plant-derived bioaerosol materials contributing to ambient PM levels in Texas and California, and by analogy, other areas of the western U.S. where air quality is affected by biomass burning emissions (e.g., from controlled burns on agricultural land, forest fires, or residential fireplaces/woodstoves).

Pollen

With regard to pollen, important new insights are beginning to emerge concerning: (a) factors influencing the occurrence of asthmatic or other allergic responses to certain types of common, widespread pollens; and (b) the likelihood that such bioaerosol-related asthma events are enhanced by the presence in ambient air of other types of non-bioaerosol airborne particles. More specifically, researchers in several countries have demonstrated links between epidemics of "thunderstorm asthma" (characterized by notable increases in asthma attacks and upsurges in hospital visits/admissions for asthma within hours after such storms) and increased levels of grass pollen allergens among respirable airborne bioaerosol components (Bellomo et al., 1992; Ong, 1994; Venables et al., 1997; Rosas et al., 1998; Newson et al., 1997; Schäppi et al., 1999; Girgis, et al., 2000).

Anemophilous plants (wind-pollinated plants) produce copious amounts of pollen, making pollen from these plants the most abundant in the atmosphere and the most important in terms of human exposure. Typically, exposure to pollen has been thought to only play a role in allergic rhinitis because they are too large to penetrate into the lower airways. However, in more recent years, there is evidence which indicates that pollen may in fact be associated with exacerbation of asthma through the release of pollen allergens small enough in size to penetrate into lower respiratory airways and/or via the binding of these allergens to other respirable size particles (Suphioglu et al., 1992; Burge and Rogers, 2000; Knox et al., 1997; Schäppi et al., 1999). More specifically, although intact (unruptured) pollen grains are typically so large (often > 10 - 20 μm) that, when inhaled, they mainly deposit in upper airways (nasopharyngeal areas), grass pollen allergens are found in the cytoplasm of the pollen grains (Taylor et al., 1994); and, upon the rupture of mature pollen grains, they are released as cytoplasmic fragments that comprise respirable (~0.1 to 5.0 μm) particles (Schäppi et al., 1999; Grote et al., 2000; Taylor et al., 2002).

The release of allergens from the pollen grains is moisture dependent (Suphioglu et al., 1992; Schäppi et al., 1997, 1999). Suphioglu et al. (1992) reported the release of a major allergen (*Lol plX*) from the intracellular starch granules of rye grass when pollen grains were ruptured during a rain storm. The allergen was small enough (< 3 μm) to penetrate the lower airways. The atmospheric concentration of the allergen showed a 50% increase on days following a rain event. Asthmatic volunteers were exposed to the starch granules or the pollen grain extracts. Asthmatic volunteers (4) that underwent inhalation challenge showed a typical

early response, characterized by the authors as a striking bronchial constriction following exposure to the starch granules. The effect was not noted in volunteers exposed to pollen grain extracts.

Taylor and colleagues (2002) confirmed that the key trigger for rupture of rye grass and Bermuda grass pollen is pollen grain contact with water, e.g., with the moistening of such pollen by dew, fog, rainfall, or lawn watering. They also further provided evidence on the specific sequence of events (and time periods) leading to appearance of the allergen-containing cytoplasmic material in airborne respirable aerosols. Taylor et al. (2002) reported that, upon drying within 1-6 hours after rye grass or Bermuda grass pollen were moistened with water and grain rupture occurred, allergen-containing cytoplasmic fragment particles could be entrained into the air by blowing air across the grass flowers or shaking them, with many thousands of such fragments in the 0.1 to 4.7 μm size range (most below 0.4 μm) being collected by a Cascade impactor. The dispersal of such allergen-laden particles following cycles of wetting and drying of grass pollen, it was noted by Taylor et al. (a) may occur in response to such disturbances as wind, lawn mowing, and recreational activities; (b) likely account for marked increases in asthma attacks after thunderstorms; and (c) may also account for increased asthmatic symptoms during grass flowering season after any moist weather conditions. Also, more recently, Taylor et al. (2003) employed analogous experimental wetting/drying procedures, collection and measurement of wind-released cytoplasmic fragments of birch tree pollen in the 0.03 to 4 μm size range, and found them to contain Bet v 1 allergens.

Taylor et al. (2002) also highlighted possible bases for interactions between aerosolized allergen-laden pollen debris and other types of ambient airborne particles. They noted, for example, that diesel emission particles are a major contributor to urban respirable aerosols mass, e.g., 18% in Pasadena, CA (Scheuer et al., 1996), and have been implicated as a cause of allergic rhinitis and asthma in mice and humans (Nel et al., 1998; Bayram et al., 1998; and Diaz-Sanchez, et al., 2000). Taylor et al. further noted (a) that fine combustion particles and aerosols of pollen allergens, because of their small size, may deposit in similar respiratory tract regions; and (b) that synergistic combinations of allergen-laden pollen debris and polycyclic hydrocarbons found in fine combustion aerosols may explain the notable increased prevalence of pollen-induced asthma during the past 50 years.

Further possibilities exist with regard to possible ways that the copresence of grass pollens and diesel particulate matter (or perhaps other airborne particles) may contribute jointly to enhanced probability of asthma symptoms occurring in susceptible human population groups. More specifically, the EPA Health Assessment Document for Diesel Engine Exhaust (U.S. Environmental Protection Agency, 2002) noted that Ormstad et al. (1998) investigated the potential for DPM (as well as other suspended PM) to act as a carrier for allergens into the airways and found both Can f 1 (dog) and Bet v 1 (birch pollen) on the surface of airborne PM collected inside homes. In an extension of the study, they found that DPM adhered to polycarbonate filters could bind both of these allergens as well as Fel d 1 (cat) and Der p 1 (house mite) allergens. The authors concluded that soot particles in indoor air house dust may act as a carrier for several allergens in indoor air. The EPA Diesel Document (2000) also noted that Knox et al. (1997) investigated whether free grass pollen allergen molecules, released from pollen grains by osmotic shock (Suphioglu et al., 1992) and dispersed in microdroplets of water in aerosols, can bind to DPM mounted on copper grids in air. Using natural highly purified Lol p 1 (the major grass pollen allergen), immunogold labeling with specific monoclonal antibodies, and a high-voltage transmission electron-microscopic imaging technique, Knox et al. found binding of Lol p 1 to DPM in vitro. They concluded that binding of Lol p 1 with DPM might be a mechanism by which allergens can become concentrated in air and trigger asthma attacks.

In addition to suggesting that airborne diesel exhaust particles can act as carriers of biological aerosols producing an enhanced allergic response (Knox et al., 1997; Diaz-Sanchez et al., 1997; Fujinaki et al., 1994), some studies suggest that allergen carriers (e.g., pollen grains) may incorporate other atmospheric pollutants that alter the pollen surface, leading to altered protein and allergen release (Behrendt et al., 1992, 1995, 1997, 2001). Pollen grains from an industrial region with high polyaromatic hydrocarbons were shown to be agglomerated with airborne particles. In vitro exposure of grass pollen to particles demonstrated ultrastructural changes at the surface of the pollen and within the protoplasm, such as exocytosis of granular proteinaceous material and increased allergen release (Behrendt et al., 1997).

Fujimaki et al. (1994) examined the effect of intratracheal instillation of a mixture of diesel exhaust particles and Japanese cedar pollen on IgE antibody production and lymphokine production in mice. IgE antibody production and IL-4 production in mediastinal lymph nodes

were significantly increased in mice instilled with the diesel exhaust particles and the cedar pollen compared with the cedar pollen alone. There was a slight increase seen in IL-2 production. Measurable levels of birch pollen-specific human IgE was noted in hu-PBL-SCID mice previously stimulated with birch pollen. When the mice were exposed i.p. to the 25 μg birch pollen plus 500 μg of diesel exhaust particles, IgE levels were twice as high as those for birch pollen exposure only. Ormstad et al. (1998) found that Fel d 1 (cat), Can f 1 (dog), Der p 1 (house dust mite) and Bet v 1 (birch pollen) allergens bind with soot particles from diesel exhaust in the < 2.5 μm size range. When the particle mixture was injected in the footpad of mice, adjuvant activity was noted on the production of IgE antibodies to ovalbumin (Ormstad et al., 2000). The authors suggested that it is likely that the soot particles alone were responsible for some of the adjuvant activity. However, the particles may increase the IgE production to allergens by modulating the immune response.

Diaz-Sanchez et al. (1997) studied possible synergistic relationships between diesel exhaust particles and ragweed allergen. Inconsistent and low levels of mucosal cytokine mRNAs were found in ragweed sensitized subjects following intranasal challenge with ragweed allergen alone. When the subjects were challenged with ragweed allergen and diesel exhaust particles there was a decrease in Th1-type cytokines (IFN-γ and IL-2) expression but an elevated expression of mRNA for other cytokines (IL-4, IL-5, IL-6, IL-10, IL-13). Ragweed allergen and diesel exhaust particles also produced a 16-fold increase in ragweed-specific IgE but not total IgE levels or IgE-secreting cell numbers. Total and specific IgG-4 levels were enhanced , while total IgG levels were not. Subject were given short ragweed Amb a I allergen, starting at 10 allergen units and increasing in 10-fold units until symptoms were noted. Diesel exhaust particles were administered for a total of 0.3 mg in 200 μL of saline. Clones of deleted switch circular DNA (Sε/Sμ), representing switching from μ to ε from the nasal lavage cells, also were detected (Fujieda et al., 1998).

Brunekreef et al. (2000) suggested that airborne pollen associated with allergic responses may pose more serious effects than previously thought. They evaluated the relationship between the daily number of deaths in the Netherlands for the period of 1986 to 1994 and air pollution, meteorological factors, and airborne pollen concentrations (analysed as categorical variables). The relationship between mortality and airborne pollen concentration was modeled using Poisson regression with generalized additive models. The pollen mortality associations were

adjusted for long-term and seasonal trend, influenza morbidity, ambient temperature, humidity, and indicators for the day of the week and holidays. The average number of daily deaths for the study period was 332.5 (total), including 141.8 cardiovascular related deaths, 15.8 COPD related deaths, and 9.8 pneumonia related deaths. Pollen concentrations were only weakly associated with air pollution and there was no confounding by particles < 10 μm, black smoke, sulphate and nitrate aerosols, nitrogen dioxide, sulphur dioxide, or ozone. *Poaceae* pollens were associated with daily deaths due to COPD and pneumonia. Other pollens, especially *Betula* and *Rumex* were also positively correlated with mortality. Information was not included on whether this association was with daily deaths due to cardiovascular disease, COPD, and/or pneumonia. The authors suggested that acute exacerbations of allergic inflammation associated with high pollen exposures may also precipitate death due to cardiovascular disease, COPD, or pneumonia in individuals already suffering from these disorders.

Rosas et al. (1998) reported an association between asthma hospital admissions and grass pollen exposure for children, adults, and seniors in Mexico City. The effects were noted for both the wet (May through October) and dry (November through April) seasons. The number of hospital admissions increased by a factor of 2 to 3 for children and adults on day when the grass pollen concentrations were above 20 grains/m^3. There was no association between asthma exacerbation and tree pollen.

An association between asthma and emergency room visits was reported by Celenza et al. (1996). During a two month study period, the daily average number of emergency room visits was 2.25 patients. However, following a thunderstorm, the emergency room visits increased to 40 patients. There was a peak in pollen concentration approximately nine hours before the peak in asthma emergency room visits. Three hours following the storm, the pollen count increased from 37 to 130 grains/L. There was no evidence that vehicle exhaust pollutants were related to the increase in asthma emergency room visits.

Hastie and Peters (2001) studied the effect of ragweed allergen exposure on ciliary activity in nonallergic subjects with mild inflammatory response, allergic subjects with mild inflammatory response, and allergic subjects with severe inflammatory response. Nonallergic subjects showed a minimal ragweed allergen effect on ciliary activity, a slight increase in bronchoalveolar cells, and a nonsignificant increase in albumin concentration. Allergic subjects with mild inflammatory changes had significant increase in albumin concentration and a two-

fold increase in bronchoalveolar cell concentration. The allergic subjects with severe inflammatory changes had a 12-fold increase in albumin concentration and a 9-fold increase in bronchoalveolar cell concentration.

Delfino et al. (1997,1996) conducted several studies evaluating the association between asthma incidence and exposure to various air pollutants and fungal spores and pollen. There was an association between exposure to air pollutants and fungal spores and symptom severity as measured by inhaler usage. Inhaler puffs increased by 1.1/100 ppb O_3 (14 to 87 ppb; 12-h daytime average) and by up to 1.2/1,000 fungal spores/m^3 (648 to 7,512 spores/m^3) depending on the species. The greatest increase in symptom severity was caused by basidiospores (Delfino et al., 1996). Delfino et al. (1997) found an association between asthma severity (asthma symptom scores and inhaler use) and peak expiratory flow rate (PEFR) and total fungal spores. Symptom severity was more strongly associated with basidiospore concentrations, especially during the period of sporulation. There was no detected association between O_3 exposure and asthma severity as seen in the Delfino et al. (1996) study. The authors suggested that there may have been problems with O_3 measurements. There was also no significant relationship between asthma severity and PM_{10} and pollen exposure. However, their concentrations during the study period were low, 26 µg/m^3 and 216 grains/m^3, respectively.

In summary, newly available information indicates release of allergen-laden material from pollen-spores in respirable-sized aerosols and suggests possible ways by which binding of such material to other airborne particles (e.g., DPM) may concentrate such allergens in ambient air or, once inhaled, jointly exacerbate allergic reactions in susceptible human populations. It should also be noted that pollen itself may act as a carrier for other allergenic materials. Spiewak et al. (1996a) found Gram-bacteria and endotoxin on the surface of pollens; and Spiewak et al. (1996b) found concentrations of several immunotoxicant allergens (Gram+ and Gram– bacteria, thermophilic actinomycetes, fungi) to range from 0 to 10,000 cfu/g of pollen from several grasses or trees in Poland.

Fungi and Their Byproducts

The fungal spore is a known cause of allergic diseases. All fungi may be allergenic depending on the dose. Once an individual is sensitized to the fungi, small concentrations can trigger an asthma attack or some other allergic response (Yang and Johanning, 2002). Unlike the

fungal induced allergic responses, fungal toxic inflammatory responses are dependent on airborne concentrations and the responses are similar for most individuals. Concentrations of fungi are usually higher in the indoor environment. However, outdoor airbornes spores are often the source of indoor fungal contamination (Koch et al., 2000), as noted earlier.

Fungi produce a variety of byproducts, including mycotoxins and volatile organic compounds. Mycotoxins have low volatility making inhalation of volatile mycotoxins unlikely. However, mycotoxins are an integral part of the fungus. Volatile organic compounds (derivatives of alcohols, ketones, hydrocarbons, and aromatices) are produced when the fungi are actively growing. Generally concentrations of these VOCs are quite low and the relationship between exposure and health effects is unclear (Yang and Johanning, 2002).

A number of studies have suggested a relationship between exposure to fungi and their byproducts in respiratory illnesses and immune pathology (Hodgeson et al., 1998; Tuomi et al., 2000; Yang and Johanning, 2002). Some fungal byproducts have been shown to stop ciliary activity in vitro and may act to produce general intoxication of macroorganisms through the lung tissue or to enhance bacterial or viral infection (Pieckova and Kunova, 2002; Yang and Johanning, 2002). Larsen et al. (1996) demonstrated non-immunological histamine release from leukocytes exposed to a suspension of fungal spores and hyphal fragments. The authors suggested that the fungal suspension possessed at least two histamine releasing components; an energy-dependent release process and a cytotoxic release process.

In a study conducted by Rosas et al. (1998) there was a statistically significant increase in fungal spore exposure-related asthma hospital admissions in children in Mexico City. The effect was not seen in adults and seniors. The highest spore (ascomycetes and basidiospore) concentrations were associated with a 2 to 3 increase in hospital admissions per day. Ascomycetes and basidiospore concentration ranged from < 100 to 207 spores/m^3 and from < 100 to > 1000 spores/m^3, respectively. There was an association with hospital admissions during both the wet and dry season. There was no strong statistical association between asthma admissions and NO_2 (mean: 0.102 and 0.164 ppm),O_3 (mean: 0.204 and 0.187 ppm), SO_2 (mean: 0.074 and 0.081 ppm), TSP (mean: 78 and 156 µg/m^3) and PM_{10} (mean: 56 and 98 µg/m^3) concentrations during either the wet or dry seasons.

Airborne fungal concentrations of ≥1000 spores/m^3 were reportedly associated with asthma deaths among 5 to 34 year olds in Chicago between 1985 and 1989 (Targonski et al., 1995). The

odds of death occurring on days with airborne fungal concentrations of ≥1000 spores/m^3 were 2.16 times higher than other days. Logistic regression analysis was used to compare the probability of deaths caused by asthma as the result of tree, grass, and ragweed pollen and fungal spores. Fungal spores were counted as a single group. Asthma deaths were obtained from death certificates. The deaths were also related to personal, social, and medical access factors.

Several newly-published studies have evaluated concentrations of fungi or their viable propagules in ambient (outdoor) and/or indoor air in various areas of the United States or other countries in Europe or East Asia. In an extensive 22-mo study, Cooley et al. (1998) investigated the types of fungi found in indoor and outdoor air at 48 schools in U.S. states located along the Atlantic seaboard and Gulf of Mexico. Five fungal genera consistently found in outdoor air comprised > 95% of the outdoor air fungi detected: *Cladosporium* (81.5%); *Penicillium* (5.2%); *Chrysosporium* (4.9%); *Alternaria* (2.8%); and *Aspergillus* (1.1%). An average of ~700 colony-forming units (CFU)/m^3 of *Cladosporium* fungi were found in outdoor air (about 3 times that found indoors); whereas relatively low concentrations of *Penicillium* (~30 CFU/m^3) and the other species (ranging from < 5 to ~40 CFU/m^3) were found in ambient air (compared to analogous levels indoors, except for notably elevated average levels for samples taken from indoor "complaint areas" where markedly higher numbers of indoor air quality (IAQ)-related symptoms (nasal drainage, congestion, watery eyes, headaches, allergies, etc.) were reported among students, teachers, and other staff. Probably of most further note here was the finding of *Penicillium* being most consistently elevated in complaint areas, the growth of this rather ubiquitous species being optimized between 10 - 25 °C and predominating in complaint areas with a wide range (23 - 67%) of relative humidity. Cooley et al. noted: (a) the apparent ability of *Penicillium* to compete successfully with most conidial fungi across a wide range of water availability; (b) the need for relatively high water content for sporulation to occur; the water content of the substrate being the critical factor determining growth rate; (c) the spores being small (1 - 5 µm) and capable of entering the lower respiratory tract; and (d) evidence showing that bronchial challenges with *Penicillium* species spores cause immediate and delayed-type asthma in sensitized subjects (Licorish et al., 1985).

In a detailed study of the nature and variation of fungi inside and outside homes in the greater New Haven, CT area, Ren et al. (1999) found that fungi in living room, bedroom, and outdoor air varied across seasons but did not differ seasonally in basement air. They reported

that *Claudosporium spp.* dominated both indoor and outdoor air during summer months, whereas *Penicillium* and *Aspergillus* were dominant in indoor air in winter, but neither were dominant in outdoor air during any season. Ren et al. further noted: (a) the fungi isolated in their study are broadly the same as those found in European studies (Beaumont et al., 1984, 1985; Verhoeff et al., 1988; Hunter and Lea, 1994); (b) the seasonal trend found by them for fungal propagules indoors and outdoors were generally comparable with those reported by Hunter and Lea (1994) for British homes, i.e., lowest in winter, increasing in spring, reaching the maximum in summer, and decreasing in fall; (c) their results support current concepts that outdoor air may affect culturable fungal propagules indoors, but the presence of culturable molds in indoor air may not always reflect the presence of such molds in outdoor air, especially in problem indoor environments; and (d) no associations were found between fungal types and their concentrations in dust and in air, suggesting that types of fungi and concentrations measured in housedust do not necessarily reflect those in indoor air, with air samples likely providing a more direct and better measure of inhalation exposure to fungi. Lastly, Ren et al. (1999) noted that: 50% of the 342 air samples taken during the 1996-1997 study period had < 575 CFU/m^3 total culturable fungal propagules; 97% < 100 CFU/m^3 of Alternaria; < 28% > 50 CFU/m^3 of Aspergillus; and ~90% < 250 CFU/m^3 of Penicillium; and none had *Cladosporium spp.* over the 3000 CFU/m3 level set as an allergic threshold by Gravesan (1979).

Koch et al. (2000) obtained data on fungi concentrations in a study that evaluated if differences in types of seasonal variations in concentrations of fungi in indoor and/or outdoor air occur and could perhaps account for lower prevalence of allergies and asthma in Western than in Eastern Germany. During 1995-1997, 405 homes in Hamburg (West) and Erfurt (East) Germany were visited twice and samples of settled dust taken by vacuuming from carpets in the living room. No significant differences were found between the two cities for total genera or single fungi species (*Alternaria, Aspergillus, Claudosporium,* and *Penicillium*) with regard to concentrations of viable fungi detected in settled housedust. Similar seasonal variations were observed for outdoor air and indoor dust, i.e., with a late summer peak detected in outdoor air (~2400 CFU/m^3 viable fungi in August) and a parallel peak in such concentrations in housedust. Koch et al. also noted: (a) that recent studies indicate that outdoor air spora influence the presence of fungi in indoor environments, but indoor air levels of fungi in indoor environments do not simply reflect the presence of fungi or spora in outdoor air; and (b) that the genera

commonly isolated in housedust (e.g., *Claudosporium, Penicillium, Alternaria, Aspergillus*) reflect their relative occurrence in outdoor spore counts.

Takahashi (1997) evaluated fungal types and concentrations in indoor and outdoor air in Yokahama, Japan and found the number of outdoor total fungal colony-forming units to vary from < 13 to 2750 CFU/m^3. *Claudosporium spp.* again was found to predominate in outdoor air, followed by *Alternaria spp.* and *Penicillium spp.*, with fungal concentrations peaking in September. Outdoor fungal concentrations were significantly correlated with maximum, minimum, and average temperature of the day, as well as average wind velocity of the day, relative humidity, and precipitation for the month. The ranges of concentrations of fungi in outdoor air were reported by Takahashi to be the same as reported for many European, North American countries, and Israel — with most showing peak levels during the summer and early fall (July to October) and lowest means during winter months (January to February). They also noted that the daily maximum of total outdoor airborne fungal CFU (mostly *Claudosporium spp.* and *Alternaria spp.*) peaked around 1700 h (5 pm), as seen in European and American studies.

In another East Asia study, Su et al. (2001) compared concentrations of airborne fungi, endotoxin, and housedust mite allergens in the homes of asthmatic and non-asthmatic children in southern Taiwan, where temperature and relative humidity are high throughout the year. With regard to fungi, the results obtained paralleled those of other studies noted above in many respects, except for some differences in seasonal variations — not too surprisingly given the more constant high temperature/humidity conditions in this study area. The most predominant indoor genera were *Claudosporium, Aspergillus, Penicillium, Alternaria,* and yeast. Cladosporium ranked highest, it being in ~85% of the colonies from indoor samples and its highest CFU/m^3 concentration in winter and other seasonal variation patterns also applying for the other types of fungi. Outdoor air *Claudosporium* levels were significantly correlated with indoor air values during all seasons; and the indoor/outdoor concentrations for the other fungi were also significantly and positively correlated during the spring. This suggests that outdoor levels of fungi and/or their spores are important determinants of indoor air levels of fungi in southern Taiwan.

Endotoxins

Endotoxins and lipopolysaccharides (LPS; chemically purified version of endotoxin) are present in the outer cell membrane of all Gram-negative (Gram-) bacteria. Endotoxins are toxic to most mammals. When released into the blood stream, it is thought that endotoxins/LPS interact with receptors on monocytes and macrophages and other types of receptors on endothelial cells, triggering the production of cytokines, which in turn stimulate production of prostaglandins and leukotrienes, arachidonic acid metabolites (e.g., prostacyclin and thromboxane A_2, and nitric oxide). These mediators can induce physiological changes, e.g., inflammation, smooth muscle constriction, and vasodilatation (Young et al., 1997).

Some of the more recent inhalation studies on endotoxin exposure are summarized in Table 7-7. In vitro studies on particle-associated endotoxin are discussed in Section 7.5.2.2. Heedrik et al. (2000) note that animal feces and plant materials contaminated with bacteria contribute most to organic dust-related endotoxin exposure. Although there is strong evidence that inhaled endotoxin plays a major role in the toxic effects of bioaerosols encountered in the work place (Castellan et al., 1984, 1987; Rose et al., 1998; Vogelzang et al., 1998; Zock et al., 1998), it is not clear as to what extent typical ambient concentrations of endotoxin are sufficient to produce toxic pulmonary or systemic effects in healthy or compromised individuals.

Several new occupational exposure studies have yielded potentially useful information for estimating exposure-response relationships for health effects associated with exposure to airborne endotoxin. For example, Vogelzang et al. (1998) evaluated exposure-response relationships for lung function decline in relation to endotoxin exposure of pig farmers in The Netherlands. Long-term average exposure to endotoxin and dust was evaluated via personal monitoring during summer and winter for a cohort of 171 pig farmers over a three-year period. Mean age at start was 39.6 yrs and mean number of years worked in pig farming was 16.7 yrs. Linear regression analyses were used to analyze relationships between declines in FEV_1 or FVC (based on measures taken in the 1st or 3rd years of the studies) and dust concentrations or endotoxin levels in the inhalable dust. Statistically significant ($p < .05$) associations (correcting for age, baseline values, and smoking) were found by regression analysis between estimated long-term average exposure (typically $\geq$ 5 h/day) to endotoxin (105 ng/m^3) and annual decline in $FEV_{1.0}$ (73 ml/yr) and FVC (55 ml/year). The FVC, but not the $FEV_{1.0}$, declines were also significantly correlated with inhalable dust concentrations (long-term average = 2.63 mg/m^3).

TABLE 7-7. RESPIRATORY EFFECTS OF INHALED ENDOTOXIN-LADENED AMBIENT BIOAEROSOLS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Humans (pig farmers), 82 symptomatic & 89 asymptomatic n = 171	Dust Endotoxin	Inhalation	2.63 mg/m^3 σg = 1.3 105 ng/m^3 σg = 1.5	N/A	5 h/day average lifetime exposure	Large decline in FEV_1 (73 mL/year) and FVC (55 mL/year) was significantly associated with estimated long-term average exposure to endotoxin at 105 ng/m^3.	Vogelzang et al. (1998)
Humans (healthy); 32 M, 32 F, 16 to 50 years old	Indoor pool water spray Endotoxin	Inhalation	N/A	0.1-7.5 µm	N/A	Recurring outbreaks of pool-associated granulomatous pneumonitis (n = 33); case patients had higher cumulative work hours. Analysis indicated increased levels of endotoxin in pool air and water.	Rose et al. (1998)
Humans (potato plant workers), low (37 M) and high (20 M) exposures	Endotoxin	Inhalation	low: 21.2 EU/m^3, σg = 1.6 high: 55.7 EU/m^3, σg = 2.1	N/A	8 h	Concentration-related decreased FEV_1, FVC, and MMEF over the work shift; endotoxin effects on lung function can be expected above 53 EU/m^3 (≈ 4.5 ng/m^3) over 8 h.	Zock et al. (1998)
Humans (healthy); 5 M, 4 F, 24 to 50 years old	LPS [1] (endotoxin)	Inhalation	0.5 µg 5.0 µg 50 µg	1 - 4 µm MMAD	30 min	Significant decrease in PMN luminol-enhanced chemiluminescence with 0.5 µg LPS; increase in blood CRP and PMNs, and increase in sputum PMNs, monocytes, and MPO with 5.0 µg LPS; increase in body blood PMNs, temperature, blood and urine CRP, sputum PMNs, lymphocytes, monocytes, TNFα, and ECP with 50 µg LPS.	Michel et al. (1997)
Rats (Fischer 344), 8 wks to 22 mo old, N = 3/group	LPS [1] (endotoxin)	Inhalation	70 EU	0.72 µm σg = 1.6	12 min	Significant increase in PMNs in bronchoalveolar lavage (BAL) in LPS exposed animals. LPS significantly affected the reactive oxygen species activity in BAL. Effects were age-dependent.	Elder et al. (2000a,b)

[1] LPS = lipopoly saccharide.

The $FEV_{1.0}$ annual average decline is large in relation to the expected age-related decline of 29 ml/yr but equal to that of 73 ml/yr reported by Iverson et al. (1994) based on a 5-yr study of farmers. The least exposed pig farmers in the Vogelzang et al. study showed an average $FEV_{1.0}$ decline similar to the expected age-related decline, whereas the predicted decline for the most exposed pig farmers ranged up to 100 ml/yr. The authors noted that their results support the selection of the lower of two proposed (Clark, 1986; Palchak et al., 1988) occupational exposure threshold levels of 30 or 100 ng/m^3 for airborne endotoxin. Some health effects have been reported for occupational exposure to complex aerosols containing endotoxin at concentrations likely more relevant to ambient levels. Zock et al. (1998) reported a decline in FEV_1 (≈ 3%) across a shift in a potato processing plant with up to 56 endotoxin units (EU)/m^3 in the air. Rose et al. (1998) reported a high incidence (65%) of BAL lymphocytes in lifeguards working at a swimming pool where endotoxin levels in the air were on the order of 28 EU/m^3. Although these latter two studies may point towards possible pulmonary changes at low concentrations (˜ 25-50 EU[3]) of airborne endotoxin, it is not possible to rule out the contribution to observed effects by other agents present in the complex airborne organic aerosols in the occupational settings studied.

In another European study, Heinrich et al. (2003) recently carried out temperal-spatial analyses of endotoxin in fine ($PM_{2.5}$) and coarse ($PM_{10-2.5}$) particle mass of ambient aerosols from two East German towns about 80 km apart. The authors noted that one town, Hettstedt, showed consistently higher prevalence of hay fever and strong allergic sensitization for children than the prevalence rates seen in the other town, Zerbst, even into the late 1990's when levels of ambient air pollutants (TSP, SO_2) had converged in areas earlier differing in such air pollution levels (Heinrich, et al. 2000a, b). From January to June 2002, weekly $PM_{2.5}$ and $PM_{10-2.5}$ samples were taken by dichotomous samplers in each of the two towns and analyzed for endotoxin in the collected ambient PM. The arithmetic mean for the $PM_{2.5}$ sample mass average 10.2 and 12.4 µg/m^3 for Hettstedt and Zerbst, respectively; and $PM_{10-2.5}$ sample mass 6.1 and 6.8 µg/m^3, respectively. Comparable ranges for Hettstedt and Zerbst were 0.3-25.8 and 4.2-26.3 µg/m^3 for $PM_{2.5}$ and 1.2-10.6 and 3.0-10.7 µg/m^3 for $PM_{10-2.5}$. Mass levels for both particle size fractions showed notable week-to-week fluctuations (mostly closely parallel for both towns), with weekly means in each town being highest in late March/early April. Airborne endotoxin concentrations for both towns tended to show strong seasonality in parallel patterns for both the fine and the

coarse particle fractions with endotoxin mass concentrations generally being low during late winter/early spring in comparison to such levels generally increasing from late April to highest points seen in early June (except for a brief episode of elevated endotoxin in fine PM seen in Hettstedt in late January/early February). Fine PM endotoxin mass concentrations for Hettstedt (1.2 EU/mg^3 arith. mean) were not statistically significantly different from such concentrations for Zerbst (1.1 EU/mg^3 arith. mean), but endotoxin levels expressed per mg^3 were significantly higher in Zerbst, suggesting that there may be a higher biogenic content or more bioactive particles in the Zerbst fine PM fraction. The endotoxin levels in the coarse fraction were about 10 times those in the fine fraction whether expressed in EU/mg dust or EU/m^3 air and were not statistically significantly different between the two towns. The range of endotoxin concentrations for Hettsted were 0.2-3.6 EU/mg dust and 0.002-0.21 EU/m^3 air for $PM_{2.5}$ versus 4.0-25.2 EU/mg dust and 0.01-0.24 EU/m^3 air for $PM_{10-2.5}$. The comparable concentrations for Zerbst were 0.2-4.3 EU/mg dust and 0.004-0.031 EU/m^3 for $PM_{2.5}$ versus 3.1-24.2 EU/mg dust and 0.02-0.17 EU/m^3 air for $PM_{10-2.5}$. The authors concluded that, given the generally similar levels and patterns in seasonal variations of endotoxin concentrations in Hettstedt and Zerbst, it was unlikely that differential exposures to endotoxin could explain differences in hay fever or allergic reaction prevalence between the two towns.

The levels of endotoxin concentrations found in Hettsted and Zerbst are similar to those reported for other ambient or rural aerosols and dusts, with those in coarse PM fractions typically notably exceeding those in fine fractions, as noted by Heinrich et al. (2003). They also noted that measurements in livestock buildings (poultry, pig, cattle) often show endotoxin concentrations up to several thousand EU/mg dust, with levels in the inhalable PM_{10} fraction being higher by ~10-fold than in the fine PM. The finding of notably higher concentrations and absolute mass amounts of endotoxin in coarse-mode particle samples versus fine particle samples thus appears to hold, in general, across a number of geographic areas and for both occupational and environmental situations. The authors also noted the seasonal variation observed in their study with increased airborne levels of endotoxin in May and June apparently following increased growth of fungi, other plants, and presumably of microbes due to increasing outdoor spring temperatures under moderate climatic conditions in Germany. They further noted that increased levels of plant-related materials and leaf surfaces (Rylander, 2002), as well as pollen surfaces (Spievak et al., 1996), may provide additional sources of growth of Gram-

bacteria (from which endotoxin is derived). The seasonal variation in endotoxin concentrations observed by Heinrich et al. appear to parallel those seen in other studies for ambient airborne endotoxin levels, their being lower in winter and high during warmer weather in late spring/summer.

Park et al. (2000) investigated endotoxin levels in indoor dust of 20 homes, indoor air of 15 homes, and outdoor air at two locations in the Boston, MA, area. They reported that endotoxin levels in indoor dust (from the bed and bedroom/kitchen floors) were not significantly associated with indoor airborne endotoxin concentrations. The airborne endotoxin levels were, however, significantly associated with absolute humidity; and a significant seasonal effect for kitchen dust (spring > fall) and indoor airborne endotoxin (spring > winter) was observed, as was a significant seasonal pattern for outdoor airborne endotoxin (summer > winter). The authors indicated that, overall, the indoor airborne endotoxin levels (geom. mean = 0.64 EU/m^3) appeared to be higher than outdoor concentrations (geom. Mean = 0.46 EU/m^3); but seasonal variations were evident in that indoor airborne endotoxin levels were generally higher than outdoor airborne endotoxin levels during September-April and lower than outdoor levels during the late spring/summer (May-August). Outdoor airborne endotoxin levels showed significant seasonality, varying by more than 4-fold across seasons, with decreases in outdoor levels beginning at the end of summer/early fall and remaining at lowest levels during winter before starting to increase again with the onset of the growing season in late spring. The authors noted that this pattern is consistent with data suggesting that outdoor Gram-bacteria (and thus airborne) endotoxins are shed from leaves of growing plants (Edmonds, 1979; Andrews, 1992). Further, the overall mean outdoor airborne endotoxin levels at an urban sampling location (geom. mean = 0.51 EU/m^3) were somewhat (but not statistically significantly) higher than at a suburban location (geom. mean = 0.39 EU/m^3).

Thorn and Rylander (1998b) examined the effect of endotoxin inhalation on inflammatory response in 21 healthy subjects from 20 to 30 years old. All subjects were known smokers, currently did not have a respiratory infection, no self-reported allergies or chronic bronchitis, and no physician diagnosed asthma. Subjects were examined before exposure to up to 40 µg LPS. Cell counts, ECP, and MPO were monitored in the blood and sputum before and 24 h following exposure. Myeloperoxidase was significantly increased in both the blood and sputum following inhalation of the LPS. Eosinophilic cationic protein was increased but the increase was only

significant in the sputum. The ratio of MPO and neutrophils was significantly decreased in blood and sputum. Spirometric testing demonstrated a significant decrease in FEV_1 and FVC values following LPS inhalation. Subjects experienced throat irritation, dry cough, breathlessness, unusual tiredness, headache, and heaviness in the head. The symptoms developed 4 to 6 h following exposure and persisted for 6 to 8 h.

Michel et al. (1997) examined the dose-response relationships for effects of inhaled lipopolysaccharide (LPS: the purified derivative of endotoxin) in normal healthy volunteers exposed to 0, 0.5, 5, and 50 µg of LPS. Inhalation of 5 or 50 µg of LPS resulted in increased PMNs in blood and sputum. At the higher concentration, a slight (3%) but nonsignificant decrease in FEV_1 was seen.

Other controlled exposure studies of laboratory animals (rat) by Elder et al. (2000a,b) indicate that priming of the respiratory tract by inhaled endotoxin increases the effect of inhaled ultrafine surrogate particles and ozone (as discussed in more detail in Section 7.6).

In vitro studies of potential endotoxin contributions to toxic effect of ambient PM are also discussed later (in Section 7.4.2).

(1→3)-β-D-Glucan

Studies from different countries have reported relationships between damp/humid indoor environments and various symptoms in both adults and children (Meklin et al., 2002b). Such symptoms consist of eye, nose, and throat irritation, dry cough, headache, tiredness, and sometime skin problems. Fungi and their byproducts (discussed above) and bacteria commonly present in damp/humid indoor environments contain several substances that have known inflammatory properties. Of the substances associated with these symptoms, (1→3)-β-D-glucan, a polyglucose compound in the cell walls of fungi, certain Gram+ bacteria, and plants, has begun to be accorded increasing attention.

The (1 → 3)-β-D-glucan can induce several biological responses in vertebrates, including stimulation of the reticulo-endothelial system, activation of neutrophils, macrophages, and complement, and possibly activation of eosinophils. T-lymphocyte activation and proliferation have been reported in experimental animals (Heederik et al., 2000). Rylander (1996) suggested that an acute exposure to (1 → 3)-β-D-glucan can produce symptoms of airway inflammation in normal subjects without a history of airway reactivity after exposing subjects to 210 ± 147 ng/m^3

(1 → 3)-β-D-glucan for 3 separate 4 h sessions 5 to 8 days apart. Exposure to (1 → 3)-β-D-glucan alone did not significantly impact FEV_1 values; but there was a slight decrease in FEV_1 values following administration of the two highest doses of methacholine (MCh). Methacholine was administered in increasing doses in 3 min intervals for a total of 1.25 mg. Forced vital capacity (FVC) and FEV_1/FVC were also unchanged following (1 → 3)-β-D-glucan exposure and MCh challenge. There was a significant, negative correlation between MCh-induced decrease in FEV_1 values and the intensity of throat irritation after 1 h exposure. The intensity of nasal irritation and stuffy nose and throat irritation was increased at 1 and 4 h. Dry cough, cough with phlegm, chest tightness and wheezy chest was not affected. No effects on airway responsiveness or inflammatory symptoms were noted in subjects exposed to endotoxins (9.9 ng/m^3) under the same exposure conditions.

Thorn and Rylander (1998a) examined the relationship between exposure to airborne (1 → 3)-β-D-glucan and airways inflammation. The study was conducted on a group of 75 houses in Gothenburg, Sweden where there had been numerous complaints about dampness and respiratory symptoms, fatigue, and mold odors. Measurements of (1 → 3)-β-D-glucan and endotoxins in airborne dust were made with Limulus lysates. Study participants included 67 females and 62 males 18 to 83 yr old and included 34 smokers and 9 physician-diagnosed asthmatics. The average number of years the subjects lived in their house was 18 yr (range 2 to 36 yr). Study participants provided questionnaire information for assessment of organic dust-induced effects. The questionnaire inquired about existing diseases states; occupation; length of time the subject had lived in the house; the presence of pets; and the occurrence of cough (dry or with phlegm); shortness of breath; nose, throat, and eye irritation; nasal and chest congestion; and joint and muscle pains, headache, fatigue, and dermal disorders. Other questions addressed subjective airway reactivity, chronic bronchitis, asthma, and episodes of fever and influenza-like symptoms gone the next day. Chronic bronchitis was defined as a cough with sputum for at least 3 mo a year for a period of at least 2 yr. Asthma was defined as physician-diagnosed asthma. Spirometry was performed on test subjects to exclude subjects with less than 70% of predicted values in FEV_1 and/or FEV_1/FVC. Airway responsiveness was assessed using MCh for a total of 1.2 mg MCh, administered in increasing doses at 3-min intervals. Serum eosinophilic cationic protein (ECP), myeloperoxidase (MPO), and C-reactive protein (CRP) were measured. Atopy

was determined using the Phadiatop test to measure the concentration of specific IgE antibodies against airborne allergens.

No detectable levels of endotoxin were found in the homes, but (1 → 3)-β-D-glucan levels ranged from 0 to 19 ng/m^3. Of 75 homes studied, 20 had (1 → 3)-β-D-glucan concentrations below 1 ng/m^3 and 13 homes had levels above 6 ng/m^3. Twenty-four subjects had positive Phadioatop test; but there was no significant correlation between exposure and atopy. However, when evaluated by age, there was a significantly larger number of atopic subjects in the > 65 yr old group exposed to > 3 ng/m^3 (1 → 3)-β-D-glucan. There was a significant inverse correlation between baseline FEV_1 and number of years the subjects lived in the house when controlled for age, gender, cigarette smoking status, asthma, atopy, and pets among male subjects < 65 yr old that was not seen in the female subjects < 65 yr old and in > 65 yr old subjects. The relationship was present only for those male subjects exposed to > 1 ng/m^3 (1 → 3)-β-D-glucan. Atopic subjects exposed to > 1 ng/m^3 (1 → 3)-β-D-glucan had significantly higher serum MPO. Serum ECP and CRP were also higher in these subjects but not significantly so.

Douwes et al. (1998) examined the relationship between exposure to (1 → 3)-β-D-glucan and endotoxins and peak expiratory flow (PEF) in children (ages 7 to 11 y) with and without chronic respiratory symptoms. The children were monitored twice a day for PEF variability. House dust samples from living room and bedroom floors and the children's mattresses were taken during the PEF monitoring period. As indicated by linear regression analysis (adjusting for dust mite allergen levels, the presence of pets, and the type of flooring in the home), peak expiratory flow variability in the children with chronic respiratory symptoms was strongly associated with (1→3)-β-D-glucan levels in dust from living room floors when expressed in micrograms per square meter. The association was strongest for atopic children with asthma.

7.4 PARTICULATE MATTER PATHOPHYSIOLOGY AND TOXICITY: IN VITRO EXPOSURES

7.4.1 Introduction

Toxicological studies play an integral role in providing evidence by which to evaluate the biological plausibility of health effects associations with ambient PM exposure observed in epidemiologic studies. At the time of 1996 PM AQCD (U.S. Environmental Protection Agency, 1996a) little was known about potential mechanisms that could explain the morbidity and mortality observed in human populations exposed to ambient airborne PM. One of the difficulties in trying to sort out possible mechanisms is the nature of ambient PM mixes. Ambient PM has diverse physicochemical properties (Table 7-8) ranging from physical characteristics of the particles to chemical components in or on the surface of the particles. Any one of these properties could change at any time in the ambient exposure atmosphere, making it hard to replicate the actual properties in a controlled experiment. As a result, controlled exposure studies have not as yet been able to clearly identify those particle properties and specific mechanisms by which ambient PM may affect biological systems. Despite these underlying difficulties, a number of toxicological studies have become available since 1996 to help explain how ambient particles may exert toxic effects on the respiratory and cardiovascular systems. The following section discusses the more recently published studies that provide an approach toward identifying potential mechanisms by which PM mediates health effects. The remaining sections discuss potential mechanisms in relation to PM characteristics based on these available data.

7.4.2 Experimental Exposure Data

In vitro exposure is a useful technique by which to obtain information on potentially hazardous PM constituents and mechanisms of PM injury, especially when only limited amounts of PM test material are available. Respiratory epithelial cells lining the airway lumen have been featured in numerous studies involving airborne pollutants and show inflammatory responses similar to that of human primary epithelial cultures. Limitations of in vitro studies include possible alterations in physiochemcial characteristics of PM because of the collection and resuspension processes, exposure conditions that do not fully simulate air-cell interface conditions within the lungs, and difficulties in estimating comparable dosage delivered to target

TABLE 7-8. EXAMPLES OF IMPORTANT PHYSICOCHEMICAL PROPERTIES OF PARTICLES OFTEN FOUND IN AMBIENT AEROSOLS

Physical Characteristics	Chemical Components
• particle mass (size, shape, density)	• sulfates
• particle number	• nitrates
• surface area	• elemental and organic carbon
• surface chemistry	• semivolatile organics
• surface charge	• metals (Fe, Cd, Co, Cu, Mn, Ni, Pb, Ti, V, Zn)
• acidity	• biologicals (e.g., pollen, fungi, microbes)

cells in vivo. Also, doses delivered in vitro, like intratracheal administration, are very high on a cellular basis, thus requiring much caution in attempting to extrapolate the in vitro findings to in vivo exposure conditions. It would be useful if in vitro studies included, in addition to the high doses, doses comparable to environmental doses predicted to occur at the cellular level under in vivo conditions. Even with these limitations, however, in vitro studies do provide an approach to by which to explore potential cellular and molecular mechanisms by which PM mediates health effects, allowing mechanisms identified in vitro to later be evaluated in vivo.

7.4.2.1 Ambient Particles

Numerous newly available studies have exposed airway epithelial cells, alveolar macrophages, or blood monocytes and erythrocytes to aqueous extracts of ambient PM to investigate cellular processes, e.g., oxidant generation and cytokine production, that may contribute to pathophysiological responses seen in vivo. In vitro studies published since the 1996 PM AQCD are summarized in Table 7-9. Types of ambient PM examined include samples collected from: Boston, MA (Goldsmith et al., 1998); North Provo, UT (Ghio et al., 1999a,b); St. Louis, MO (SRM 1648, Dong et al., 1996; Becker and Soukup, 1998); Washington, DC (SRM 1649, Becker and Soukup, 1998); Ottawa, Canada (EHC-93, Becker and Soukup, 1998); Dusseldorf and Duisburg, Germany (Hitzfeld et al., 1997), Mexico City (Bonner et al., 1998), Terni, Italy (Fabiani et al., 1997); and Rome, Italy (Diociaiuti et al., 2001).

TABLE 7-9. IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Human bronchial epithelial cells, asthmatic (ASTH) nonasthmatic (NONA)	DPM		10-100 µg/mL	0.4 µm	2, 4, 6, 24 h	DPM caused no gross cellular damage. Ciliary beat frequency was attentuated at all doses. DPM caused IL-8 release at 10 µg/m^3 in ASTH and at 50 µg/mL in NONA. Higher concentrations (50 and 100 µg/mL) DPM suppressed IL-8, and GM-CSF, in ASTH cells.	Bayram et al. (1998a)
Human bronchial epithelial cells (smokers)	DPM		10-100 µg/mL in culture medium 50 µg/mL filtered solution	0.4 µm	24 h	DPM attenuated ciliary beating. Release of IL-8 protein increased by exposure to ≥ 50 µg/mL DPM in culture medium, but 10-fold higher increase by DPM filtered solution. GM-CSF and CAM-1 increased after 50-100 µg/mL.	Bayram et al. (1998b)
Human and rat AM	Four Urban air particles (UAP): ROFA DPM Volcanic ash Silica	2.5×10^5 cells/mL	Urban and DPM: 12, 27, 111, 333, or 1000 µg/mL SiO_2 and TiO_2: 4, 12, 35, 167, or 500 µg/mL Fe_2O_3: 1:1, 3:1; 10:1 particles/cell ratio	Urban particles: 0.3-0.4 µm DPM: 0.3 µm ROFA: 0.5 µm Volcanic ash: 1.8 µm Silica: 05-10 µm TiO_2: < 5 µm Latex: 3.8 µm	2 h for cytotoxicity, 16-18 h for cytokine assay; chemiluminescence at 30 minutes	UAP-induced cytokine production (TNF, IL-6) in AM of both species that is not related to respiratory burst or transition metals but may be related to LPS (blocked by polymyxin B but not DEF). The effects were seen in human AM at concentrations of ≥ 56 µg/mL and in rat AM at all exposures. ROFA induced strong chemiluminescence (all conc. in humans and ≥ 35 µg/mL in rats) but had no effects on TNF production.	Becker et al. (1996)
Human AM and blood monocytes M and F 20 - 35 yr	Urban air particles (UAP): St. Louis SRM 1648; Washington, DC, SRM 1649; Ottawa, Canada, EHC-93	2×10^5 cells/mL	33 or 100 µg/mL	0.2 to 0.7 µm	3, 6, or 18-20 h	Phagocytosis was inhibited by exposure to 100 µg/mL UAP for 18 h. UAP caused decreased expression of β_2-integrins involved in antigen presentation and phagocytosis in the AMs exposed to 100 µg/mL.	Becker and Soukup (1998)
Rat AM	PM_{10} Mexico City 1993; volcanic ash (MSHA)		10 µg/cm^2	< 10 µm	24 h	PM_{10} stimulated alveolar macrophages to induce up-regulation of PDGF ∝ receptor on myofiboroblasts. Endotoxin and metal components of PM_{10} stimulate release of IL-β. This is a possible mechanism for PM_{10}-induced airway remodeling.	Bonner et al. (1998)

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
NHBE cells	ROFA		0, 5, 50, or 200 µg/mL (actual dose delivered 1.6 – 60 µg/cm^2)	< 10 µm	2 or 24 h	Increase in expression of the cytokines IL-6 and IL-8 at all exposure concentrations; TNF-α increased at ≥ 50 µg/mL; inhibition by DMTU or deferoxamine.	Carter et al. (1997)
Human erythrocytes; mouse monocyte-macrophage cell RAW 264.7	$PM_{10-2.5}$; $PM_{2.5}$ from Rome, Italy	1 × 10^6 cells/mL	50 ± 45 µg/m^3 31 ± 24 µg/m^3 19 ± 20 µg/m^3	PM_{10} $PM_{2.5}$ $PM_{10-2.5}$	1 h 24 h	Oxidative stress on cell membranes is related to PM surface per volume unit of suspension; $PM_{2.5}$ caused dose-dependent decrease in viability and increased markers of inflammation.	Diociaiuti et al. (2001)
Supercoiled DNA	PM_{10} from Edinburgh, Scotland		996.2 ± 181.8 µg/filter in 100 µL	PM_{10}	8 h	PM_{10} caused damage to DNA; mediated by hydroxyl radicals (inhibited by mannitol) and iron (inhibited by DEF). Clear supernatant has all of the suspension activity. Free radical activity is derived either from a fraction that is not centrifugeable on a bench centrifuge or that the radical generating system is released into solution.	Donaldson et al. (1997)
Rat AM	UAP (St. Louis) DPM	1 × 10^6 or 3 × 10^6 cells/mL	25 to 200 µg/mL	DPM: 1.1 – 1.3 µm	2 h incubation; supernatant collected following 18 h of culture	Dose-dependent increase in TNF-α, IL-6, CINC, MIP-2 gene expression by UAP but not DPM TNF-α increase at all doses with peak at 200 µg/mL). Cytokine production not related to ROS; cytokine production inhibited by polymyxin B; LPS detected on UAP but not DPM. Endotoxin responsible for cytokine gene expression induced by UAP in AM. Increase in gene expression determined semi-quantitatively.	Dong et al. (1996)
Primary cultures of RTE	ROFA	3 × 10^4 cells/cm^2	30 µg/cm^2	1.95 µm MMAD	6 h	No gross alterations in cellular morphology, adhesion, or cytotoxicity.	Dye et al. (1997)
			5, 10, or 20 µg/cm^2		24 h	Dose-dependent particle induced epithelial cell detachment and lytic cell injury; alterations in the permeability of the cultured RTE cell layer; increase in LDH, G-6-PDH, gluathione reductase, glutathione S-transferase; mechanism of ROFA-induced RTE cytotoxicity and pulmonary cellular inflammation involves the development of an oxidative burden. Effects seen at all exposure levels.	

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Primary cultures of RTE	ROFA; metal solutions		5, 10, or 20 µg/cm^2	1.95 µm MMAD	Analysis at 24 h	ROFA, V, or Ni + V (at ≥10 µg/cm^2), but not Fe or Ni, increased epithelial permeability, decreased cellular glutathione, cell detachment, and lytic cell injury; treatment with DMTU inhibited expression of MIP-2 and IL-6 genes.	Dye et al. (1999)
Peripheral blood monocytes	Organic extract of TSP, Italy	1×10^4 cells/mL	5.3, 10.6, 21.2, 42.5, 85, 340 µg residue/m^3 (acetone)	N/A, collected from high-volume sampler (60 m^3/h)	2 h	Superoxide anion generation was inhibited at a particulate concentration of 0.17 mg/mL (340 µg) when stimulated with PMA; dose-dependent increase in LDH; at 0.17 mg/mL LDH increased 50%; disintegration of plasma membrane.	Fabiani et al. (1997)
BEAS-2B	Provo PM_{10} extract		125, 250, 500 µg/mL	PM_{10}	2 and 24 h	Dose-dependent increase in IL-6 and IL-8 induced at all doses after 24 h for cells by particles collected while steel mill in operation (years 1 and 3). Increase noted for year 2 for particles taken during plant closure, but not dose-dependent; and particles collected during plant closure had the lowest concentrations of soluble Fe, Cu, and Zn. Cytotoxicity seen at 500 µg/mL.	Frampton et al. (1999)
Rat AM	ROFA, iron sulfate, nickel sulfate, vanadyl sulfate Latex particles with metal complexed on the surface	$0.5 – 1.0 \times 10^6$ cells/mL	0.01–1.0 mg/mL	3.6 µm MMAD (dust) 0.945 µm (latex beads)	Up to 400 min	At all concentrations, increased chemiluminescence, inhibited by DEF and hydroxyl radical scavengers; solutions of metal sulfates and metal-complexed latex particles similarly elevated chemiluminescence. Effects were generally dose-dependent, with largest dose creating effects over the shortest period of exposure.	Ghio et al. (1997a)
NHBE BEAS-2B	ROFA		5, 50, 200 µg/mL	3.6 µm	2 and 24 h	mRNA for ferritin did not change; ferritin protein increase at ≥ 50 µg/mL; mRNA for transferrin receptor decreased at ≥50 µg/mL; mRNA for lactoferrin increased; transferrin decreased at ≥ 50 µg/mL, whereas lactoferrin increased at ≥ 50; deferoxamine alone increased lactoferrin mRNA; effects significant for two highest exposure following 24 h exposure.	Ghio et al. (1998c)
BEAS-2B respiratory epithelial cells	ROFA		100 µg/mL	3.6 µm	5 min – 1 h	Lactoferrin binding with PM metal occurred within 5 min. V and Fe $^{(III)}$, but not Ni, increased the concentration of lactoferrin receptor.	Ghio et al. (1999b)
BEAS-2B	Provo TSP soluble and insoluble extract		500 µg/mL	TSP	24 h	Water soluble fraction caused greater release of IL-than insoluble fraction. The effect was blocked by deferoxamine and presumably because of metals (Fe, Cu, Zn, Pb).	Ghio et al. (1999a)

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
ØX174 RF1 DNA	PM_{10} from Edinburgh, Scotland		3.7 or 7.5 µg/assay	PM_{10}	8 h	Significant free radical activity on degrading supercoiled DNA at both concentrations; mainly because of hydroxyl radicals (inhibited by mannitol); Fe involvement (DEF-B conferred protection); more Fe^{3+} was released compared to Fe^{2+}, especially at pH 4.6 than at 7.2.	Gilmour et al. (1996)
Hamster AM	ROFA or CAPs	0.5×10^6 cells/mL	ROFA: 0, 25, 50, 100, or 200 µg/mL CAPs: 1:5, 1:10, 1:20 (described as 4, 10, 20 µg/mL)	CAPs: 0.1–2.5 µm (from Harvard concentrator) TiO_2: 1 µm	30 min incubation, analysis immediately following	Dose-dependent increase in AM oxidant stress with both ROFA and CAPs (at 4 µg/mL). Increase in particle uptake; Mac-type SR mediate a substantial proportion of AM binding; particle-associated components (e.g., transition metals) are likely to mediate intracellular oxidant stress and proinflammatory activation.	Goldsmith et al. (1997)
Hamster AM Mouse AM	CAPs, ROFA, and their water-soluble and particulate fractions	0.5×10^6 cells/mL	ROFA: 25, 50, 100, 200 µg/mL, 50 and 250 µg/mL, and 100, 200, 400 µg/mL CAPs: 38-180 µg/mL	CAPs = 0.1-2.5 µm ROFA = 1.0 µm	30 min	ROFA (particles –50, 100, and 200 µg/mL and water soluble components –200 µg/mL only dose tested) and CAPs (all doses for particulate fraction and 150-180 µg/mL for soluble fraction - only dose tested) caused increases in DCFH oxidation; CAPs samples and components showed substantial day-to-day variability in their oxidant effects; ROFA increased MIP-2 in hamster AMs exposed to 50 or 250 µg/mL and TNF-α production in mouse AM exposed to 100, 200, 400 µg/mL. Effects inhibited by NAC.	Goldsmith et al. (1998)
AMs from female CD rats	Vanadyl chloride sodium metavanadate	$2 – 2.5 \times 10^6$ cells/mL	10-1000 µM metavanadate 0.5 and 0.78 mg/mL (aqueous extracts) dust mL	N/A	30 min	Metavanadate caused increased production of ROS. The LOEL was 50 µM.	Grabowski et al. (1999)
Human PMN	Aqueous and organic extracts of TSP in Dusseldorf and Duisburg, Germany	1×10^6 cells/mL	0.51 and 0.78 mg/mL (aqueous extracts) 0.03 – 0.08 µg/mL (organic extracts)	Collected by high volume sampler, 90% < 5 µm, 50% < 1µm, maximum at 0.3-0.45 µm Extracted using water and then dichloromethane to yield aqueous and organic extracts	Up to 35 min	PM extract alone significantly stimulated the production and release of ROS in resting but not in zymosan-stimulated PMN. The effects of the PM extracts were inhibited by SOD, catalase and sodium azide (NaN_3); Zymosan-induced LCL is inhibited by both types of extracts, but aqueous extracts have a stronger inhibitory effect. Phagocytosis is not affected.	Hitzfeld et al. (1997)

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Human AM	UAP (#1648, 1649) Volcanic ash ROFA	1×10^6 cells/mL	0, 25, 100, or 200 µg/mL	Volume median diameter: ROFA 1.1 µm #1648: 1.4 µm #1649: 1.1 µm volcanic ash 2.3 µm	24 h	ROFA highly toxic; urban PM toxic at 200 µg/mL; ROFA produced significant apoptosis as low as 25 µg/mL; UAP produced apoptosis at 100 µg/mL; UAP and ROFA also affect AM phenotype: increased immune stimulatory, whereas decreased immune suppressor phenotype.	Holian et al. (1998)
Primary GPTE cells	ROFA DOFA STL WDC OT MSH	$2 – 5 \times 10^5$ cells/cm^2	6.25, 12.5, 25, and 50 µg/cm^2	N/A	4, 8, and 24 h	ROFA was the most toxic particle (effects seen at 12.5 µg/cm^2), enhancing mucin secretion at 50 µg/cm^2 and causing toxicity, assessed by LDH release at ≥ 25 µg/cm^2. DOFA produced significant effect at 25 µg/cm^2. Several other particles toxic at highest exposure dose for 24 h.	Jiang et al. (2000)
BEAS-2B	TSP collected in Provo	2×10^5 cells/mL	TSP filter samples (36.5 mg/mL) agitated in deionized H_2O_2 for 96 h, centrifuged at 1200 g for 30 min, lyophylized and resuspended in deionized H_2O_2 or saline	N/A (TSP samples, comprised 50 to 60% PM_{10})	Sacrificed at 24 h	Provo particles caused cytokine-induced neutrophil-chemoattractant-dependent inflammation of rat lungs; Provo particles stimulated IL-6 at 500 µg/mL and IL-8 at ≥ 200 µg/mL, increased IL-8 mRNA at 500 µg/mL and ICAM-1 at 100 µg/mL in BEAS-2B cells, and stimulated IL-8 secretion at ≥ 125 µg/mL in primary cultures of BEAS-2B cells; cytokine secretion was preceded by activation of NF-κB and was reduced by SOD, DEF, or NAC; quantities of Cu^{2+} found in Provo particles replicated the effects	Kennedy et al. (1998)
Human lung mucoepidermoid carcinoma cell line, NCI-H292	ROFA	1×10^6 cells/mL	10, 30, 100 µg/mL	N/A	6 and 24 h	Epithelial cells secreted increased mucin at ≥ 10 µg/mL and lysozyme ≥ 30 µg/mL; effect time- and concentration-dependent; effects significant for mucin at the lowest exposure dose for both exposure periods; effects on lysozyme only significant at highest dose for 6 h exposure and two highest doses for 24 h exposure; caused by V-rich fraction (18.8%).	Longphre et al. (2000)
BEAS-2B	ROFA	5×10^6 cells/mL	0, 0.5, or 2.0 mg in 10 mL	1.95 µm	1 h	ROFA induced production of acetaldehyde in dose-dependent fashion. No effects on cell viability.	Madden et al. (1999)
Male (Wistar) rat lung macrophages	Urban dust SRM 1649, TiO_2, quartz	2×10^5 cells/mL	0-100 µg/mL	0.3 – 0.6 µm	18 h	Cytotoxicity ranking was quartz > SRM 1649 > TiO_2, based on cellular ATP decrease and LDH, acid phosphatase, and β-glucuronidase release. Effects were noted at the lowest exposure dose.	Nadeau et al. (1996)

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Human blood monocytes and neutrophils (PMN)	Ambient air particles, carbon black, oil fly ash, coal fly ash	2×10^5 cells/ 0.2 mL	100 µg 25, 50, 100, 150, 200 µg	N/A	40 min.	ROS generation, measured by LCL increase in PMN and monocytes; PMN effects were correlated with Si, Fe, Mn, Ti, and Co content but not V, Cr, Ni, and Cu. Deferoxamine, a metal ion-chelator, and did not affect LCL in PMN, suggesting that metal ions are not related to the induction of LCL. Effects were generally dose-dependent with effects seen at lowest dose.	Prahalad et al. (1999)
BEAS-2B	ROFA		0, 6, 12, 25, or 50 µg/mL	1.96 µm	1 to 24 h	Transient activation at 50 µg/mL of IL-6 gene by NF-κB activation and binding to specific sequences in promoter of IL-6 gene at all dose levels; inhibition of NF-κB activation by DEF and NAC; activation NF-B may be a critical first step in the inflammatory cascade following exposure to ROFA particles.	Quay et al. (1998)
BEAS-2B	ROFA		2, 20, or 60 µg/cm^2	1.96 µm	2 or 24-h exposure	Epithelial cells exposed to ROFA at ≥ 20 µg/cm^2 for 24 h secreted substantially increased amounts of the PHS products prostaglandins E_2 and $F_{2\alpha}$; ROFA-induced increase in prostaglandin synthesis was correlated with a marked increase in PHS activity.	Samet et al. (1996)
BEAS-2B	ROFA Synthetic ROFA (soluble Ni, Fe, and V)		ROFA: 0–200 µg/mL Synthetic ROFA (100 µg/mL): Ni, 64 µM Fe, 63 µM V, 370 mM	ROFA: 1.96 µm Synthetic ROFA: N/A (soluble)	5 min to 24 h	Tyrosine phosphatase activity, which was known to be inhibited by vanadium ions, was markedly diminished after ROFA treatment at ≥ 50 µg/mL; effects were dose- and time-dependent; ROFA exposure induces vanadium ion-mediated inhibition of tyrosine phosphatase activity, leading to accumulation of protein phosphotyrosines in cells.	Samet et al. (1997)
Human airway epithelium-derived cell lines BEAS-2B	Particle components As, Cr, Cu, Fe, Ni, V, and Zn		500 µM of As, F, Cr (III), Cu, V, Zn	N/A (soluble)	20 min; analyses conducted 6 and 24 h following exposure	Noncytotoxic concentrations of As, V, and Zn induced a rapid phosphorylation of MAPK in cells; activity assays confirmed marked activation of ERK, JNK, and P38 in cells exposed to As, V, and Zn. Cr and Cu exposure resulted in a relatively small activation of MAPK, whereas Fe and Ni did not activate MAPK under these conditions; the transcription factors c-Jun and ATF-2, substrates of JNK and P38, respectively, were markedly phosphorylated in cells treated with As, Cr, Cu, V, and Zn; acute exposure to As, V, or Zn that activated MAPK was sufficient to induce a subsequent increase in IL-8 protein expression in cells. Most effects seen by 6 h postexposure.	Samet et al. (1998)

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Human lung epithelial (A549) cells ØX174 RFI DNA	Urban particles: SRM 1648, St. Louis SRM 1649, Washington, DC	20,000 cells/cm^2	100 µg/cm^2 for Fe mobilization assay	SRM 1648: 50% < 10 µm SRM 1649: 30% < 10 µm	Up to 25 h	Single-strand breaks in DNA were induced by PM only in the presence of ascorbate, and correlated with amount of Fe that can be mobilized; ferritin in A549 cells was increased with treatment of PM suggesting mobilization of Fe in the cultured cells.	Smith and Aust (1997)
Human AMs	Provo PM_{10} extract	2×10^5 cells/mL	500 µg	PM_{10}	24 h	AM phagocytosis of (FITC)-labeled Saccharomyces cerevisiae inhibited 30% by particles collected before steel mill closure.	Soukup et al. (2000)
Human AMs	Chapel Hill PM extract; both H_20 soluble(s) and insoluble(is)	2×10^7 cells/mL	100 µg/mL	$PM_{2.5}$ $PM_{10\text{-}2.5}$	24 h	Increased cytokine production (IL-6, TNFα, MCP-1); $isPM_{10} > sPM_{10} > isPM_{2.5}$; $sPM_{2.5}$ was inactive; endotoxin was partially responsible.	Soukup and Becker (2001)
Rat (Wistar) AM RAM cells (a rat AM cell line)	TiO_2	1×10^6 cells/mL	20, 50, or 80 µg/mL	N/A	4 h	Opsonization of TiO_2 with surfactant components resulted in a modest dose-dependent increase in AM uptake compared with that of unopsonized TiO_2 at ≥ 50 µg/mL; surfactant components increase AM phagocytosis of particles.	Stringer and Kobzik (1996)
Human lung epithelial (A549) cells	ROFA, α-quartz, TiO_2	2.5×10^5 cells/mL	25-200 µg/mL	N/A	60 min	Exposure of A549 cells to ROFA at ≥ 50 µg/mL, α-quartz at ≥ 25 µg/mL, but not TiO_2, caused increased IL-8 production in TNF-α primed cells.	Stringer and Kobzik (1998)
Human lung epithelial (A549) cells	TiO_2, Fe_2O_3, CAP, and the fibrogenic particle α-quartz	3×10^5 cells/mL	TiO_2 [40 µg/mL], Fe_2O_3 [100 µg/mL], α-quartz [200 µg/mL], or CAP [40 µg/mL]	N/A	24 h	$TiO_2 > Fe_2O_3 >$ α-quartz > CAP in particle binding; binding of particle was found to be calcium-dependent for TiO_2 and Fe_2O_3, while α-quartz binding was calcium-independent; scavenger receptor, mediate particulate binding; α-quartz caused a dose-dependent production of IL-8 (≥ 26.6 µg/cm^2). Il-8 was not present in TiO_2 and CAPs treated cells.	Stringer et al. (1996)
RLE-6TN cells (type II like cell line)	$PM_{2.5}$, Burlington, VT; Fine/ultrafine TiO_2	1×10^6 cells/mL	α-quartz, [0-200 µg/mL] 1, 2.5, 5, 10 µg/mL $PM_{2.5}$, or up to 5 µg/mL TiO_2	$PM_{2.5}$: 39 nm Fine TiO_2: 159 nm UF TiO_2: 37 nm	24 and 48 h exposure	PM increases in c-Jun kinase activity at ≥ 10 µg/mL, levels of phosphorylated c-Jun immunoreactive protein at ≥ 5 µg/mL; and transcriptional activation of activator protein-1-dependent gene expression; elevation in number of cells incorporating 5'-bromodeoxyuridine at ≥ 1 µg/mL.. UF TiO_2 increased c-Jun kinase activity compared to fine TiO_2.	Timblin et al. (1998)

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Rat, Long Evans epithelial cells	CFA PFA α-quartz.	1×10^4 cells/100 µL		1.5-3.0 µm 17.7 µm 2.5 µm	3 h	CFA produced highest level of hydroxyl radicals; no relationship between hydroxy/radical generation and CFA particle size, surface area, quartz, or iron content, but positive correlation noted with iron mobilization.	Van Maanen et al. (1999)
BEAS-2B	ROFA Birmingham, AL. 188 mg/g of VO		100 µg/mL	N/A	2-6 h	ROFA caused increased intracellular Ca^{++}, IL-6, IL-and TNF-α through activation of capsicin- and pH-sensitive receptors; effects seen at the lowest dose tested.	Veronesi et al. (1999a)
NHBE BEAS-2B	Utah Valley PM_{10} extract		50, 100, 200 µg/mL	PM_{10}	24 h	Dose-dependent increase in expression of IL-8 produced at ≥ 50 µg/mL by particles collected when the steel mill was in operation; effects seen at lowest dose tested.	Wu et al. (2001)
Human AM from smokers (mean age 68) and non-smokers (mean age 72), male and female	EHC-93 ROFA latex beads carbon particles	$0.5x10^6$ cells/ml	0.01-0.1 mg/ml	<10 µm 0.1,1, and 10 µm	2,4,8,12, and 24h (only 24 h data shown)	TNF α increased at 0.01 to 0.1 mg/ml EHC-93 and at 0.1 mg/ml latex, carbon and ROFA. EHC93 at 0.1 mg/ml increases levels of IL6, IL-1β, MIP-1 α, and GM-CSF	Van Eeden, et al. (2001)
Human AM from age 62± 5 smokers Rabbit AM 6 wk old	EHC-93	$0.5x10^6$ cells/ml	0.01-0.1 mg/ml	4-5 µm mass median diameter	2,4,8,12, and 24h (only 24 h data shown)	0.1 mg/ml produced significant increase in TNF α. Instillation of supernatants from human and rabbit PM-exposed AMs into the lungs of rabbits caused increases in circulating PMNs and circulating band cells and shortening the transit time of PNMs through mitotic and postmitotic bone narrow pools.	Mukae et al. (2000)
Rat AM and AM primed with LPS	$PM_{2.5}$ Indoor and outdoor	$1x10^6$ cells/ml	100 µg/ml	<2.5 µm	20h	Increased TNF production in both indoor and outdoor exposures. LPS-primed AMs had greater responses. Indoor $PM_{2.5}$ caused significantly more TNF production than outdoor $PM_{2.5}$.	Long et al. (2001)
Rat AM and AM primed with LPS	SRM1649, iron oxide, carbon black, diesel dust, Boston CAPS, separated in soluble and insoluble fractions	$2.4x10^6$ cells/ml	100 µg/ml	Fe, CV and DD all <1 µm, UAP was 30% larger	20h	Priming enhanced AM release of TNF and MIP-2 in response to UAP and some CAPs samples. CB, DD, Fe and others CAPs did not induce cytokines. Toxicity associated with insoluble fractions. The activation state of the AM determines which particle-associated components are most bioactive.	Imrich et al. (2000)

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Mouse AM	Boston CAPs	$1x10^6$ cells/ml	~5-120 μg/ml	≤2.5 μm	5h	Soluble and insoluble CAPs caused MIP-2 and TNF α production. Cytokine induction and endotoxin content was associated with the insoluble fraction. PB neutralization of endotoxin abrogated >80% of TNF α induction, but inhibited MIP-2 production by only approximately 40%.	Ning et al. (2000)
Rat AM	Switzerland PM collected during the four seasons.	$4x10^5$/ml		<10 μm	40h	All exposures produced significant toxicity in MTT assay. Spring and summer samples induced the most TNF α. Oxidative response was greatest in non-winter months.	Monn et al. (2003)
Mouse monocytes and mouse mesenchymal cells	PM from Northern and Southeastern Mexico City		20, 40 or 80 $\mu g/cm^2$	10 or 2.5 μm	24h	Southeastern PM_{10} had most endotoxins and induced the most TNF α and IL-6 at all doses. Cytokine release was reduced 50-75% by rENP. Northern Pms most cytotoxic.	Osornio-Vargas, et al. (2003)
Human AM from healthy males and females, 20-35 yrs old. CHO expressing CD14 and TLR2 or TLR4	EHC-93, Mt. St.Helens Volcanic Ash, ROFA, silica, PM from Chapel Hill, NC, bacteria collected from Chapel Hill ambient air	$2-3x10^5$ cells/ml	PM - 30 μg/ml; bacteria - 10^3 - $2x10^6$/tube	2.5-10 μm	overnight	Three times more gram+ bacteria were required to elicit the same level of cytokine induction as gram- bacteria. This induction was inhibited by anti-CD14 and required serum. TLR4 was involved in $PM_{25.-10}$ and gram- induced activation. TLR2 activation was induced by both gram + and - bacteria and by PM.	Becker et al. (2002)
Human AM	Urban PM from Netherlands	$3x10^5$ cells/ml	770 pg/ml 1781 pg/ml 20411 pg/ml	<0.1 μm 0.1-2.5 μm 2.5-10 μm	18-20 h	IL-6 levels induced by $PM_{2.5-10}$ were 10x higher than $PM_{0.1-2.5}$. Levels induced by $PM_{0.1-2.5}$ were 2-3x higher than $PM_{<0.1}$. Induction wws inhibited by antibody to CD14. Phagaocytosis of osonized yeast and yeast-induced oxidative burst were inhibited by larger PM. Larger PM decreased CD11b expression more.	Becker et al. (2003)

TABLE 7-9 (cont'd). IN VITRO EFFECTS OF PARTICULATE MATTER AND PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc.[a]	Particle or Constituent[b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Mouse monocyte-macrophage cell line RAW 264.7	PM from Taiwan		40 μg/ml	<2.5 μm 2.5-10 μm	16h	PM 2.5-10 had greater endotoxin content and greater TNFα production which was inhibited by polymyxinB.	Huang et al. (2002)

[a]Cell types: RTE = Rat tracheal epithelial cells; GPTE = Guinea pig tracheal epithelial cells; NHBE = Normal human bronchial epithelial; A549 = Human lung epithelial cell line.
[b]DEF = Deferoxamine
ROFA = Residual oil fly ash
UAP = Urban air particulates
TSP = Total suspended particles
CAP = Concentrated air particles
DOFA = Domestic oil fly ash
VO = Vanadate oxide
CFA = Coal fly ash
PFA = Pulverized fuel ash
TiO_2 = Titanium oxide

Because soluble metals from ROFA-like particles have been associated with biological effect and toxicity, several new studies have investigated whether the soluble components of ambient PM may have the same biological activities. Extracts of ambient PM samples collected from North Provo, UT, (during 1981 and 1982) were used to test whether the soluble components or ionizable metals, which accounted for approximately 0.1% of the mass, are responsible for the biological activity of the extracted PM components. Release of IL-8 from BEAS-2B cells, oxidant generation (thiobarbituric acid reactive products), and PMN influx in rats exposed to these samples correlated with sulfate content and the ionizable concentrations of metals in these PM extracts (Ghio et al., 1999a,b). In addition, these extracts stimulated IL-6 and IL-8 production as well as increased IL-8 mRNA and enhanced expression of intercellular adhesion molecule-1 (ICAM-1) in BEAS-2B cells (Kennedy et al., 1998). Cytokine secretion was preceded by activation of nuclear factor kappa B (NF-κB) and was reduced by treatment with superoxide dismutase (SOD), Deferoxamine (DEF), or N-acetylcysteine. The addition of similar quantities of Cu^{+2} as found in the Provo extract replicated the biological effects observed with particles alone. When normal constituents of airway lining fluid (mucin or ceruloplasmin) were added to BEAS cells, particulate-induced secretion of IL-8 was modified. Mucin reduced IL-8 secretion; whereas ceruloplasmin significantly increased IL-8 secretion and activation of NF-κB. The authors suggest that copper ions may cause some of the biologic effects of inhaled PM in the Provo region and may provide an explanation for the sensitivity of asthmatics to Provo PM seen in epidemiologic studies.

Molinelli et al. (2002) also exposed human airway epithelial cell line (BEAS-2B) cultures for 24 h to an aqueous extract of PM collected in the Utah Valley. A portion of the extract was treated with Chelex, an agent that removes transition metals from solution. Cells incubated with the untreated extract showed a significant concentration-dependent increase in the inflammatory mediator interleukin-8 (IL-8) when compared to the control cells. However, cells incubated with Chelex-treated extract produced no change (relative to control) in IL-8. They exposed rats in vivo for 24 h to the same treatments as the in vitro cells and found significant increases in lactate dehydrogenase (LDH) and total protein in the rats exposed to the untreated extract and to the Chelex-treated extract with metals added back to achieve original concentrations. There was an attenuation of the observed LDH and total protein increases in the rats instilled with the Chelex-treated extract. The authors concluded that removal of metal cations attenuates cellular

responses to the aqueous extract and suggest a role for transition metal involvement in PM-associated increases in morbidity and mortality.

Frampton et al. (1999) examined the effects of the same ambient PM samples collected from Utah Valley in the late 1980s (see Section 7.2.1). Aqueous extracts of the filters were analyzed for metal and oxidant production and added to cultures of human respiratory epithelial cells (BEAS-2B) for 2 or 24 h. Particles collected in 1987, when the steel mill was closed had the lowest concentrations of soluble iron, copper, and zinc and showed the least oxidant generation. Ambient PM collected before and after plant closing induced expression of IL-6 and IL-8 in a dose-response relationship (125, 250, and 500 μg/mL). Ambient PM collected after reopening of the steel mill also caused cytotoxicity, as demonstrated by microscopy and LDH release at the highest concentration used (500 μg/mL).

Soukup et al. (2000) used similar ambient PM extracts as Frampton et al. (1999) to examine effects on human alveolar macrophages (AM). The phagocytic activity and oxidative response of AMs was measured after segmental instillation of aqueous extracts from the Utah Valley or after overnight in vitro cell culture. Ambient PM collected before closure of the steel mill inhibited AM phagocytosis of (FITC)-labeled *Saccharomyces cerevisiae* by 30%; no significant effect on phagocytosis was seen with the other two extracts. Furthermore, although extracts of ambient PM collected before and after plant closure inhibited oxidant activity of AMs when incubated overnight in cell culture, only the former particles caused an immediate oxidative response in AMs. Host defense effects were attributed to apoptosis which was most evident in particles collected before plant closure. Interpretation of loss of these effects by chelation removal of the metals was complicated by the observed differences in apoptosis despite similar metal contents of ambient PM collected during the steel mill operation.

Wu et al. (2001) investigated intracellular signaling mechanisms related to pulmonary responses to Utah Valley PM extracts. Human primary airway epithelial cells were exposed to aqueous extracts of PM collected from the year before, during, and after the steel mill closure in Utah Valley. Transfection with kinase-deficient extracellular signal-regulated kinase (ERK) constructs partially blocked the PM-induced interleukin (IL)-8 promoter reporter activity. The mitogen-activated protein kinase/ERK kinase (MEK) activity inhibitor PD-98059 significantly abolished IL-8 released in response to the PM, as did the epidermal growth factor (EGF) receptor kinase inhibitor AG-1478. Western blotting showed that the PM-induced

phosphorylation of EGF receptor tyrosine, MEK1/2, and ERK1/2 could be ablated with AG-1478 or PD-98059. The results indicate that the potency of Utah Valley PM collected during plant closure was lower than that collected while the steel mill was in operation and imply that Utah Valley PM can induce IL-8 expression partially through the activation of the EGF receptor signaling.

There are regional as well as daily variations in the composition of ambient PM and, hence, its biological activities. For example, concentrated ambient PM (CAP), from Boston urban air has substantial day-to-day variability in its composition and oxidant effects (Goldsmith et al., 1998). Similar to Utah PM, the water-soluble component of Boston CAPs significantly increased AM oxidant production and inflammatory cytokine (MIP2 and TNFα) production over negative control values. These effects could be blocked by metal chelators or antioxidants, suggesting important roles for metals in contributing to the observed Boston particle effects on AM function.

Becker and Soukup (1998) found interesting differences between biological activity of PM materials drawn from urban air particle (UAP) sources (baghouse collection in St. Louis and Ottawa), ROFA samples from a power plant, and Mt. St. Helens volcanic ash (VA) stored since 1980. Exposure of human alveolar macrophages (AM) and blood-derived monocytes (MO) to 100 µg/ml of UAP (0.2 to 0.7 µm MMAD originally) from both Boston and St. Louis reduced expression of certain receptors (important for recognition of microbial entities), the phagocytosis of bioparticles (yeast cell walls), and oxidant generation (an important bactericidal mechanism) in both AM and MO. All of these were little affected at 33 µg/ml of UAP. Exposure to 100 µg/ml of ROFA (0.5 µm MMAD originally) also significantly decreased AM (but not MO) phagocytosis (likely due to ROFA cytotoxic effects on AM), but VA had little effect on phagocytosis. The oxidative burst response was significantly decreased by ROFA in both AM and MO, but only in AM by VA. Administration of 10 mg/ml of lipopolysaccaride (LPS), the active endotoxin component, reduced AM receptor expression similar to UAP, but did not reduce all the same receptor expression as UAP in MO. The authors noted that their results indicated (a) differences in biological activity between urban air-related particles (both baghouse collected and ROFA) and the more inert Mt. St. Helens volcanic ash particles (that had little effect on any of the receptors or phagocytosis functions studied); and (b) that UAP endotoxin content may be an important effector in UAP-modulation of some, but certainly not all,

macrophage functions. The findings of Dong et al. (1996) also suggest that biological activity of some ambient PM materials may result from the presence of endotoxin on the particles. Using the same urban particles (SRM 1648), cytokine production (TNF-α, IL-1, Il-6, CINC, and MIP-2) was increased in macrophages following treatment with 50 to 200 μg/mL of urban PM (Dong et al., 1996). The urban particle-induced TNF-α secretion was abrogated completely by treatment with polymyxin B (an antibiotic that blocks LPS-associated activities), but not by antioxidants.

The potential involvement of endotoxin, at least partially, in some PM-induced biological effects has been explored further by Bonner et al. (1998) and Soukup and Becker (2001). Bonner et al. (1998) used urban PM_{10} collected from north, south, and central regions of Mexico City with SD rat AM to examine PM effects on platelet-derived growth factor (PDGF) receptors on lung myofibroblasts. Mexico City PM_{10} (but not volcanic ash) stimulated secretion of upregulatory factors for the PDGF α receptor, possibly via IL-1β. In the presence of an endotoxin-neutralizing protein, the Mexico City PM_{10} effect on PDGF was blocked partially, suggesting that LPS was partly responsible for the PM_{10} effect. In addition, both LPS and vanadium (both present in the PM_{10}) acted directly on lung myofibroblasts, even though the ambient vanadium levels in Mexico City PM_{10} were probably not high enough to exert an independent effect. The authors concluded that PM_{10} exposure could lead to airway remodeling by enhancing myofibroblast replication and chemotaxis.

Soukup and Becker (2001) used a dichotomous sampler to collect fresh $PM_{2.5}$ and $PM_{10-2.5}$ from the ambient air of Chapel Hill, NC, and compared the activity of these two particle size fractions. Both water soluble and insoluble components were assessed for cytokine production, inhibition of phagocytosis, and induction of apoptosis. The insoluble $PM_{10-2.5}$ fraction was the most potent in terms of inducing cytokines and increasing oxidant generation, thus suggesting the importance of the coarse fraction in contributing to ambient PM health effects. Endotoxin appeared to be responsible for much of the cytokine production, whereas inhibition of phagocytosis was induced by other moieties in the coarse material. None of the activities were inhibited by the metal chelator deferoxamine.

In another study, the effects of water soluble as well as organic components (extracted in dichloromethane) of ambient PM were investigated by exposing human PMN to PM extracts (Hitzfeld et al., 1997). PM was collected with high-volume samplers in two German cities,

Dusseldorf and Duisburg; these sites have high traffic and high industrial emissions, respectively. Organic, but not aqueous, extracts of PM alone significantly stimulated production and release of ROS in resting human PMN. The effects of the PM extracts were inhibited by SOD, catalase, and sodium azide (NaN_3). Similarly, the organic fraction (extractable by acetone) of ambient PM from Terni, Italy, was shown to produce cytotoxicity, superoxide release in response to PMA and zymosan in peripheral monocytes (Fabiani et al., 1997).

Diociaiuti et al. (2001) compared the in vitro toxicity of coarse ($PM_{10-2.5}$) and fine ($PM_{2.5}$) particulate matter, collected in an urban area of Rome. The in vitro toxicity assays used included human red blood cell hemolysis, cell viability, and nitric oxide (NO) release in the RAW 264.7 macrophage cell line. There was a dose-dependent hemolysis in human erythrocytes when they were incubated with fine and coarse particles. The hemolytic potential was greater for the fine particles than for the coarse particles in equal mass concentration. However, when data were expressed in terms of PM surface area per volume of suspension, the hemolytic activity of the fine fraction was equal to the coarse fraction. This result suggested that the oxidative stress induced by PM on the cell membranes could be due mainly to the interaction between the particle surfaces and the cell membranes. Although RAW 264.7 cells challenged with fine and coarse particles showed decreased viability and an increased release of NO, a key inflammatory mediator, both effects were not dose-dependent in the tested concentration range. The fine particles were the most effective in inducing these effects when the data were expressed as mass concentration or as surface area per unit volume. The authors concluded that these differences in biological activity were due to the differing physicochemical nature of the particles.

7.4.2.2 Comparison of Ambient and Combustion-Related Surrogate Particles

In vitro toxicology studies utilizing alveolar macrophages as target cells (Imrich et al., 2000; Long et al., 2001; Ning et al., 2000; Mukae et al., 2000, 2001; Van Eeden et al., 2001) have found that urban air particles are much more potent for inducing cellular responses than individual combustion particles such as diesel and ROFA. Similar to the results described above in Section 7.5.2.1, these studies also show that when cytokine responses are measured, LPS/endotoxin is found to be responsible for most of the activity. Metals, on the other hand, do not seem to affect cytokine production, as confirmed by studies showing that ROFA does not induce macrophage cytokine production. These results are important because LPS is an

important component associated with both coarse and fine particles (Menetrez et al., 2001). In fact, in one study (Long et al., 2001), cytokine responses in the alveolar macrophages were correlated with LPS content and more LPS was found associated with indoor $PM_{2.5}$ than outdoor $PM_{2.5}$.

Imrich et al. (2000) found that, when mouse alveolar macrophages were stimulated with CAPs ($PM_{2.5}$), the resulting TNF responses could be inhibited by an endotoxin neutralizing agent [e.g., polymyxin-B (PB)]. Because the MIP-2 response (IL-8) was only partly inhibited by PB, however, the authors concluded that endotoxin primed AM cells to respond to other particle components. In a related study (Ning et al., 2000), the use of PB showed that particle-absorbed endotoxin in CAPs suspensions caused activation of normal (control) AMs, while other (nonendotoxin) components were predominantly responsible for the enhanced cytokine release observed for primed AMs incubated with CAPs. The non-LPS component was not identified in this study; however, the AM biological response did not correlate with any of several elements quantified within the insoluble CAPs samples (e.g., Al, Cd, Cr, Cu, Fe, Mg, Mn, Ni, S, Ti, V).

Van Eeden et al. (2001) compared ROFA, the atmospheric dust sample EHC-93, and different size latex particles for cytokine induction by human alveolar macrophages. The EHC-93 particles produced greater than 8-fold induction of various cytokines, including IL-1, TNF, GMCSF; the other particles induced these cytokines approximately 2-fold. Using the same EHC-93 particles, Mukae et al. (2000, 2001) found that inhalation exposure stimulated bone marrow band cell-granulocyte precursor production. They also found that the magnitude of the response was correlated with the amount of phagocytosis of the particles by alveolar macrophages. These results may indicate that macrophages produce factors which stimulate bone marrow, including IL-6 and GMCSF. In fact, alveolar macrophages exposed in vitro to these particles released cytokines; and when the supernatant of PM-stimulated macrophages was instilled into rabbits, the bone marrow was stimulated.

In a series of studies using the same ROFA samples, several in vitro experiments have investigated the biochemical and molecular mechanisms involved in ROFA induced cellular injury. Prostaglandin metabolism in cultured human airway epithelial cells (BEAS-2B and NHBE) exposed to ROFA was investigated by Samet et al. (1996). Epithelial cells exposed to 200 $\mu g/m^3$ ROFA for 24 h secreted substantially increased amounts of prostaglandins E2 and F2 α. The ROFA-induced increase in prostaglandin synthesis was correlated with a marked

increase in activity of the prostaglandin H synthase-2 (PHS-2) as well as mRNA coded for this enzyme. In contrast, expression of the PHS1 form of the enzyme was not affected by ROFA treatment of airway epithelial cells. These investigators further demonstrated that the ROFA induced a significant dose- and time-dependent increase in protein tyrosine phosphate, an important index of signal transduction activation leading to a broad spectrum of cellular responses. ROFA-induced increases in protein phosphotyrosines were associated with its soluble fraction and were mimicked by V-containing solutions but not iron or nickel solutions (Samet et al., 1997).

ROFA also stimulates respiratory cells to secrete inflammatory cytokines such as IL-6, IL-8, and TNF. Normal human bronchial epithelial (NHBE) cells exposed to ROFA produced significant amounts of IL-8, IL-6, and TNF, as well as mRNAs coding for these cytokines (Carter et al., 1997). Increases in cytokine production were dose-dependent. The cytokine production was inhibited by the addition of metal chelator, DEF, or the free radical scavenger dimethylthiourea (DMTU). Similar to the data of Samet et al. (1997), V but not Fe or Ni compounds were responsible for these effects. Cytotoxicity and decreased cellular glutathione levels in primary cultures of rat tracheal epithelial (RTE) cells exposed to suspensions of ROFA indicated that respiratory cells exposed to ROFA were under oxidative stress. Treatment with buthionine sulfoxamine (an inhibitor of γ-glutamyl cysteine synthetase) augmented ROFA-induced cytotoxicity; whereas treatment with DMTU that inhibited ROFA-induced cytoxicity further suggested that ROFA-induced cell injury may be mediated by hydroxyl-radical-like reactive oxygen species (ROS) (Dye et al., 1997). Using BEAS-2B cells, a time- and dose-dependent increase in IL-6 mRNA induced by ROFA was shown to be preceded by the activation of nuclear proteins, for example, nuclear factor-κB (NF-κB) (Quay et al., 1998). Taken together, exposure to ROFA in high doses increases oxidative stress, perturbs protein tyrosine phosphate homeostasis, activates NF-κB, and up-regulates inflammatory cytokine and prostaglandin synthesis and secretion to produce lung injury.

Stringer and Kobzik (1998) observed that “primed” lung epithelial cells exhibited enhanced cytokine responses to PM. Compared to normal cells, exposure of tumor necrosis factor (TNF)-α-primed A549 cells to ROFA or α -quartz caused increased IL-8 production in a concentration-dependent manner for particle concentrations ranging from 0 to 200 μg/mL. Addition of the antioxidant N-acetylcysteine (NAC) (1.0 mM) decreased ROFA and α -quartz-

mediated IL-8 production by approximately 50% in both normal and TNF-α-primed A549 cells. Exposure of A549 cells to ROFA caused an increase in oxidant levels that could be inhibited by NAC. These data suggest that (1) lung epithelial cells primed by inflammatory mediators show increased cytokine production after exposure to PM and (2) oxidant stress is an important mechanism for this response.

Osornio-Vargas et al. (2003) compared exposures of mouse monocytes to $PM_{2.5}$ or to PM_{10} collected in either southeastern or northern Mexico City, and characterized as to metal and endotoxin content. Tumor necrosis factor-α and IL-6 were measured from exposures both with and without recombinant endotoxin-neutralizing protein (rENP). The southeastern PM_{10} samples had the highest endotoxin levels, which correlated with greater cytokine secretion. rENP reduced cytokine secretion by 50-75%, suggesting to the authors that the fine PM utilized an endotoxin-independent, transition metal-dependent mechanism for cytotoxicity, whereas an endotoxin-dependent mechanism was responsible for the proinflammatory response in PM_{10}.

Rat AM exposed to PM_{10} collected from both rural and urban sites in Switzerland during all four seasons demonstrated increased cytotoxicity from all PM samples (Monn et al., 2003). TNF α and oxidative radical release were highest with PM collected during non-winter months. ENP inhibited cytotoxic effects and oxidative radical release, suggesting that endotoxin possibly modulates macrophage activity.

In central Taiwan, Huang et al. (2002) collected $PM_{2.5}$ and PM_{10} samples which were then exposed at 40 µg/ml to RAW 264.7 cells, a mouse monocyte-macrophage cell line. After a 6 hour exposure, either with or without polymyxin B, TNF-α levels were assayed. PM_{10}-exposed cells stimulated higher TNF-α secretion, and polymyxin B inhibited TNF-α by 42% and 32% in PM_{10} and $PM_{2.5}$ exposures, respectively, suggesting that endotoxin is a greater factor in TNF-α stimulation in larger particles.

Becker et al. (2002) hypothesized that PM activates receptors involved in recognition of microbial cell structures. They coated model pollution particles with either gram-negative (Pseudomonas) or gram-positive (Staphloccocus or Streptococcus) bacteria. Three times more gram+ bacteria were required to elicit the same level of cytokine induction as gram- bacteria. This inhibition was inhibited by anti-CD14 and required serum. This study further found a suggested role of Toll-like receptors (TLR) in PM recognition, thus implicating bacterial components as a factor in PM-induced inflammatory responses in AM.

Becker et al. (2003) exposed human AM to ultrafine (< 0.1 μm), fine ($PM_{0.1-2.5}$) or coarse ($PM_{2.5-10}$) particles collected in two urban sites in the Netherlands. IL-6 induction levels and reductions in CD11b phagocyte receptor expression were positively correlated with particle size. Induction of IL-6 was inhibited by an antibody to CD14. Yeast-induced oxidative burst and inhibition of phagocytosis of opsonized yeast was also correlated with size, with the ultrafine particles having no effect. The authors concluded that human AM recognize microbial cell structures, which are more prevalent in larger particles, and that exposure to PM is associated with inflammatory events and decreased pulmonary defenses.

In summary, exposure of lung epithelial cells to ambient PM or ROFA leads to increased production of cytokines and the effects may be mediated, at least in part, through production of ROS. Day-to-day variations in the components of PM, such as soluble transition metals (which may be critical to eliciting the response) are suggested. The involvement of organic components (e.g., endotoxins) in ambient PM was also suggested by some studies.

7.4.2.3 Mutagenicity/Genotoxicity Effects

The majority of newly-published PM research since the 1996 PM AQCD have focused on acute cardiopulmonary effects associated with short-term exposure to ambient PM or selected constituents. However, new epidemiologic analyses by Pope et al. (2002) not only substantiate associations between long-term exposure to ambient PM and increases in cardiopulmonary mortality but also provide the strongest evidence yet linking such PM exposures to lung cancer effects. A limited number of new in vitro studies have examined the mutagenic and/or other genotoxicity potential of ambient PM; and, in general, they have shown some degree of evidence that appears to support the biologic plausibility of lung cancer effects being causally related to long-term exposure to ambient PM, as implied by the epidemiologic findings. These in vitro studies, listed in Table 7-10, have focused mainly on the ability of the organic fraction of ambient PM to induce mutagenic effects in mammalian cell lines and bacteria.

The World Health Organization (1993) has found that the induction of sister chromatid exchanges (SCE) to be a sensitive cytogenic endpoint for the demonstration of genotoxic activity of environmental mutagens and carcinogens. In vitro SCE assays using various types of human or laboratory animal cells have been used in new studies, along with other techniques, to evaluate the genotoxic potential of ambient PM samples or ambient PM constituents.

TABLE 7-10. MUTAGENIC/CARCINOGENIC EFFECTS OF PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle or Constituent	Exposure Technique	Mass Concentration (µg/mL) or (µg/m3)	Particle Characteristics Size (µm); μ_g	Exposure Duration	Effects of Particles on Mammalian Cells or Bacteria	Reference
Cultured tracheal epithelial cells from Hamster, Syrian golden, young	Ambient PM	in vitro	Not given	Dichloromethane extraction of high volume samples.	Dilutions of extracted organic phase of particles incubated with cells for 48 hours.	Dose-related increases in sister chromatid exchanges were observed.	Hornberg et al. (1996)
Human bronchioepithelial cell line (BEAS-2B)	Ambient PM_{10} and $PM_{2.5}$ collected in industrial and rural regions	in vitro	Not given in µg/mL	Dichloromethane extraction of coarse (PM_{10}) and fine ($PM_{2.5}$) fractions.	Dilutions of extracted organic phase of size-segregated particles incubated with cells for 72 hours.	Significant increases in sister chromatid exchanges were greater in $PM_{2.5}$ from all sampling sites. Extraction phase of coarse particles produced fewer sister chromatid exchanges than did the fine particles.	Hornberg et al. (1998)
Kidney cells from hamster, Syrian golden, 8-10 weeks old	Ambient PM	in vitro	Not given	Dichloromethane extraction of high volume samples.	Dilutions of extracted organic phase of particles incubated with cells for 18 hours followed by infection with simian virus SV-40.	Significantly greater SV-40-induced transformation of hamster kidney cells pre-treated with organic extractions of urban particles.	Seemayer and Hornberg (1998)
Cultured hepatoma cells	Ambient PM	in vitro	Not given	Acetone/dichloromethane extraction of high volume samples.	Dilutions of extracted organic phase of particles incubated with cells for 6 or 48 hours.	Extracts of ambient PM both upwind and downwind of highway have genotoxic effects although PAH content was greater in downwind samples.	Hamers (2000)
Liver tumor cell line (HEPA1c1c7)	Ambient particles from diesel exhaust Rubber, metal industries biologic sources (poultry/swine farming) compost	in vitro	6 - 12 µg 17 - 37 µg 36 - 47 µg 32 - 175 µg 81 - 137 µg 42 µg	Aqueous and organic extraction of particles collected with high volume samplers.	Not given.	Inhibition of gap-junctional intercellular communication was significant only in cells treated with aqueous extract of diesel, compost, or rubber particles.	Alink (1998)
Ames assay with and without activation	Diesel exhaust particles	in vitro	Not given	Dichloromethane extraction of particles collected from diesel engine run with diesel fuels with low or high sulfur and 2 plant oil fuels.	48 hours incubation with TA98 and TA100 strains.	Revertants were 2 to 10-fold higher with high sulfur diesel fuel particles.	Bunger (2000)

For example, Hornberg et al. (1996) evaluated genotoxic effects on cultured rodent (rat; Syrian golden hamster) tracheal epithelium cells exposed in vitro to ambient PM collected on hi-vol (TSP) sampler filters during Winter 1991 in a heavily industrialized city (Duisburg) of the Rhine-Rhur area of Germany or in another area (Düsseldorf) dominated by high density vehicular traffic. Exposure to ambient PM extracted (by dichloromethane) from filters from both types of locations induced highly significant dose-dependent increases in SEC in the tracheal cells of both rodent species. The authors noted that it was remarkable that even quantities of chemical substances equivalent to airborne PM from 0.11 to 3.56 m^3 air for the samples from the heavy industry area and from 0.16 to 10.22 m^3 for the heavy traffic area induced significant genotoxic effects (i.e., 2 to 2.25-fold increases in SCE).

Hornberg et al. (1998) evaluated the genotoxic effects on human tracheal epithelial cells of fine ($PM_{2.5}$) and coarse (PM_{10}) fractions of ambient PM collected during Winter 1996 on dichotomous sampler filters in an urban area (Düsseldorf), an industrial area (Duisburg) and a rural area (Borken) of the Rhine-Rhur region of Germany. Both coarse fraction (PM_{10}) and especially fine fraction ($PM_{2.5}$) ambient PM extracted (by dichloromethane) from filters for all three areas significantly increased SCE in the human bronchioepithelial cell line (BEAS-2B) cultured in vitro. The authors noted that the fine fraction PM exerted stronger genotoxic activity than the corresponding coarse fraction from a given area and that, whereas the Düsseldorf and Duisburg ambient PM materials had comparable genotoxic activity, samples from the rural area (Borken) showed lower genotoxicity. The fine fraction $PM_{2.5}$ exerted strong genotoxicity (equivalent to airborne PM substances) from < 0.5 m^3 of air. Concentrations of fine ($PM_{2.5}$) and coarse (PM_{10}) fraction PM from the filters were 18.4 and 4.8 $\mu g/m^3$ for Düsseldorf; 45 and 24.1 $\mu g/m^3$ for Duisburg, and 21.8 and 10 $\mu g/m^3$ for Borken, respectively.

Based on the above results, Hornberg et al. (1996, 1998) concluded that the increases observed in SCE of tracheo epithelium cells with in vitro exposures to ambient PM materials are indicative of genotoxic activity of such materials and increased risks for humans due to such genotoxicity activity. They also note that the tracheobronchial epithelium is the site of the most common cancer in humans, i.e., bronchogenic carcinoma (Tomatis, 1990).

Further evidence for the likely carcinogenic potential of ambient PM in addition to the above SCE findings, is derived from a study by Seemayer and Hornberg (1998), which employed a bioassay for enhancement of malignment cell transformation in vitro. Seemayer and

Hornberg (1998) exposed exponentially growing cell cultures from the Syrian golden hamster for 18 hr to varying concentrations of PM materials extracted (by dichloromethane) from hi-vol sampler filters that collected ambient PM from Düsseldorf or Duisburg, Germany in the Winter of 1990. Control and PM-exposed cultures were then infected with the papovivarus simian virus (SV-40). There was a strong dose-dependent enhancement of cell transformation frequency in the kidney cell cultures as a function of varying pretreatment concentrations of ambient PM extracts. Inoculation of transformed cells into syngeneic animals produced a high percentage of malignant tumors, mostly sarcomas, as noted by the authors. Positive control cultures pretreated with benzo-a-pyrene (BaP) showed similar dose-dependent enhancement of malignant cell transformations. They also noted that the human papovaruses BK and JC are ubiquitous and infect a large proportion of human populations worldwide (Monini et al., 1995); and the interaction of environmental carcinogens (particularly from airborne PM) and viruses was to be considered in human carcinogenesis.

Using a different type of bioassay from that used by Hornberg and colleagues, Hamers et al. (2000) evaluated the genotoxicity of ambient PM collected by hi-vol sampler at sites in The Netherlands: (1) a site next to a highway traffic point (density = 63×10^3 vehicle passages/day); (2) another site next to a higher density (93×10^3 vehicle passages/day) highway traffic point; and (3) a site in a natural conservation area (with extensive non-manured grasslands and cattle grazing) and thought to have background levels of diffuse air pollution. Extracts of PM filter materials, collected from each of these sites in 1997 and/or 1998, were tested for genotoxic activity in the umu-assay (using a strain of Salmonella, S. typhineuriun). Arylhydrocarbon-receptor activation was also assessed by DR-CALUX-assay, using a stable transfected H4IIE hepatoma cell line. Ambient PM collected downwind from the highway (west-wind) traffic points had increased genotoxicity that appeared to be attributable at least in part to polycyclic aromatic hydrocarbons (PAHs) from traffic exhaust. The extracts of ambient PM collected upwind of the highway (eastern wind) had a different composition of compounds (probably including some transported in from nearby Germany) with higher genotoxicity less related to highway-emitted PAH-like compounds. Of interest, even the rural site ambient PM extracts showed some genotoxic activity. The authors concluded that their results demonstrated the presence of pollutants with genotoxic or PAH-like characteristics pose an undesirable mutagenic risk.

Alink, et al. (1998) compared gap-junctional intercellular communications (GJIC) effects in liver tumor (HEPA1c1c7) cells in vitro due to exposures to PM materials from rubber and metal industry, diesel exhaust, urban air, and biological sources (i.e., poultry, pig farming, compost industry). Only diesel and rubber sample extract suspensions significantly inhibited GJIC, with up to 83% of the inhibition being attributable to the particles per se. More active organics were reported to have been extracted from the rubber industry particles than from the diesel particles by organic solvents. The authors interpreted their results as suggesting that cancer promoting potential (as measured by GJIC inhibition) may vary widely depending on particle source, possibly due to the particles per se or to surface-bound bio-active material.

Additional evidence for cytotoxic and mutagenic effects of particles emitted from diesels comes from a study by Bunger et al. (2000). Filter sample particles, collected from diesel emissions generated by a tractor engine during conventional fossil diesel fuel or diesel fuel containing rapeseed oil methyl ester (RME), used as a "green fuel" in some countries, were extracted (by dichloromethane) and their cytotoxicity evaluated by the neutral red assay and their mutagenicity by the S. typhinuiam assay. The fossil diesel fuel emissions had much higher numbers of smaller particles than the RME emissions. However, 4-fold stronger toxic effects on mouse fibroblast cells were exerted by RME extracts from filters taken at "idling" but not at "rated" power load modes. Both types of extracts were significantly mutagenic at both load modes in both the TA98 and TA100 strain bioassays, but the fossil diesel fuel extracts had 4-fold more mutagenic effect in the TA98, and 2-fold more in the TA100 strain assays than did RME extracts. The authors attributed the lower mutagenic potency of the RME diesel emissions to lower emissions of polycyclic aromatic compounds.

The above studies, collectively, appear to demonstrate significant mutagenic and/or tumorgenic effects of ambient PM drawn from industrialized urban areas and/or vehicular traffic-dominated areas. Also, several specifically link such effects to diesel exhaust and or DPM contained therein. Such results add further to an extensive database on diesel-related mutagenicity that was thoroughly reviewed in an U.S. EPA Diesel Health Assessment Document (U.S. EPA 2002) alluded to earlier. Important information drawn from that document's evaluation of diesel-related mutagenic properties is recapitulated below (at times verbatim) with particular emphasis on findings bearing on the role of PM components of diesel exhaust.

As noted in the 2002 Diesel Document, the use of mutagenicity data as one approach to evaluating potential carcinogenicity of diesel emissions is based on the premise that genetic alterations are found in all cancers and that several of the chemicals found in diesel emissions possess mutagenic activity in a variety of genetic assays. These genetic alterations can be produced by gene mutations, deletions, translocations, aneuploidy, or amplification of genes; hence no single genotoxicity assay should be expected to predict carcinogenicity. Also, because of the inherent biological differences of measured endpoints, both within genotoxicity assays and between genotoxicity assays and cancer bioassays, a direct extrapolation should not be expected. Indeed, most genotoxicity data are generated with in vitro assays that frequently employ concentrations of test agent that may be orders of magnitude greater than encountered in environmental situations. With diesel emissions or other mixtures, other complications arise due to the complexity of the materials tested.

Since 1978, more than 100 publications have appeared in which genotoxicity assays were used with diesel emissions, the volatile and particulate fractions (including extracts), or individual chemicals found in diesel emissions. The interest in the contribution of mutagens to carcinogenicity was high in the early 1980s and the lack of long- term rodent carcinogenicity information on diesel emissions led to use of semiquantitative mutagenicity (and in vitro cell transformation) data from diesel emissions to augment epidemiology studies of diesel-related carcinogenic effects.

The number of chemicals in diesel emissions is very large; and many of these have been determined to exhibit mutagenic activity in a variety of assay systems (see Claxton, 1983). Among some of the mutagenically active compounds found in the gas phase are ethylene, benzene, 1,3-butadiene, acrolein and several PAHs. Of the diesel particle-associated chemicals, several PAHs and nitro-PAHs have been the focus of mutagenic investigations both in bacteria and in mammalian cell systems.

Gene Mutations

Huisingh et al. (1978) demonstrated that dichloromethane extracts from DPM were mutagenic in strains TA1537, TA1538, TA98, and TA100 of *S. typhimurium*, both with and without rat liver S9 activation, based on data from several fractions as well as DPM from different vehicles and fuels. Similar results with diesel extracts from various engines and fuels

have been reported by a number of investigators using the salmonella frameshift-sensitive strains TA1537, TA1538, and TA98 (Siak et al., 1981; Claxton, 1981; Dukovich et al., 1981; Brooks et al., 1984). Similarly, mutagenic activity was seen in salmonella forward mutation assays measuring 8-azaguanine resistance (Claxton and Kohan, 1981) and in *E. coli* mutation assays (Lewtas, 1983).

One approach to identifying significant mutagens in chemically complex environmental samples such as diesel exhaust or ambient particulate extracts is the combination of short-term bioassays with chemical fractionation (Scheutzle and Lewtas, 1986). The analysis is most frequently carried out by sequential extraction with increasingly polar or binary solvents. Fractionation by silica-column chromatography separates compounds by polarity or into acidic, basic, and neutral fractions. The resulting fractions are too complex to characterize by chemical methods, but the bioassay analysis can be used to determine fractions for further analysis. In most applications, salmonella strain TA98 without the addition of S9 has been used as the indicator for mutagenic activity.

Generally, a variety of nitrated polynuclear aromatic compounds have been found that account for a substantial portion of the mutagenicity (Liberti et al., 1984; Schuetzle and Frazer, 1986; Schuetzle and Perez, 1983). However, not all bacterial mutagenicity has been identified in this way, and the identity of the remaining mutagenic compounds remains unknown. The nitrated aromatics thus far identified in diesel engine exhaust (DE) were the subject of review in an IARC monograph on DE (International Agency for Research on Cancer, 1989). In addition to qualitative identification of mutagenic chemicals, several investigators have used numerical data to express mutagenic activity as activity per distance driven or mass of fuel consumed. These types of calculations have been the basis for estimates that the nitroarenes (both mono- and dinitropyrenes) contribute a significant amount of the total mutagenic activity of the whole extract (Nishioka et al., 1982; Salmeen et al., 1982; Nakagawa et al., 1983). More recently, Crebelli et al. (1995) used salmonella to examine the effects of different fuel components. They reported that although mutagenicity was highly dependent on aromatic content, especially di- or triaromatics, there was no clear effect of sulfur content of the fuel. Later, however, Sjögren et al. (1996), using multivariate statistical methods with ten diesel fuels, concluded that the most influential chemical factors in salmonella mutagenicity were sulfur contents, certain PAHs (1-nitropyrene) and naphthenes.

Matsushita et al. (1986) tested particle-free DE gas and of benzene nitroderivatives and polycyclic aromatic hydrocarbons (PAHs), identified as components of DE gas. The particle-free exhaust gas was positive in both TA100 and TA98, but only without S9 activation. Of the 94 nitrobenzene derivatives tested, 61 were mutagenic, and the majority showed greatest activity in TA100 without S9; whereas 28 of 50 PAHs tested were mutagenic, all required the addition of S9 for detection, and most appeared to show a stronger response in TA100. When 1,6-dinitropyrene was mixed with various PAHs or an extract of heavy-duty (HD) DE, the mutagenic activity in TA98 was greatly reduced when S9 was absent but was increased significantly when S9 was present. These latter results suggested that caution should be used in estimating mutagenicity (or other toxic effects) of complex mixtures from the specific activity of individual components.

Mitchell et al. (1981) reported mutagenic activity of DPM extracts of diesel emissions in the mouse lymphoma L5178Y mutation assay. Positive results were seen both with and without S9 activation in extracts from several different vehicles, with mutagenic activity only slightly lower in the presence of S9. These findings have been confirmed in a number of other mammalian cell systems using several different genetic markers. Casto et al. (1981), Chescheir et al. (1981), Li and Royer (1982), and Brooks et al. (1984) all reported positive responses at the HPRT locus in Chinese hamster ovary (CHO) cells. Morimoto et al. (1986) used the APRT and Ouar loci in CHO cells; Curren et al. (1981) used Ouar in BALB/c 3T3 cells. In all of these studies, mutagenic activity was observed without S9 activation. Liber et al. (1981) used the thymidine kinase (TK) locus in the TK6 human lymphoblast cell line and observed induced mutagenesis only in the presence of rat liver S9 when testing a methylene chloride extract of DE. Barfknecht et al. (1982) also used the TK6 assay to identify some of the chemicals responsible for this activation-dependent mutagenicity and they suggested that 1-methylphenanthrene, 9-methylphenanthrene, and fluoranthene could account for over 40% of the observed activity.

Balisario et al. (1984) applied the Ames test to urine from SD rats exposed to single applications of DPM administered by gastric intubation, i.p. injection, or s.c. geletin capsules. In all cases, dose-related increases were taken in TA98 (without and with S9) from urine concentrations taken 24 h after particle administration. Urine from Swiss mice exposed by inhalation to filtered exhaust (particle concentration 6 to 7 mg/m^3) for 7 weeks (Pereira et al.,

1981a) or Fischer 344 rats exposed to DPM at a concentration of 1.9 mg/m^3 for 3 months to 2 years (Ong et al., 1985) was negative in salmonella strains.

Schuler and Niemeier (1981) exposed drosophila males in a stainless steel chamber connected to the 3 m^3 chamber used for the chronic animal studies at EPA (see Hinners et al., 1980 for details). Flies were exposed for 8 h and mated to untreated females 2 days later. Although the frequency of sex-linked recessive lethals from treated males was not different from that of controls, the limited sample size precluded detecting less than a threefold increase over controls. The authors noted that, because there were no signs of toxicity, the flies might tolerage exposures to higher concentrations for longer time periods.

Specific-locus mutations were not induced in (C3H × 101)F1 male mice exposed to DE 8 h/day, 7 days/week for either 5 or 10 weeks (Russell et al., 1980). The exhaust was a 1:18 dilution and the average particle concentration was 6 mg/m^3. After exposure, males were mated to T-stock females and matings continued for the reproductive life of the males. The results were unequivocally negative; no mutants were detected in 10,635 progeny derived from postspermatogonial cells or in 27,917 progeny derived from spermatogonial cells.

Hou et al. (1995) measured DNA adducts and *hprt* mutations in peripheral lymphocytes of 47 bus maintenance workers and 22 control individuals. All were nonsmoking men from garages in the Stockholm area and the exposed group consisted of 16 garage workers, 25 mechanics, and 6 other garage workers. There were no exposure data, but the three groups were considered to be of higher to lower exposure to diesel engine exhaust. Levels of DNA adducts determined by 32P-postlabeling were significantly higher in workers than controls (3.2 versus 2.3 × 10-8), but *hprt* mutant frequencies were not different 8.6 versus 8.4 × 10-6). Although group mean mutant frequencies were not different, both adduct level and mutagenicity were highest among the 16 most exposed and mutant frequency was significantly correlated with adduct level. All individuals were genotyped for glutathione transferase GSTM1 and aromatic amino transferase NAT2 polymorphism. Neither GSTM1 nulls nor NAT2 slow acetylators exhibited effects on either DNA adducts or *hprt* mutant frequencies.

Driscoll et al. (1996) exposed Fischer 344 male rats to aerosols of carbon black (1.1, 7.1, and 52.8 mg/m^3) or air for 13 weeks (6 hr/day, 5 days/week) and measured *hprt* mutations in alveolar type II cells in animals immediately after exposure and at 12 and 32 weeks after the end of exposure. The two higher concentrations resulted in significant increases in mutant

frequency. Whereas the mutant frequency from the 7.1 mg/m^3 group returned to control levels by 12 weeks, the mutant frequency of the high-exposure group was still higher than controls even after 32 weeks. Carbon black particles have very little adsorbed PAHs, hence a direct chemically induced mechanism is highly unlikely. Induction of *hprt* mutations were also seen for rat alveolar epithelial cells after intratracheal instillation with carbon black, quartz, and titanium dioxide (Driscoll et al., 1997). All three types of particles elicited an inflammatory response as shown by significant increases of neutrophils in bronchoalveolar lavage (BAL) fluid. Culturing the BAL from exposed rats with a rat lung epithelial cell line also resulted in elevation of *hprt* mutational response. This response was effectively eliminated when catalase was included in the incubation mixture, providing evidence for cell-derived oxidative damage. Recently, Sato et al. (2000) exposed male Big Blue transgenic F344 rats to diluted DE (1 and 6 mg/m^3 suspended particle concentration) for 4 weeks. Mutant frequency in lung DNA was significantly elevated (4.8x control) at 6 mg/m^3 but not at 1 mg/m^3. Lung DNA adduct levels measured by 32P-postlabeling and 8-hydroxydeoxyguanosine measured by HPLC were elevated at both particle concentrations, but to a lesser extent than mutant frequencies. Sequence analysis of mutants indicated that some, but not all, of the mutations could be explained by an oxidative damage mechanism.

Chromosome Effects

Mitchell et al. (1981) and Brooks et al. (1984) reported increased SCE in CHO cells exposed to DPM extracts of emissions from both LD and HD diesel engines. Morimoto et al. (1986) observed increased SCE from both LD and HD DPM extracts in PAH-stimulated human lymphocyte cultures. Tucker et al. (1986) exposed human peripheral lymphocyte cultures from four donors to direct DE for up to 3 h. Samples were taken at 16, 48, and 160 min of exposure. Cell cycle delay was observed in all cultures; and significantly increased SCE levels were reported for two of the four cultures. Structural chromosome aberrations were induced in CHO cells by DPM extracts from a Nissan diesel engine (Lewtas, 1983) but not by similar extracts from an Oldsmobile diesel engine (Brooks et al., 1984).

DPM dispersed in an aqueous mixture containing dipalmitoyl lecithin (DPL), a component of pulmonary surfactant or extracted with dichloromethane (DCM) induced similar responses in SCE assays in Chinese hamster V79 cells (Keane et al., 1991), micronucleus tests in V79 and

CHO cells (Gu et al., 1992), and unscheduled DNA synthesis (UDS) in V79 cells (Gu et al., 1994). After separating the samples into supernatant and sediment fractions, mutagenic activity was confined to the sediment fraction of the DPL sample and the supernatant of the DCM sample. These findings suggest that the mutagenic activity of DPM inhaled into the lungs could be made bioavailable through solubilization and dispersion of pulmonary surfactants. In a later study in the same laboratory, Liu et al. (1996) found increased micronuclei in V79 cells treated with crystalline quartz and a noncrystalline silica, but response was reduced after pretreatment of the particles with the simulated pulmonary surfactant.

Pereira et al. (1981a) exposed female Swiss mice to DE 8 h/day, 5 days/week for 1, 3, and 7 weeks. The incidence of micronuclei and structural aberrations was similar in bone marrow cells of both control and exposed mice. Increased incidences of micronuclei, but not SCE, were observed in bone marrow cells of male Chinese hamsters after 6 months of exposure to DE (Pereira et al., 1981b).

Guerrero et al. (1981) observed a linear concentration-related increase in SCE in lung cells cultured after intratracheal instillation of DPM at doses up to 20 mg/hamster. However, they did not observe any increase in SCE after 3 months of inhalation exposure to DE particles (6 mg/m^3).

Pereira et al. (1982) measured SCE in embryonic liver cells of Syrian hamsters. Pregnant females were exposed to DE diluted with air 1:9 to contain about 12 mg/m^3 particles from days 5 to 13 of gestation or injected intraperitoneally with diesel particles or particle extracts on gestational day 13 (18 h before sacrifice). Neither the incidence of SCE nor mitotic index was affected by exposure to DE. The injection of DPM extracts but not DPM resulted in a dose-related increase in SCE; however, the toxicity of the DPM was about twofold greater than the DPM extract.

In the only studies with mammalian germ cells, Russell et al. (1980) reported no increase in either dominant lethals or heritable translocations in males of T-stock mice exposed by inhalation to diesel emissions. In the dominant lethal test, T-stock males were exposed for 7.5 weeks and immediately mated to females of different genetic backgrounds. There were no differences from controls in any of the parameters measured in this assay. For heritable translocation analysis, T-stock males were exposed for 4.5 weeks and mated to (SEC × C57BL/6) females, and the F1 males were tested for the presence of heritable translocations.

Although no translocations were detected among 358 progeny tested, the historical control incidence is less than 1/1,000.

Other Genotoxic Effects

A number of studies measuring DNA adducts in animals exposed to DPM, carbon black or other particles have been reported, as reviewed by Shirnamé-Moré (1995). Although modest increases in DNA adducts have been observed in lung tissue of rats after inhalation of DPM (Wong et al., 1986; Bond et al., 1990), the increases are small in comparison with those induced by chemical carcinogens present in DE (Smith et al., 1993). While Gallagher et al. (1994) found no increases in total DNA adducts in lung tissue of rats exposed to DE, carbon black, or titanium dioxide they did observe an increase in an adduct with migration properties similar to nitrochrysene and nitro-benzo(a)pyrene adducts from diesel but not carbon black or titanium dioxide exposures. The majority of the studies used the 32P postlabeling assay to detect adducts. Although this method is sensitive, chemical identity of adducts can only be inferred if an adduct spot migrates to the same location as a known prepared adduct.

DNA adducts have also been measured in humans occupationally exposed to DE. Distinct adduct patterns were found among garage workers occupationally exposed to DE when compared to nonexposed controls (Nielsen and Autrup, 1994). Furthermore, the findings were concordant with the adduct patterns observed in groups exposed to low concentrations of PAHs from combustion processes. Hemminki et al. (1994) also reported significantly elevated levels of DNA adducts in lymphocytes from garage workers with known DE exposure compared with unexposed mechanics. Hou et al. (1995) found elevated adduct levels in bus maintenance workers exposed to DE. Although no difference in mutant frequency was observed between the groups, the adduct levels were significantly different (3.2 vs. 2.3 × 10-8). Nielsen et al. (1996) reported significantly increased levels of three biomarkers (lymphocyte DNA adducts, hydroxyethylvaline adducts in hemoglobin, and 1-hydroxypyrene in urine) in DE-exposed bus garage workers.

The role of oxidative damage in causing mutations has received increasing attention. More than 50 different chemicals have been studied in rodents usually measuring the formation of 8-hydroxydeoxyguanosine (8-OH-dG), a highly mutagenic adduct (Loft et al., 1998). Dose-dependent increases in that mutagenic DNA adduct were found in mouse lung DNA after

intratracheal instillation of diesel particles (Nagashima et al., 1995). Mice fed on a high-fat diet showed an increased response, whereas the responses were partially reduced when the antioxidant, β-carotene, was included in the diet (Ichinose et al., 1997). Oxidative damage also has been measured in rat lung tissue after intratracheal instillation of quartz (Nehls et al., 1997) and in rat alveolar macrophages after in vitro treatment with silica dust (Zhang et al., 2000). Arimoto et al. (1999) demonstrated that redissolved methanol extracts of DPM also induced the formation of 8-OH-dG adducts in L120 mouse cells. The response was dependent on both DPM concentration and P450 reductase. A detailed discussion of the potential role of oxidative damage in DE carcinogenesis is presented in the U.S. EPA Diesel Document (U.S. Environmental Protection Agency, 2002).

Summary of Key Mutagenicity/Genotoxicity Findings

Extensive studies with salmonella have demonstrated mutagenic activity in both particulate and gaseous fractions of DE. In most studies using salmonella, DPM extracts and individual nitropyrenes exhibited the strongest responses in strain TA98 when no exogenous activation was provided. Gaseous fractions reportedly showed greater response in TA100, whereas benzo[*a*]pyrene and other unsubstituted PAHs are mutagenic only in the presence of S9 fractions. The induction of gene mutations has been reported in several in vitro mammalian cell lines after exposure to extracts of DPM. Note that only the TK6 human cell line did not give a positive response to DPM extracts in the absence of S9 activation. Dilutions of whole diesel exhaust did not induce sex-linked recessive lethals in drosophila or specific-locus mutations in male mouse germ cells.

Structural chromosome aberrations and SCE in mammalian cells have also been induced by DE particles and extracts. Whole exhaust induced micronuclei, but not SCE or structural aberrations, in bone marrow of male Chinese hamsters exposed to whole diesel emissions for 6 mo. In a shorter exposure (7 weeks), neither micronuclei nor structural aberrations were increased in bone marrow of female Swiss mice. Likewise, whole DE did not induce dominant lethals or heritable translocations in male mice exposed for 7.5 and 4.5 weeks, respectively.

Exercises that combined the salmonella mutagenic potency with the total concentration of mutagenic chemicals deposited in the lungs could not account for the observed tumor incidence in exposed rats (Rosenkranz, 1993; Goldstein et al., 1998). However, such calculations ignored

the contribution of gaseous phase chemicals which have been estimated to contribute from less than 50% (Rannug et al., 1983) to over 90% (Matsushita et al., 1986) of the total mutagenicity. This wide range is partly reflective of the differences in material tested, semivolatile extracts in the former and whole gaseous emission in the latter. Of greater importance is that these calculations are based on a reverse mutation assay in bacteria with metabolic processes strikingly different from mammals. This is at least partly reflected in the observations that different nitro-PAHs give different responses in bacteria and in CHO cells (Li and Dutcher, 1983) or in human hepatoma-derived cells (Eddy et al., 1986).

The above studies provide qualitative evidence for mutagenic/genotoxic potential of ambient PM and some fuel combustion (e.g., diesel emission) products. However, these published in vitro studies generally fail to provide details regarding the dose of PM extract delivered to the cells in vitro. In general, equal volumes of air or amounts of time were sampled and reported, but only limited, if any, characterization of the amount of PM mass or size appeared to be done or reported in most studies. Thus, quantitative extrapolation of the reported findings is quite difficult. Nevertheless, they collectively do appear to provide extensive credible evidence substantiating the biologic plausibility of, and/or elucidating potential mechanisms underlying, reported associations between long-term exposure to ambient PM (and DPM as one of its typical major constituents).

7.4.3 Potential Cellular and Molecular Mechanisms

The numerous studies assessed in the foregoing sections provide evidence for various types of PM effects on cardiopulmonary system components and functions. Considerable interest and research attention has been accorded to effects aimed at characterizing specific cellular and molecular mechanisms underlying PM effects. The ensuing sections highlight information derived in part from in vivo, but more so, from in vitro, studies that supports identification of several general types of mechanisms as mediating various PM-induced pathophysiological responses affecting cardiopulmonary and other functions. This includes, in particular, evidence for important involvement in mediating PM effects of (a) reactive oxygen species; (b) intracellular signaling mechanisms; and (c) other types of mechanisms (e.g., impacts on sensory nerve receptors).

7.4.3.1 Reactive Oxygen Species

Ambient particulate matter contains transition metals, such as iron (most abundant), copper, nickel, zinc, vanadium, and cobalt. These metals are capable of catalyzing the one-electron reductions of molecular oxygen necessary to generate reactive oxygen species (ROS). These reactions can be demonstrated by the iron-catalyzed Haber-Weiss reactions that follow.

$$\text{Reductant}^{n} + \text{Fe(III)} \rightarrow \text{Reductant}^{n+1} + \text{Fe(II)} \quad (1)$$

$$\text{Fe(II)} + O_2^- \rightarrow \text{Fe(III)} + O_2^- \quad (2)$$

$$HO_2^- + O_2^- + H^+ \rightarrow O_2 + H_2O_2 \quad (3)$$

$$\text{Fe(II)} + H_2O_2 \rightarrow \text{Fe(III)} + {}^{\bullet}OH + HO^- \text{ (Fenton Reaction)} \quad (4)$$

Iron will continue to participate in the redox cycle in the above reactions as long as there is sufficient O_2 or H_2O_2 and reductants.

Soluble metals from inhaled PM dissolved into the fluid lining of the airway lumen can react directly with biological molecules (acting as a reductant in the above reactions) to produce ROS. For example, ascorbic acid in the human lung epithelial lining fluid can react with Fe(III) from inhaled PM to cause single strand breaks in supercoiled plasmid DNA, ϕX174 RFI (Smith and Aust, 1997). The DNA damage caused by some PM_{10} samples can be inhibited by mannitol, an hydroxyl radical scavenger, further confirming the involvement of free radicals in these reactions (Gilmour et al., 1996; Donaldson et al., 1997; Li et al., 1997). Because the clear supernatant of the centrifuged PM_{10} suspension contained all of the suspension activity, the free radical activity is derived either from a fraction that is not centrifugable (10 min at 13,000 rpm on a bench centrifuge) or the radical generating system is released into solution (Gilmour et al., 1996; Donaldson et al., 1997; Li et al., 1997).

In addition to measuring the interactions of ROS and biomolecules directly, the role of ROS in PM-induced lung injury also can be assessed by measuring the electron spin resonance (ESR) spectrum of radical adducts or fluorescent intensity of dichlorofluorescin (DCFH), an intracellular dye that fluoresces on oxidation by ROS. Alternatively, ROS can be inhibited using free radical scavengers, such as dimethylthiourea (DMTU); antioxidants, such as glutathione or N-acetylcysteine (NAC); or antioxidant enzymes, such as superoxide dismutase (SOD). The diminished response to PM after treatment with these antioxidants may indicate the involvement of ROS; however, some antioxidants (e.g., thiol-containing) can interact with metal ions directly.

As described earlier, Kadiiska et al. (1997) used the ESR spectra of 4-POBN [α-(4-pyridyl 1-oxide)-N-tert-butylnitrone] adducts to measure ROS in rats instilled with ROFA and demonstrated the association between ROS production within the lung and soluble metals in ROFA. Using DMTU to inhibit ROS production, Dye et al. (1997) had shown that systemic administration of DMTU impeded development of the cellular inflammatory response to ROFA, but did not ameliorate biochemical alterations in BAL fluid. Goldsmith et al. (1998), as described earlier, showed that ROFA and CAPs caused increases in ROS production in AMs. The water-soluble component of both CAPs and ROFA significantly increased AM oxidant production over negative control values. In addition, increased PM-induced cytokine production was inhibited by NAC. Li et al. (1996, 1997) instilled rats with PM_{10} particles (collected on filters from an Edinburgh, Scotland, monitoring station). Six hours after intratracheal instillation of PM_{10}, they observed a decrease in glutathione (GSH) levels in the BAL fluid. Although this study does not describe the composition of the PM_{10}, the authors suggest that changes in GSH, an important lung antioxidant, support the contention that the free radical activity of PM_{10} is responsible for its biological activity in vivo.

In addition to ROS generated directly by PM, resident or newly recruited AMs or PMNs also are capable of producing these reactive species on stimulation. The ROS produced during the oxidative burst can be measured using a chemiluminescence (CL) assay. With this assay, AM CL signals in vitro have been shown to be greatest with ROFA containing primarily soluble V and were less with ROFA containing Ni plus V (Kodavanti et al., 1998a). As described earlier, exposures to Dusseldorf and Duisburg PM increased the resting ROS production in PMNs, which could be inhibited by SOD, catalase, and sodium azide (Hitzfeld et al., 1997).

Stringer and Kobzik (1998) showed that addition of NAC (1.0 mM) decreased ROFA-mediated IL-8 production by approximately 50% in normal and TNF-α-primed A549 cells. In addition, exposures of A549 cells to ROFA caused a substantial (and NAC inhibitable) increase in oxidant levels as measured by DCFH oxidation. In human AMs, Becker et al. (1996) found a CL response for ROFA, but not urban air particles (Ottawa and Dusseldorf) or volcanic ash.

Metal compounds of PM are the most probable species capable of catalyzing ROS generation on exposure to PM. To determine elemental content and solubility in relation to their ability to generate ROS, PMN or monocytes were exposed to a wide range of ambient air particles from divergent sources (one natural dust, two types of oil fly ash, two types of coal fly ash, five different ambient air samples, and one carbon black sample), and CL production was measured over a 20-min period postexposure (Prahalad et al., 1999). Percent of sample mass accounted for by XRF detectable elements was 1.2% (carbon black); 22 to 29% (natural dust and ambient air particles); 13 to 22% (oil fly ash particles); and 28 to 49% (coal fly ash particles). The major proportion of elements in most of these particles were aluminosilicates and insoluble iron, except oil derived fly ash particles in which soluble vanadium and nickel were in highest concentration, consistent with particle acidity as measured in the supernatants. All particles induced CL response in cells, except carbon black. The CL response of PMNs in general increased with all washed particles, with oil fly ash and one urban air particle showing statistical differences between deionized water washed and unwashed particles. These CL activities were significantly correlated with the insoluble Si, Fe, Mn, Ti, and Co content of the particles. No relationship was found between CL and soluble transition metals such as V, Cr, Ni, and Cu. Pretreatment of the particles with a metal ion chelator, deferoxamine, did not affect CL activities. Particle sulfate content and acidity of the particle suspension did not correlate with CL activity.

Soluble metals can be mobilized into the epithelial cells or AMs to produce ROS intracellularly. Size-fractionated coal fly ash particles (2.5, 2.5 to 10, and < 10 μm) of bituminous b (Utah coal), c (Illinois coal), and lignite (Dakota coal) were used to compare the amount of iron mobilization in A549 cells and by citrate (1 mM) in cell-free suspensions (Smith et al., 1998). Iron was mobilized by citrate from all three size fractions of all three coal types. More iron, in Fe(III) form, was mobilized by citrate from the < 2.5-μm fraction than from the > 2.5-μm fractions. In addition, the amount of iron mobilized was dependent on the type of coal

used to generate the fly ash (Utah coal > Illinois coal = Dakota coal) but was not related to the total amount of iron present in the particles. Ferritin (an iron storage protein) levels in A549 cells increased by as much as 12-fold in cells treated with coal fly ash (Utah coal > Illinois coal > Dakota coal). More ferritin was induced in cells treated with the < 2.5-µm fraction than with the > 2.5-µm fractions. Mossbauer spectroscopy of a fly ash sample showed that the bioavailable iron was assocated with the glassy aluminosilicate fraction of the particles (Ball et al., 2000). As with the bioavailability of iron, there was an inverse correlation between the production of IL-8 and fly ash particle size, with the Utah coal fly ash being the most potent.

Using ROFA and colloidal iron oxide, Ghio et al. (1997b; 1998a,b,c; 1999c; 2000c) have shown that exposures to these particles disrupted iron homeostasis and induced the production of ROS in vivo and in vitro. Treatment of animals or cells with metal-chelating agents such as DEF with an associated decrease in response has been used to infer the involvement of metal in PM-induced lung injury. Metal chelation by DEF (1 mM) caused significant inhibition of particulate-induced AM oxidant production, as measured using DCFH (Goldsmith et al., 1998). DEF treatment also reduced NF-κB activation and cytokine secretion in a human bronchial epithelial cell line (BEAS-2B cells) exposed to Provo PM (Kennedy et al., 1998). However, treatment of ROFA suspension with DEF was not effective in blocking leachable metal induced acute lung injury (Dreher et al., 1997). Dreher et al. (1997) indicated that DEF could chelate Fe(III) and V(II), but not Ni(II), suggesting that metal interactions played a significant role in ROFA-induced lung injury.

Other than Fe, several V compounds have been shown to increase mRNA levels for selected cytokines in BAL cells and induce pulmonary inflammation (Pierce et al., 1996). $NaVO_3$ and $VOSO_4$, highly soluble forms of V, tended to induce pulmonary inflammation and inflammatory cytokine mRNA expression more rapidly and more intensely than the less soluble form, V_2O_5, in rats. Neutrophil influx was greatest following exposure to $VOSO_4$ and lowest following exposure to V_2O_5. However, metal components of fly ash have not been shown to consistently increase ROS production from bovine AM treated with combustion particles (Schlüter et al., 1995). For example, As(III), Ni(II), and Ce(III), which are major components of fly ash, had been shown to inhibit the secretion of superoxide anions (O_2^-) and hydrogen peroxide. In the same study, O_2^- were lowered by Mn(II) and Fe(II); whereas V(IV) increased O_2^- and H_2O_2. In contrast, Fe(III) increased O_2^- production, demonstrating that the oxidation state

of metal may influence its oxidant generating properties. Other components of fly ash, such as Cd(II), Cr(III), and V(V), had no effects on ROS.

It is likely that a combination of several metals rather than a single metal in PM is responsible for the PM-induced cellular response. For example, V and Ni+V but not Fe or Ni alone (in saline with the final pH at 3.0) resulted in increased epithelial permeability, decreased cellular glutathione, cell detachment, and lytic cell injury in rat tracheal epithelial cells exposed to soluble salts of these metals at equivalent concentrations found in ROFA (Dye et al., 1999). Treatment of V-exposed cells with buthionine sulfoximine further increased cytotoxicity. Conversely, treatment with radical scavenger dimethyl thiourea inhibited the effects in a dose-dependent manner. These results suggest that soluble metal or combinations of several metals in ROFA may be responsible for these effects.

Similar to combustion particles such as ROFA, the biological response to exposure to ambient PM also may be influenced by the metal content of the particles. Human subjects were instilled with 500 µg (in 20 mL sterile saline) of Utah Valley dust (UVD1, 2, 3, collected during 3 successive years) on the left segmental bronchus and on the right side with sterile saline as control. A second bronchoscopy was performed 24 hours post-instillation and phagocytic cells were obtained from the segmental bronchi on both sides. Alveolar macrophage from subjects instilled with UVD, obtained by bronchoaveolar lavage 24 h post-instillation, were incubated with fluoresceinated yeast (Saccharomyces cerevisiae) to assess their phagocytic ability. Although the same proportion of AMs that were exposed to UVD phagocytized yeast, AMs exposed to UVD1, which were collected while a local steel mill was open, took up significantly less particles than AMs exposed to other extracts (UVD2 when the steel mill was closed and UVD3 when the plant reopened). AMs exposed to UVD1 also exhibited a small decrease in oxidant activity (using dihydrorhodamine-123, DHR). AMs from healthy volunteers were incubated in vitro with the various UVD extracts to assess whether similar effects on human AMs function could be observed to those seen following in vivo exposure. The percentage of AMs that engulfed yeast particles was significantly decreased by exposure to UVD1 at 100 µg/mL, but not at 25 µg/mL. However, the amount of particles engulfed was the same following exposure to all three UVD extracts. AMs also demonstrated increased oxidant stress (using chemiluminescence) after in vitro exposure to UVD1, and this effect was not abolished with pretreatment of the extract with the metal chelator deferoxamine. As with the AMs exposed to

UVD in vivo, AM exposed to UVD in vitro had a decreased oxidant activity (DHR assay). UVD1 contains 61 times and 2 times the amount of Zn compared to UVD 2 and UVD3, respectively; whereas UVD3 contained 5 times more Fe than UVD1. Ni and V were present only in trace amounts. Using similarly extracted samples, Frampton et al. (1999) exposed BEAS-2B cells for 2 and 24 h. Similar results were observed for oxidant generation in these cells (i.e., UVD 2, which contains the lowest concentrations of soluble iron, copper, and zinc, produced the least response). Only UVD 3 produced cytotoxicity at a dose of 500 µg/mL. UVD 1 and 3, but not 2, induced expression of IL-6 and 8 in a dose-dependent fashion. Taken together, the above results showed that the biological response to ambient particle extracts is heavily dependent on the source and, hence, the chemical composition of PM.

7.4.3.2 Intracellular Signaling Mechanisms

In has been shown that the intracellular redox state of the cell modulates the activity of several transcription factors, including NF-κB, a critical step in the induction of a variety of proinflammatory cytokine and adhesion-molecule genes. NF-κB is a heterodimeric protein complex that in most cells resides in an inactive state in the cell cytoplasm by binding to inhibitory kappa B alpha (IκBα). On appropriate stimulation by cytokines or ROS, IκBα is phosphorylated and subsequently degraded by proteolysis. The dissociation of IκBα from NF-κB allows the latter to translocate into the nucleus and bind to appropriate sites in the DNA to initiate transcription of various genes. Two studies in vitro have shown the involvement of NF-κB in particulate-induced cytokine and intercellular adhesion molecule-1 (ICAM-1) production in human airway epithelial cells (BEAS-2B) (Quay et al., 1998; Kennedy et al., 1998). Cytokine secretion was preceded by activation of NF-κB and was reduced by treatment with antioxidants or metal chelators. These results suggest that metal-induced oxidative stress may play a significant role in the initiation phase of the inflammatory cascade following PM exposure.

A second well-characterized human transcription factor, AP-1, also responds to the intracellular ROS concentration. AP-1 exists in two forms, either in a homodimer of c-jun protein or a heterodimer consisting of c-jun and c-fos. Small amounts of AP-1 already exist in the cytoplasm in an inactive form, mainly as phosphorylated c-jun homodimer. Many different oxidative stress-inducing stimuli, such as UV light and IL-1, can activate AP-1. Exposure of rat

lung epithelial cells to ambient PM in vitro resulted in increases in c-jun kinase activity, levels of phosphorylated c-jun immunoreactive protein, and transcriptional activation of AP-1-dependent gene expression (Timblin et al., 1998). This study demonstrated that interaction of ambient particles with lung epithelial cells initiates a cell signaling cascade related to aberrant cell proliferation.

Early response gene transactivation has been linked to the development of apoptosis, a potential mechanism to account for PM-induced changes in cellular response. Apoptosis of human AMs exposed to ROFA (25 µg/mL) or urban PM was observed by Holian et al. (1998). In addition, both ROFA and urban PM upregulated the expression of the $RFD1^+$ AM phenotype; whereas only ROFA decreased the $RFD1^+7^+$ phenotype. It has been suggested that an increase in the AM phenotype ratio of $RFD1^+/RFD1^+7^+$ may be related to disease progression in patients with inflammatory diseases. These data showed that ROFA and urban PM can induce apoptosis of human AMs and increase the ratio of AM phenotypes toward a higher immune active state and may contribute to or exacerbate lung inflammation.

Inhaled fine and coarse particles are trapped in the epithelial lining of the nasal and tracheal airways. Somatosensory neurons located in the dorsal root ganglia (DRG) innervate the upper thoracic region of the airways and extend their terminals over and between the epithelial lining of the lumen. Given this anatomical proximity, the sensory fibers and the tracheal epithelial cells that they innervate encounter inhaled pollutants, such as PM, early during inhalation. The differential responses of these cell types to PM derived from various sources (i.e., industrial, residential, volcanic) were examined with biophysical and immunological endpoints (Veronesi et al., 2002a). Although the majority of PM tested stimulated IL-6 release in both BEAS-2B epithelial cells and DRG neurons in a receptor-mediated fashion, the degree of these responses was markedly higher in sensory neurons. Epithelial cells are damaged or denuded in many common health disorders (e.g., asthma, viral infections), allowing PM particles to directly encounter the sensory terminals and their acid-sensitive receptors.

Another intracellular signaling pathway that could lead to diverse cellular responses such as cell growth, differentiation, proliferation, apoptosis, and stress responses to environmental stimuli, is the phosphorylation-dependent, mitogen-activated protein kinase (MAPK). Significant dose- and time-dependent increases in protein tyrosine phosphate levels have been seen in BEAS cells exposed to 100 µg/mL ROFA for periods ranging from 5 min to 24 h (Samet

et al., 1997). In a subsequent study, the effects of As, Cr, Cu, Fe, Ni, V, and Zn on the MAPK, extracellular receptor kinase (ERK), c-jun N-terminal kinase (JNK), and P38 in BEAS cells were investigated (Samet et al., 1998). Arsenic, V, and Zn induced a rapid phosphorylation of MAPK in BEAS cells. Activity assays confirmed marked activation of ERK, JNK, and P38 in BEAS cells exposed to As, V, and Zn; Cr and Cu exposure resulted in a relatively small activation of MAPK; whereas Fe and Ni did not activate MAPK. Similarly, the transcription factors c-Jun and ATF-2, substrates of JNK and P38, respectively, were markedly phosphorylated in BEAS cells treated with As, Cr, Cu, V, and Zn. The same acute exposure to As, V, or Zn that activated MAPK was sufficient to induce a subsequent increase in IL-8 protein expression in BEAS cells. All exposures were non-cytotoxic based on measurement of lactate dehydrogenase release and microscopic examination of trypan blue or propidium iodide exclusion (Samet et al., 1996). These data suggest that MAPK may mediate metal-induced expression of inflammatory proteins in human bronchial epithelial cells. The ability of ROFA to induce activation of MAPKs in vivo was demonstrated by Silbajoris et al. (2000; see Table 7-3). In addition, Gercken et al. (1996) showed that the ROS production induced by PM was markedly decreased by the inhibition of protein kinase C as well as phospholipase A_2. Comparisons of in vitro and in vivo exposures of ROFA to airway epithelial cells requires consideration of in vivo dosimetry and ambient concentrations. Therefore, such extrapolations must be made with caution.

The major cellular response downstream of ROS and the cell signaling pathways described above is the production of inflammatory cytokines or other reactive mediators. In an effort to determine the contribution of cyclooxygenase to the pulmonary responses to ROFA exposure in vivo, Samet et al. (2000) intratracheally instilled Sprague-Dawley rats with ROFA (200 or 500 µg in 0.5 mL saline). These animals were pretreated ip with 1 mg/kg NS398, a specific prostaglandin H synthase 2 (COX2) inhibitor, 30 min prior to intratracheal exposure. At 12 h after intratracheal instillations, ip injections (1 mL of NS398 in 20% ethanol in saline) were repeated. ROFA treatment induced a marked increase in the level of PGE_2 recovered in the BAL fluid, which was effectively decreased by pretreating the animals with the COX2 inhibitor. Immunohistochemical analyses of rat airway showed concomitant expression of COX2 in the proximal airway epithelium of rats treated with soluble fraction of ROFA. This study further showed that, although COX2 products participated in ROFA induced lung inflammation, the

COX metabolites are not involved in IL-6 expression nor the influx of PMN influx into the airway. However, the rationale for the use of intraperitoneal challenge was not elaborated.

The production of cytokines and mediators also has been shown to depend on the type of PM used in the experiments. A549 cells (a human airway epithelial cell line) were exposed in vitro to several particulate materials: carbon black (CB, Elftex-12, Cabot Corp.), diesel soot from two sources (ND from NIST, LD produced from General Motors LH 6.2 V8 engine at light duty cycle), ROFA (from the heat exchange section of the Boston Edison), OAA (Ottawa ambient air PM, EHC-93), SiO_2, and Ni_3S_2 at 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 100, 300, 1,000 $\mu g/cm^2$ for 18 h (Seagrave and Nikula, 2000). Endpoints included loss of adherence to tissue culture substratum as evaluated by crystal violet staining, cell death measured by lactate dehydrogenase release, release of interleukin-8 (IL-8) measured by enzyme-linked immunosorbent assay, mitotic fraction and apoptosis, and release of alkaline phosphatase measured by enzymatic activity using paranitrophenol phosphate. Results indicated that (1) SiO_2 and Ni_3S_2 caused dose dependent acute toxicity and apototic changes; (2) ROFA and ND were acutely toxic only at the highest concentrations; (3) SiO_2 (30, 100, 300 $\mu g/cm^2$) and Ni_3S_2 (10, 30, 100, 300 $\mu g/cm^2$) increased IL-8 (three and eight times over the control, respectively) but suppressed IL-8 release at the highest concentration; (4) OAA and ROFA also induced IL-8 but to a lesser degree; and (5) both diesel soots suppressed IL-8 production. The authors speculated that the suppression of IL-8 release may contribute to increased respiratory disease as a result of decreased response to infectious agents. Silicon dioxide and Ni_3S_2 increased the release of alkaline phosphatase, a marker of toxic responses, only slightly. The less acutely toxic compounds caused significant release of alkaline phosphatase. The order of potency in alkaline phosphatase production is OAA > LD = ND > ROFA >> SiO_2 = Ni_3S_2. These results demonstrated that the type of particle used has a strong influence on the biological response.

Dye et al. (1999) carried out reverse transcriptase-polymerase chain reactions on RNA from rat tracheal epithelial cells to evaluate changes in steady-state gene expression of IL-6, MIP-2, and iNOS in cells exposed for 6 h to ROFA (5 $\mu g/cm^2$) and Ni, V, or Ni and V(water-soluble equivalent metal solution [pH 3.0]). Expression of MIP-2 and IL-6 genes was significantly upregulated as early as 6 h post-ROFA-exposure in rat tracheal epithelial cells; whereas gene expression of iNOS was maximally increased 24 h postexposure. Vanadium but not Ni appeared to be mediating the effects of ROFA on gene expression. Treatment with

dimethylthiourea (4 and 40 mm) inhibited both ROFA and V induced gene expression in a dose-dependent manner.

It appears that many biological responses are produced by PM whether it is composed of a single component or a complex mixture. The newly developed gene array monitors the expressions of many mediator genes that regulate complex and coordinated cellular events involved in tissue injury and repair. Using an array consisting of 27 rat genes representing inflammatory and anti-inflammatory cytokines, growth factors, adhesion molecules, stress proteins, metalloproteinases, vascular tone regulatory molecules, transcription factors, surfactant proteins and antioxidant enzymes, Nadadur et al. (2000) measured pulmonary effects in rats 3 and 24 h following intratracheal instillation of ROFA (3.3 mg/kg), $NiSO_4$ (1.3 μmol/kg), and VSO_4 (2.2 μmol/kg). Their data revealed a two- to three-fold increase in the expression of IL-6 and TIMP-1 at 24 h post-Ni exposure. The expression of cellular fibronectin (cFn-EIIIA) and iNOS increased 24 h following ROFA exposure. Cellular fibronectin, interferon, iNOS, ICAM-1 was increased 24 h following Ni exposure and IL-6 was increased 24 h postexposure in V exposed animals. There was a modest increase in the expression of SP-S and β-actin genes. There was a 2-fold increase in the expression of IL-6 24 h following exposure to ROFA, Ni, and V using the Northern blot analysis. A densitometric scan of an autoradiograph of blots stripped and reprobed with SP-A cDNA insert indicated a minimal increase in the expression of SP-A, both 3 and 24 h postexposure in all test groups. The findings in this study suggest that gene array may provide a tool for screening the expression profile of tissue specific markers following exposure to PM. However, care should be taken in reviewing such findings because of the variations in dose, instillation versus inhalation, and the time-course for gene expression.

To investigate the interaction between respiratory cells and PM, Kobzik (1995) showed that scavenger receptors are responsible for AM binding of unopsonized PM and that different mechanisms mediate binding of carbonaceous dusts such as DPM. In addition, surfactant components can increase AM phagocytosis of environmental particles in vitro, but only slightly relative to the already avid AM uptake of unopsonized particles (Stringer and Kobzik, 1996). Respiratory tract epithelial cells are also capable of binding with PM to secrete cytokine IL-8. Using a respiratory epithelial cell line (A549), Stringer et al. (1996) found that binding of particles to epithelial cells was calcium-dependent for TiO_2 and Fe_2O_3, while α-quartz binding was not calcium dependent. In addition, as observed in AMs, PM binding by A549 cells also

was mediated by scavenger receptors, albeit those distinct from the heparin-insensitive acetylated-LDL receptor. Furthermore, α-quartz, but not TiO_2 or CAPs, caused a dose-dependent production of IL-8 (range 1 to 6 ng/mL), demonstrating a particle-specific spectrum of epithelial cell cytokine (IL-8) response.

7.4.3.3 Other Potential Cellular and Molecular Mechanisms

A potential mechanism involved in the alteration of surface tension may be related to changes in the expression of matrix metalloproteinases (MMPs), such as pulmonary matrilysin and gelatinase A and B, and tissue inhibitor of metalloproteinase (TIMP) (Su et al., 2000a,b). Sprague-Dawley rats exposed to ROFA by intratracheal injection (2.5 mg/rat) had increased mRNA levels of matrilysin, gelatinase A, and TIMP-1. Gelatinase B, not expressed in control animals, was increased significantly from 6 to 24 h following ROFA exposure. Alveolar macrophages, epithelial cells, and inflammatory cells were major cellular sources for the pulmonary MMP expression. The expression of Gelatinase B in rats exposed to the same dose of ambient PM (< 1.7 μm and 1.7 to 3.7 μm) collected from Washington, DC, was significantly increased as compared to saline control; whereas the expression of TIMP-2 was suppressed. Ambient PM between 3.7 and 20 μm also increased the Gelatinase B expression. Increases in MMPs, which degrade most of the extracellular matrix, suggest that ROFA and ambient PM can similarly increase the total pool of proteolytic activity to the lung and contribute in the pathogenesis of PM-induced lung injury. Since no control particles were used in this study, the results must be interpreted with caution because it is possible that any particle administered in high doses could have a similar effect.

The role of sensory nerve receptors in the initiation of PM inflammation has been delineated in a series of recent studies. Neuropeptide and acid-sensitive sensory irritant (i.e., capsaicin, VR1) receptors were first identified on human bronchial epithelial cells (i.e., BEAS-2B). To address whether PM could initiate airway inflammation through these acid sensitive sensory receptors, BEAS-2B cells were exposed to ROFA and responded with an immediate increase in $[Ca^{+2}]_i$ followed by a concentration-dependent release of inflammatory cytokine (i.e., IL-6, IL-8, TNFα) and their transcripts (Veronesi et al., 1999b). To test the relevance of neuropeptide or capsaicin VR1 receptors to these changes, BEAS-2B cells were pretreated with neuropeptide receptor antagonists or capsazepine (CPZ), the antagonist for the capsaicin (i.e.,

VR1) receptor. The neuropeptide receptor antagonists reduced ROFA-stimulated cytokine release by 25%-50%. However, pretreatment of cells with CPZ inhibited the immediate increases in $[Ca^{+2}]_i$, diminished transcript (i.e., IL-6, IL-8, TNFα) levels and reduced IL-6 cytokine release to control levels (Veronesi et al., 1999a). The above studies suggested that ROFA inflammation was mediated by acid sensitive VR1 receptors located on the sensory nerve fibers that innervate the airway and on epithelial target cells.

Colloidal particles carry an inherently negative surface charge (i.e., zeta potential) that attracts protons from their vaporous milieu. These protons form a neutralizing, positive ionic cloud around the individual particle (Hunter, 1981). Since VR1 irritant receptors respond to acidity (i.e., protonic charge), experiments were designed to determine if the surface charge carried by ROFA and other PM particles could biologically activate cells and stimulate inflammatory cytokine release. The mobility of ROFA particles was measured in an electrically charged field (i.e., micro-electrophoresis) microscopically and their zeta potential calculated. Next, synthetic polymer microspheres (SPM) (i.e., polymethacrylic acid nitrophenylacrylate microspheres) were prepared with attached carboxyl groups to yield SPM particles with a geometric diameter of 2 ± 0.1 and 6 ± 0.3 μm and with zeta potentials similar to ROFA (−29 + 0.9 mV) particles. These SPM acted as ROFA surrogates with respect to their size and surface charge, but lacked all other contaminants thought to be responsible for its toxicity (e.g., transition metals, sulfates, volatile organics and biologicals). Similar concentrations of SPM and ROFA particles were used to test BEAS-2B cells and mouse dorsal root ganglia (DRG) sensory neurons, both targets of inhaled PM. Equivalent degrees of biological activation (i.e., increase in intracellular calcium, $[Ca^{+2}]_i$, IL-6 release) occurred in both cell types in response to either ROFA or SPM, and both responses could be reduced by antagonists to VR1 receptors or acid-sensitive pathways. Neutrally charged SPM (i.e., zeta potential of 0 mV), however, failed to stimulate increases in $[Ca^{+2}]_i$ or IL-6 release (Oortgiesen et al., 2000). To expand on these data, a larger set of PM was obtained from urban (St. Louis, Ottawa), residential (wood stove), volcanic (Mt. St. Helen), and industrial (oil fly ash, coal fly ash) sources. Each PM sample was described physicochemically (i.e., size and number of particles, acidity, zeta potential) and used to test BEAS-2B epithelial cells. The resulting biological effect (i.e., increases in $[Ca^{+2}]_i$, IL-6 release) was related to their physicochemical characteristics. When examined by linear regression analysis, the only measured physicochemical property that correlated with increases in $[Ca^{+2}]_i$

and IL-6 release was the zeta potential of the visible particles ($r2 > 0.97$) (Veronesi et al., 2002b).

Together, the above studies demonstrate a plausible neurogenic basis for PM inflammation by which the proton cloud associated with negatively-charged colloidal PM particles can activate acid-sensitive VR1 receptors found on human airway epithelial cells and sensory terminals. This activation results in an immediate influx of calcium and release of inflammatory cytokines and neuropeptides, which proceed to initiate and sustain inflammatory events in the airways through the pathophysiology of neurogenic inflammation (Veronesi and Oortgiesen, 2001).

7.4.4 Specific Particle Size and Surface Area Effects

Most particles used in laboratory animal toxicology studies are greater than 0.1 μm in size. However, the enormous number and huge surface area of ultrafine particles highlight the likely importance of considering the size of the particle in assessing response. Ultrafine particles with a diameter of 20 nm, when inhaled at the same mass concentration, have a number concentration that is approximately 6 orders of magnitude higher than for a 2.5-μm diameter particle; particle surface area is also greatly increased (Table 7-11).

TABLE 7-11. 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	2400000	3016
0.1	19100	600
0.5	153	120
1	19	60
2.5	1.2	24

Source: Oberdörster (1996a).

Many studies assessed in 1996 PM AQCD (U.S. Environmental Protection Agency, 1996a), as well as here, suggest that the surface of particles or substances released from the surface (e.g., transition metals, organics) interact with biological substrates, and that surface-associated free radicals or free radical-generating systems may be responsible for toxicity. Thus, if ultrafine particles were to cause toxicity by a transition metal-mediated mechanism, for example, then the relatively large surface area for a given mass of ultrafine particles would imply high concentrations of transition metals being available to cause oxidative stress to cells.

Two groups have examined toxicity differences between fine and ultrafine particles, with the general finding that ultrafine particles show a significantly greater response at similar mass doses (Oberdörster et al., 1992; Li et al., 1996, 1997, 1999). However, only a few studies have investigated the ability of ultrafine particles to generate a greater oxidative stress when compared to fine particles of the same material. Studies by Gilmour et al. (1996) have shown that, at equal mass, ultrafine TiO_2 caused more plasmid DNA strand breaks than fine TiO_2. This effect could be inhibited with mannitol. Osier and Oberdörster (1997) compared the response of rats (F344) exposed by intratracheal inhalation to "fine" (~250 nm) and "ultrafine" (~21 nm) TiO_2 particles.

Consistent with these in vivo studies, Finkelstein et al. (1997) has shown that exposing primary cultures of rat Type II cells to 10 µg/mL ultrafine TiO_2 (20 nm) causes increased TNF and IL-1 release throughout the entire 48-h incubation period. In contrast, fine TiO_2 (200 nm) had no effect. In addition, ultrafine polystyrene carboxylate-modified microspheres (UFP, fluorospheres, molecular probes 44 ± 5 nm) have been shown to induce a significant enhancement of both substance P and histamine release after administration of capsaicin (10^{-4} M), to stimulate C-fiber, and carbachol (10^{-4} M), a cholinergic agonist in rabbit intratracheally instilled with UFP (Nemmar et al., 1999). A significant increase in histamine release also was recorded in the UFP-instilled group following the administration of both Substance P (10^{-6} M) plus thiorpan (10^{-5} M) and compound 48/80 (C48/80, 10^{-3} M) to stimulate mast cells. Bronchoalveolar lavage analysis showed an influx of PMN, an increase in total protein concentration, and an increase in lung wet weight/dry weight ratio. Electron microscopy showed that both epithelial and endothelial injuries were observed. The pretreatment of rabbits in vivo with a mixture of either SR 140333 and SR 48368, a tachykinin NK_1 and NK_2 receptor antagonist, or a mixture of terfenadine and cimetidine, a histamine H_1 and H_2 receptor antagonist, prevented UFP-induced PMN influx and increased protein and lung WW/DW ratio.

It is believed that ultrafine particles cause greater cellular injury because of the relatively large surface area for a given mass. In addition, the fate of ultrafines after deposition is also different in that they interact more rapidly with epithelial target cells rather than to be phagocytized by alveolar macrophages. However, in a study that compared the response to carbon black particles of two different sizes, Li et al. (1999) demonstrated that in the instillation model, a localized dose of particle over a certain level causes the particle mass to dominate the response, rather than the surface area. Ultrafine carbon black (ufCB, Printex 90), 14 nm in diameter, and fine carbon black (CB, Huber 990), 260 nm in diameter, were instilled intratracheally in rats, and BAL profile at 6 h was assessed. At mass of 125 µg or below, ufCB generated a greater response (increase LDH, epithelial permeability, decrease in GSH, TNF, and NO production) than fine CB at various times postexposure. However, higher doses of CB caused more PMN influx than the ufCB. In contrast to the effect of CB, which showed dose-related increasing inflammatory response, ufCB at the highest dose caused less of a neutrophil influx than at the lower dose, confirming earlier work by Oberdörster et al. (1992). Moreover, when the PMN influx was expressed as a function of surface area, CB produced greater response than ufCB at all doses used in this study. Although particle insterstitialization with a consequent change in the chemotatic gradient for PMN was offered as an explanation, these results need further scrutiny. Moreover, these findings imply that mass is relatively less important than surface area and that the latter metric may be more useful for assessing PM toxicity. However, it is unclear if this finding is restricted to the particular endpoints addressed and/or carbon black, the PM compound studied.

Oberdörster et al. (2000) reported on a series of studies in rats and mice using ultrafine particles of various chemical composition. In rats sensitized with endotoxin (70 EU) and exposed to ozone (1 ppm) plus ultrafine carbon particles (~100 µg/m^3), they found a nine-fold greater release of reactive oxygen species in old rats (20 mo) than in similarly treated young rats (10 wk). Exposure to ultrafine PM alone in sensitized old rats also caused an inflammatory response.

Although the potential mechanisms of ultrafine-induced lung injury remain unclear, it is likely that ultrafine particles, because of their small size, are not effectively phagocytized by alveolar macrophages and can easily penetrate the airway epithelium, gaining access to the interstitium. Using electron microscopy, Churg et al. (1998) examined particle uptake in rat

tracheal explants. Explants were submerged in a 5 mg/mL suspension of either fine (0.12 μm) or ultrafine (0.021 μm) TiO_2 particles in Dulbecco's minimal Eagle's medium, without serum and examined after 3 or 7 days. They found both size particles in the epithelium at both time points; but, in the subepithelial tissues, only at day 7. The volume proportion (the volume of TiO_2 over the entire volume of epithelium or subepithelium area) of both fine and ultrafine particles in the epithelium increased from 3 to 7 days. It was greater for ultrafine at 3 days but was greater for fine at 7 days. The volume proportion of particles in the subepithelium at day 7 was equal for both particles, but the ratio of epithelial to subepithelial volume proportion was 2:1 for fine and 1:1 for ultrafine. Ultrafine particles persisted in the tissue as relatively large aggregates; whereas the size of fine particle aggregates became smaller over time. Ultrafine particles appeared to enter the epithelium faster and, once in the epithelium, a greater proportion of them were translocated to the subepithelial space compared to fine particles. However, the authors assumed that the volume proportion is representative of particle number and the number of particles reaching the interstitial space is directly proportional to the number applied (i.e., there is no preferential transport from lumen to interstitium by size). These data are in contrast to the results of instillation or inhalation of fine and ultrafine TIO_2 particles reported earlier (Ferin et al., 1990, 1992). However, the explant and intratracheal instillation test systems differ in many aspects, making direct comparisons difficult. Limitations of the explant test system include traumatizing the explanted tissue, introducing potential artifacts through the use of liquid suspension for exposure, the absence of inflammatory cells, and possible overloading of the explants with dust.

Only two studies examined the influence of specific surface area on biological activity (Lison et al., 1997; Oettinger et al., 1999). The biological responses to various MnO_2 dusts with different specific surface area (0.16, 0.5, 17, and 62 m^2/g) were compared in vitro and in vivo (Lison et al., 1997). In both systems, the results show that the amplitude of the response is dependent on the total surface area that is in contact with the biological system, indicating that surface chemistry phenomena are involved in the biological reactivity. Freshly ground particles with a specific surface area of 5 m^2/g also were examined in vitro. These particles exhibited an enhanced cytotoxic activity that was almost equivalent to that of particles with a specific surface area of 62 m^2/g, indicating that undefined reactive sites produced at the particle surface by mechanical cleavage also may contribute to the toxicity of insoluble particles.

In another study (Oettinger et al., 1999), two types of carbon black particles were used: (1) Printex 90 or P90 (formed by controlled combustion and consisting of defined granules with specific surface area of 300 m^2/g and particle size of 14 nm) is predominantly loaded with metallic components (< 100 ppm Fe; < 50 ppm Pb; < 10 ppm Se; < 10 ppm As; < 10 ppm Zn); and (2) soot FR 101 (with specific surface area of 20 m^2/g, particle size of < 95 nm) has the ability to adsorb polycyclic and other carbons. Exposure of AMs to 100 $\mu g/10^6$ cells of FR 101 and P90 resulted in a 1.4- and 2.1-fold increase in ROS release, respectively. These exposures also caused a fourfold up-regulation of NF-κB gene expression. This suggests that carbon particles with larger surface area produce greater biological response than carbon particles with smaller surface area. Another study by Schluter et al. (1995), showed that by exposing bovine AMs to metal oxide coated silica particles, most of the metal coatings (As, Ce, Fe, Mn, Ni, Pb, and V) had no effect on ROS production by these cells. However, coating with CuO markedly lowered the O_2^- and H_2O_2, whereas V(IV) increases both reactive oxygen intermediates (ROI). This study demonstrated that, in addition to specific surface area, chemical composition of the particle surface also influences its cellular response.

Thus, ultrafine particles apparently have the potential to significantly contribute to the adverse effects of PM. These studies, however, have not considered the portion of ambient ultrafine particles that are not solid in form. Droplets (e.g., sulfuric acid droplets) and organic based ultrafine particles do exist in the ambient environment; they can spread, disperse, or dissolve after contact with liquid surface layers and may thereby contribute further to PM-related effects.

7.5 FACTORS AFFECTING SUSCEPTIBILITY TO PARTICULATE MATTER EXPOSURE EFFECTS

Susceptibility of an individual to adverse health effects of PM can vary depending on a variety of host factors such as age, physiological activity profile, genetic predisposition, or preexistent disease. The potential for preexistent disease to alter pathophysiological responses to toxicant exposure is widely acknowledged but poorly understood. Epidemiologic studies have demonstrated that the effects of PM exposure tend to be more evident in populations with pre-existing disease; and it is logical that important mechanistic differences may exist among these populations. However, because of inherent variability (necessitating large numbers of subjects) and ethical concerns associated with using diseased subjects in clinical research studies, a solid database on human susceptibilities is lacking. For more control over both environmental and host variables, animal models are often used. Many laboratory studies have demonstrated alterations in a variety of endpoints in experimental animals following exposure to laboratory-generated particles. These findings (e.g., increased pulmonary inflammation, increased airway resistance, and decrements in pulmonary host defenses) may be of limited value because of uncertainties in extrapolating between the laboratory-generated particles and actual ambient air particle mixes. Thus, care must be taken in extrapolation from animal models of human disease to humans. Rodent models of human disease, their use in toxicology, and the criteria for judging their appropriateness as well as their limitations must be considered (Kodavanti et al., 1998b; Kodavanti and Costa, 1999; Costa, 2000; Conn et al., 2000; Bice et al., 2000; Mauderly et al., 2000; Muggenberg et al., 2000).

7.5.1 Pulmonary Effects of Particulate Matter in Compromised Hosts

Epidemiologic studies suggest that there may be subsegments of human populations that are especially susceptible to effects from inhaled particles (see Chapter 8). The elderly with chronic cardiopulmonary disease, those with pneumonia and possibly other lung infections, and those with asthma (at any age) appear to be at higher risk than healthy people of similar age. Apropos to this, although many of the newly available toxicology studies used healthy adult animals, a growing number of other newer studies examined effects of ambient or surrogate particles in compromised host models. For example, Costa and Dreher (1997) used a rat model of cardiopulmonary disease to explore the question of susceptibility and possible mechanisms by

which PM effects are potentiated. Rats with advanced monocrotaline (MCT)-induced pulmonary vasculitis/hypertension were given intratracheal instillations of ROFA (0, 0.25, 1.0, and 2.5 mg/rat). The MCT animals had a marked neutrophilic inflammation. In the context of this inflammation, ROFA induced a four- to fivefold increase in BAL PMNs. There was increased mortality at 96 h that was ROFA-dose dependent. The results of this study suggest that particles, albeit at a high concentration, may enhance mortality in MCT-compromised animals.

As discussed previously, Kodavanti et al. (1999) also studied PM effects in the MCT rat model of pulmonary disease. Rats treated with 60 mg/kg MCT were exposed to 0, 0.83 or 3.3 mg/kg ROFA by intratracheal instillation and to 15 mg/m^3 ROFA by inhalation. Both methods of exposure caused inflammatory lung responses; and ROFA exacerbated the lung lesions, as shown by increased lung edema, inflammatory cells, and alveolar thickening.

The manner in which MCT can alter the response of rats to inhaled particles was examined by Madl and colleagues (1998). Rats were exposed to fluorescent colored microspheres (1 µm) 2 weeks after treatment with MCT. In vivo phagocytosis of the microspheres was altered in the MCT rats in comparison with control animals. Fewer microspheres were phagocytized in vivo by alveolar macrophages, and there was a concomitant increase in free microspheres overlaying the epithelium at airway bifurcations. The decrease in in vivo phagocytosis was not accompanied by a similar decrease in vitro. Macrophage chemotaxis, however, was impaired significantly in MCT rats compared with control rats. Thus, MCT appeared to impair particle clearance from the lungs via inhibition of macrophage chemotaxis.

Respiratory infections are common in all individuals. The infections are generally cleared quickly, depending on the virulence of the organism; however, in individuals with immunologic impairment or lung diseases such a COPD, the residence time in the lung is extended. A variety of viral and bacterial agents have been used to develop infection models in animals. Viral infection models primarily use mice and rats. The models focus on the proliferation and clearance of the microorganisms and the associated pulmonary effect. The models range from highly virulent and lethal (influenza A/Hong Kong/8/68, H3N2) to nonlethal (rat-adapted influenza virus model [RAIV]). The lethal model terminates in extensive pneumonia and lung consolidation. Less virulent models (A/Port Chalmers/1/73 and H3N2) exhibit airway epithelial damage and immune responses. The non-lethal model exhibits airway reactivity that subsides,

with recovery being complete in about 2 weeks (Kodavanti et al., 1998b). Bacterial infection models mimic the chronic bacterial infections experienced by humans with other underlying disease conditions. The models develop signs similar to those in humans but to a milder degree. To mimic the more chronic infections, the bacteria are encased in agar beads to prevent rapid clearance. Generally, the models involve pre-exposure to the irritant followed by the bacterial challenge. More recently, bacterial infection models have involved pre-exposure by the bacteria followed by exposure to the irritant (Kodavanti et al., 1998b).

Elder et al. (2000a,b) exposed 8 week to 22 month old Fischer 344 rats and 14- to 17-month-old T_{sk} mice to 100 $\mu g/m^3$ of ultrafine carbon (UF) and/or 1.0 ppm O_3 for six hours following a 12 minute exposure to a low dose (70 EU) of endotoxin (lipopolysaccharide, LPS). The ultrafine carbon had a small effect on lung inflammation and inflammatory cell activation. The effects were enhanced in the compromised lung and in older animals. The greatest effect was in the compromised lung exposed to both ultrafine carbon and ozone.

Chronic bronchitis is the most prevalent of the COPD-related illnesses. In humans, chronic bronchitis is characterized by pathologic airway inflammation and epithelial damage, mucus cell hyperplasia and hypersecretion, airway obstruction and in advance cases, airway fibrosis. The most widely used animal models of bronchitis (rat and dog) are those produced by subchronic exposure to high concentrations of SO_2 (150 to 600 ppm) for 4 to 6 weeks. Exposure to SO_2 produces changes in the airways similar to those of chronic bronchitis in humans. There is an anatomical difference between the rat and the human in the absence of submucosal glands in the rat. However, like humans, rats exhibit increased airway responsiveness to inhaled bronchoconstricting agonists. Sulfur dioxide-induced lesions include increased numbers of epithelial mucus-producing cell, loss of cilia, airway inflammation, increased pro-inflammatory cytokine expression, and thickening of the airway epithelium. When the cause of the chronic bronchitis is removed, the pathology slowly reverses. The time course and the extent of reversal differs between the human and rodent. Consequently, care should be exercised when applying this model (Kodavanti et al., 1998b).

The sulfur dioxide (SO_2)-induced model of chronic bronchitis has been used to examine the potential interaction of PM with preexisting lung injury. Clarke and colleagues pretreated Sprague-Dawley rats for 6 weeks with air or 170 ppm SO_2 for 5 h/day and 5 days/week (Clarke et al., 1999; Saldiva et al., 2002). Exposure to concentrated ambient air particles (CAPs) for

5 h/day for 3 days to concentrations ranging from 73.5 to 733 μg/m^3 produced significant changes in both cellular and biochemical markers in lavage fluid. In comparison to control animal values, protein was increased approximately threefold in SO_2-pretreated animals exposed to concentrated ambient PM. Lavage fluid neutrophils and lymphocytes were increased significantly in both groups of rats exposed to concentrated ambient PM, with greater increases in both cell types in the SO_2-pretreated rats. Thus, exposure to concentrated ambient PM produced adverse changes in the respiratory system, but no deaths, in both normal rats and in a rat model of chronic bronchitis.

Clarke et al. (2000b) next examined the effect of concentrated ambient PM from Boston, MA, in normal rats of different ages. Unlike the earlier study that used Sprague-Dawley rats, 4- and 20-mo-old Fischer 344 rats were examined after exposure to concentrated ambient PM for 5 h/day for 3 consecutive days. They found that exposure to the daily mean concentrations of 80, 170, and 50 μg/m^3 PM, respectively, produced statistically significant increases in total neutrophil counts (over 10-fold) in lavage fluid of the young, but not the old, rats. Thus, repeated exposure to relatively low concentrations of ambient PM produced an inflammatory response, although the actual percent neutrophils in the concentrated ambient PM-exposed young adult rats was low (approximately 3%). On the other hand, Gordon et al. (2000) found no evidence of neutrophil influx in the lungs of normal and monocrotaline-treated Fischer 344 rats exposed in nine separate experiments to concentrated ambient PM from New York, NY at concentrations as high as 400 μg/m^3 for a 6-h exposure or 192 μg/m^3 for three daily 6-h exposures. Similarly, normal and cardiomyopathic hamsters showed no evidence of pulmonary inflammation or injury after a single exposure to the same levels of concentrated ambient PM. Gordon and colleagues did report a statistically significant doubling in protein concentration in lavage fluid in monocrotaline-treated rats exposed for 6 h to 400 μg/m^3 concentrated ambient PM.

Kodavanti and colleagues (1998b) also have examined the effect of concentrated ambient PM in normal rats and rats with sulfur dioxide-induced chronic bronchitis. Among the four separate exposures to PM, there was a significant increase in lavage fluid protein in bronchitic rats from only one exposure protocol in which the rats were exposed to 444 and 843 μg/m^3 PM on 2 consecutive days (6 h/day). Neutrophil counts were increased in bronchitic rats exposed to concentrated ambient PM in three of the four exposure protocols, but was decreased in the fourth

protocol. No other changes in normal or bronchitic rats were observed, even in the exposure protocols with higher PM concentrations. Thus, rodent studies have demonstrated that inflammatory changes can be produced in normal and compromised animals exposed to concentrated ambient PM. These findings are important because only a limited number of studies have used real-time inhalation exposures to actual ambient urban PM.

Pulmonary function measurements are often less invasive than other means to assess the effects of inhaled air pollutants on the mammalian lung. After publication of the 1996 PM AQCD, a number of investigators examined the response of rodents and dogs to inhaled ambient particles. In general, these investigators have demonstrated that ambient PM has minimal effects on pulmonary function. Gordon et al. (2000) exposed normal and monocrotaline-treated rats to filtered air or 181 $\mu g/m^3$ concentrated ambient PM for 3 h. For both normal and monocrotaline-treated rats, no differences in lung volumes or diffusing capacities for carbon monoxide were observed between the air or PM exposed animals at 3 or 24 h after exposure. Similarly, in cardiomyopathic hamsters, concentrated ambient PM had no effect on these same pulmonary function measurements.

Other pulmonary function endpoints have been studied in animals exposed to concentrated ambient PM. Clarke et al. (1999) observed that tidal volume was increased slightly in both control rats and rats with sulfur dioxide-induced chronic bronchitis exposed to 206 to 733 $\mu g/m^3$ PM on 3 consecutive days. No changes in peak expiratory flow, respiratory frequency, or minute volume were observed after exposure to concentrated ambient PM. In the series of dog studies by Godleski et al. (2000) (also see Section 7.3), no signficant changes in pulmonary function were observed in normal mongrel dogs exposed to concentrated ambient PM, although a 20% decrease in respiratory frequency was observed in dogs that underwent coronary artery occlusion and were exposed to PM. Thus, studies using normal and compromised animal models exposed to concentrated ambient PM have found minimal biological effects of ambient PM on pulmonary function.

Johnston et al. (1998) exposed 8-week-old mice (young) and 18-mo-old mice (old) to polytetrafluoroethylene (PTFE) fumes (0, 10, 25, and 50 $\mu g/m^3$) for 30 min. Lung lavage endpoints (PMN, protein, LDH, and β-glucuronidase) as well as lung tissue mRNA levels for various cytokines, metallothionein and for Mn superoxide dismutase were measured 6 h following exposure. Protein, lymphocyte, PMN, and TNF-α mRNA levels were increased in

older mice when compared to younger mice. These findings suggest that the inflammatory response to PTFE fumes is altered with age, being greater in the older animals. Although ultrafine PTFE fumes are not a valid surrogate for ambient ultrafine particles (Oberdörster et al., 1992), this study provides evidence supporting the hypothesis that particle-induced pulmonary inflammation differs between young and old mice. Other studies on age-related PM effects are described in Section 7.6 (Responses to PM and Gaseous Pollutant Mixtures).

Kodavanti et al. (2000b; 2001) used genetically predisposed spontaneously hypertensive (SH) rats as a model of cardiovascular disease to study PM-related susceptibility. The SH rats were found to be more susceptible to acute pulmonary injury from intratracheal ROFA exposure than normotensive control Wistar Kyoto (WKY) rats (Kodavanti et al., 2001). The primary metal constituents of ROFA, V and Ni, caused differential species-specific effects. Vanadium, which was less toxic than Ni in both strains, caused inflammatory responses only in WKY rats; whereas Ni was injurious to both WKY and SH rats (SH > WKY). This differential responsiveness of V and Ni was correlated with their specificity for airway and parenchymal injury, discussed in another study (Kodavanti et al., 1998b). When exposed to the same ROFA by inhalation (15 mg/m^3, 6 h/d, 3 days), SH rats were more sensitive than WKY rats in regards to vascular leakage (Kodavanti et al., 2000b). The SH rats exhibited a hemorrhagic response to ROFA. Oxidative stress was much higher in ROFA exposed SH rats than matching WKY rats. Also, SH rats, unlike WKY rats, showed a compromised ability to increase BAL glutathione in response to ROFA, suggesting a potential link to increased susceptibility. However, lactate dehydrogenase and n-acetylglucosaminidase activities were higher in WKY rats. Lactate dehydrogenase was slightly higher in SH rats instilled with ROFA (Kodavanti et al., 2001). Cardiovascular effects were characterized by ST-segment area depression of the ECG in ROFA-exposed SH but not WKY rats. When the same rats were exposed to ROFA by inhalation to 15 mg/m^3, 6 h/d, 3 d/wk for 1, 2, or 4 wk compared to intratracheal exposure to 0, 1.0, 5.0 mg/kg in saline (Kodavanti et al., 2002), differences in effects were dependent on the length of exposure. After acute exposure, increased plasma fibrinogen was associated with lung injury; longer-term, episodic ROFA exposure resulted in progressive protein leakage and inflammation that was significantly worse in SH rats when compared to WKY rats. These studies demonstrate the potential utility of cardiovascular disease models for the study of PM health effects and show

that genetic predisposition to oxidative stress and cardiovascular disease may play a role in increased sensitivity to PM-related cardiopulmonary injury.

On the basis of in vitro studies, Sun et al. (2001) predicted that the antioxidant and lipid levels in the lung lining fluid may determine susceptibility to inhaled PM. In a subsequent study from the same laboratory, Norwood et al. (2001) conducted inhalation studies on guinea pigs to test this hypothesis. On the basis of dietary supplementation or depletion of ascorbic acid and glutathione (GSH) the guinea pigs were divided into four groups: (+C + GSH), (+C – GSH), (–C + GSH), and (–C – GSH). All groups were exposed (nose-only) to clean air or 19-25 mg/m^3 ROFA (< 2.5 μm) for 2 h. Nasal lavage and BAL fluid and cells were examined at 0 h and 24 h postexposure. Exposure to ROFA increased lung injury in the (-C-GSH) group only (as shown by increased BAL fluid protein, LDH, and PMNs and decreased BAL macrophages) and resulted in lower antioxidant concentrations in BAL fluid than were found with single deficiencies.

In summary, although more of these studies are just beginning to emerge and are only now being replicated or followed more thoroughly to investigate underlying mechanisms, they do provide evidence suggestive of enhanced susceptibility to inhaled PM in "compromised" hosts.

7.5.2 Genetic Susceptibility to Inhaled Particles and their Constituents

A key issue in understanding adverse health effects of inhaled ambient PM is identification of which classes of individuals are susceptible to PM. Although factors such as age and health status have been studied in both epidemiology and toxicology studies, some investigators have begun to examine the importance of genetic susceptibility in the response to inhaled particles because of evidence that genetic factors play a role in the response to inhaled pollutant gases. To accomplish this goal, investigators typically have studied the interstrain response to particles in rodents. The response to ROFA instillation in different strains of rats has been investigated by Kodavanti et al. (1996, 1997a). In the first study, male Sprague-Dawley (SD) and Fischer-344 (F-344) rats were instilled intratracheally with saline or ROFA particles (8.3 mg/kg). ROFA instillation produced an increase in lavage fluid neutrophils in both SD and F-344 rats; whereas a time-dependent increase in eosinophils occurred only in SD rats. In the subsequent study (Kodavanti et al., 1997a), SD, Wistar (WIS), and F-344 rats (60 days old) were exposed to saline or ROFA (8.3 mg/kg) by intratracheal instillation and examined for up to 12 weeks. Histology

indicated focal areas of lung damage showing inflammatory cell infiltration as well as alveolar, airway, and interstitial thickening in all three rat strains during the week following exposure. Trichrome staining for fibrotic changes indicated a sporadic incidence of focal alveolar fibrosis at 1, 3, and 12 weeks in SD rats; whereas WIS and F-344 rats showed only a modest increase in trichrome staining in the septal areas. One of the isoforms of fibronectin mRNA was upregulated in ROFA-exposed SD and WIS rats, but not in F-344 rats. Thus, in rats there appears to be a genetic based difference in susceptibility to lung injury induced by instilled ROFA.

Differences in the degree of pulmonary inflammation have been described in rodent strains exposed to airborne pollutants. To understand the underlying causes, signs of airway inflammation (i.e., airway hyper-responsiveness, inflammatory cell influx) were established in responsive (BABL/c) and non-responsive (C57BL/6) mouse strains exposed to ROFA (Veronesi et al., 2000). Neurons taken from the ganglia (i.e., dorsal root ganglia) that innervate the nasal and upper airways were cultured from each mouse strain and exposed to 25 or 50 µg/mL ROFA for 4 h. The difference in inflammatory response noted in these mouse strains in vivo was retained in culture, with C57BL/6 neurons showing significantly lower signs of biological activation (i.e., increased intracellular calcium levels) and cytokine (i.e., IL-6, IL-8) release relative to BALB/c mice. RT-PCR and immunocytochemistry indicated that the BALB/c mouse strain had a significantly higher number of neuropeptide and acid-sensitive (i.e., NK1, VR1) sensory receptors on their sensory ganglia relative to the C57BL/6 mice. Such data indicate that genetically-determined differences in sensory inflammatory receptors can influence the degree of PM-induced airway inflammation.

Kleeberger and colleagues have examined the role that genetic susceptibility plays in the effect of inhaled acid-coated particles on macrophage function. Nine inbred strains of mice were exposed nose-only to very high doses of carbon particles coated with acid (10 mg/m^3 carbon with 285 µg/m^3 sulfate) for 4 h (Ohtsuka et al., 2000a). Significant inter-strain differences in Fc-receptor-mediated macrophage phagocytosis were seen with C57BL/6J mice being the most sensitive. Although neutrophil counts were increased more in C3H/HeOuJ and C3H/HeJ strains of mice than in the other strains, the overall magnitude of change was small and not correlated with the changes in macrophage phagocytosis. In follow-up studies using the same type particle, Ohtsuka et al. (2000a,b) performed a genome-wide scan with an intercross cohort derived from

C57BL/6J and C3H/HeJ mice. Analyses of phenotypes of segregant and nonsegregant populations derived from these two strains indicate that two unlinked genes control susceptibility. They identified a 3-centiMorgan segment on mouse chromosome 17 which both contains an acid-coated particle susceptibility locus. Interestingly, this quantitative trait locus (a) overlaps with those described for ozone-induced inflammation (Kleeberger et al., 1997) and acute lung injury (Prows et al., 1997) and (b) contains several promising candidate genes that may be responsible for the observed genetic susceptibility for macrophage dysfunction in mice exposed to acid-coated particles.

Leikauf and colleagues (Leikauf et al., 2000; Wesselkamper et al., 2000; McDowell et al., 2000; Prows and Leikauf, 2001; Leikauf et al., 2001) have identified a genetic susceptibility in mice that is associated with mortality following exposures to high concentrations (from 15 to 150 $\mu g/m^3$) of a "$NiSO_4$" aerosol (0.22 μm MMAD) for up to 96 h. These studies also have preliminarily identified the chromosomal locations of a few genes that may be responsible for this genetic susceptibility. This finding is particularly significant in light of the toxicology studies demonstrating that bioavailable, first-row transition metals participate in acute lung injury following exposure to emission and ambient air particles. Similar genes may be involved in human responses to particle-associated metals; but additional studies are needed to determine whether the identified metal susceptibility genes are involved in human responses to ambient levels of particulate-associated metals.

One study has examined the interstrain susceptibility to ambient particles. C57BL/6J and C3H/HeJ mice were exposed to 250 $\mu g/m^3$ concentrated ambient $PM_{2.5}$ for 6 h and examined at 0 and 24 h after exposure for changes in lavage fluid parameters and cytokine mRNA expression in lung tissue (Shukla et al., 2000). No interstrain differences in response were observed. Surprisingly, although no indices of pulmonary inflammation or injury were increased over control values in the lavage fluid, increases in cytokine mRNA expression were observed in both murine strains exposed to $PM_{2.5}$. Although the increase in cytokine mRNA expression was generally small (approximately twofold), the effects on IL-6, TNF-α, TGF-β2, and γ-interferon were consistent.

Thus, a handful of studies have begun to demonstrate that genetic susceptibility can play a role in the response to inhaled particles. However, the doses of PM administered in these studies, whether by inhalation or instillation, were extremely high when compared to ambient

PM levels. Similar strain differences in response to inhaled metal particles have been observed by other investigators (McKenna et al., 1998; Wesselkamper et al., 2000), although the concentration of metals used in these studies were also more relevant to occupational rather than environmental exposure levels. The extent to which genetic susceptibility plays as significant a role in the adverse effects of ambient PM as does age or health status remains to be determined.

7.5.3 Particulate Matter Effects on Allergic Hosts

Relatively little is known about the effects of inhaled particles on humoral (antibody) or cell-mediated immunity. Alterations in the response to a specific antigenic challenge have been observed in animal models at high concentrations of acid sulfate aerosols (above 1,000 $\mu g/m^3$) (Pinto et al., 1979; Kitabatake et al., 1979; Fujimaki et al., 1992). Several studies have reported an enhanced response to nonspecific bronchoprovocation agents, such as acetylcholine and histamine, after exposure to inhaled particles. This nonspecific airway hyperresponsiveness, a central feature of asthma, occurs in animals and human subjects exposed to sulfuric acid under controlled conditions (Utell et al., 1983; Gearhart and Schlesinger, 1986). Although, its relevance to specific allergic responses in the airways of atopic individuals is unclear, it demonstrates that the airways of asthmatics may become sensitized to either specific or nonspecific triggers that could result in increases in asthma severity and asthma-related hospital admissions (Peters et al., 1997; Jacobs et al., 1997; Lipsett et al., 1997). Combustion particles also may serve as carrier particles for allergens (Knox et al., 1997).

A number of in vivo and in vitro studies have demonstrated that diesel particles (DPM) can alter the immune response to challenge with specific antigens and suggest that DPM may act as an adjuvant. These studies have shown that treatment with DPM enhances the secretion of antigen-specific IgE in mice (Takano et al., 1997) and in the nasal cavity of human subjects (Diaz-Sanchez et al., 1996, 1997; Ohtoshi et al., 1998; Nel et al., 2001). Because IgE levels play a major role in allergic asthma (Wheatley and Platts-Mills, 1996), upregulation of its production could lead to an increased response to inhaled antigen in particle-exposed individuals.

Van Zijverden et al. (2000) and Van Zijverdan and Granum (2000) used mouse models to assess the potency of particles (diesel, carbon black, silica) to adjuvate an immune response to a protein antigen. All types of particles exerted an adjuvant effect on the immune response to co-administered antigen, apparently stimulated by the particle core rather than the attached chemical

factors. Different particles, however, stimulated distinct types of immune responses. In one model (Van Zijverden et al., 2001), BALB/c mice were intranasally treated with a mixture of antigen (model antigen TNP-Ovalbumin, TNP-OVA) and particles on three consecutive days. On day 10 after sensitization, mice were challenged with the antigen TNP-OVA alone, and five days later the immune response was assessed. Diesel particulate matter, as well as carbon black particles (CB), were capable of adjuvating the immune response to TNP-OVA as evidenced by an increase of TNP-specific antibody (IgG1 and IgE) secreting B cells antibodies in the lung-draining lymph nodes. Increased antigen-specific IgG1, IgG2a, and IgE isotypes were measured in the serum, indicating that the response resulted in systemic sensitization. Importantly, an increase of eosinophils in the bronchio-alveolar lavage was observed with CB. Companion studies with the intranasal exposure model showed that the adjuvant effect of CB particles was even more pronounced when the particles were given during both the sensitization and challenge phases; whereas administration during the challenge phase caused only marginal changes in the immune response. These data show that PM can increase both the sensitization and challenge responses to a protein antigen, and the immune stimulating activity of particles appears to be a time-dependent process, suggesting that an inflammatory microenvironment (such as may be created by the particles) is crucial for enhancing sensitization by particles.

Only a small number of studies have examined mechanisms underlying the enhancement of allergic asthma by ambient urban particles. Ohtoshi et al. (1998) reported that a coarse size-fraction of resuspended ambient PM, collected in Tokyo, induced the production of granulocyte macrophage colony stimulating factor (GMCSF), an upregulator of dendritic cell maturation and lymphocyte function, in human airway epithelial cells in vitro. In addition to increased GMCSF, epithelial cell supernatants contained increased IL-8 levels when incubated with DPM, a principal component of ambient particles collected in Tokyo. Although the sizes of the two types of particles used in this study were not comparable, the results suggest that ambient PM, or at least the DPM component of ambient PM, may be able to upregulate the immune response to inhaled antigen through GMCSF production. Similarly, Takano et al. (1998) has reported airway inflammation, airway hyperresponsiveness, and increased GMGSF and IL-5 in mice exposed to diesel exhaust.

In a study by Walters et al. (2001), PM_{10} was found to induce airway hyperresponsiveness, suggesting that PM exposure may be an important factor contributing to increases in asthma

prevalence. Naive mice were exposed to a single dose (0.5 mg/ mouse) of ambient PM, coal fly ash, or diesel PM. Exposure to PM_{10} induced increases in airway responsiveness and BAL cellularity; whereas diesel PM induced significant increases in BAL cellularity, but not airway responsiveness. On the other hand, coal fly ash exposure did not elicit significant changes in either of these parameters. Ambient PM-induced airway hyperresponsiveness was sustained over 7 days. The increase in airway responsiveness was preceded by increases in BAL eosinophils; whereas a decline in airway responsiveness was associated with increases in macrophages. Thus, ambient PM can induce asthma-like parameters in naive mice.

Several other studies have examined in greater detail the contribution of the particle component and the organic fraction of DPM to allergic asthma. Tsien et al. (1997) treated transformed IgE-producing human B lymphocytes in vitro with the organic extract of DPM. The organic phase extraction had no effect on cytokine production but did increase IgE production. In these in vitro experiments, DPM appeared to be acting on cells already committed to IgE production, thus suggesting a mechanism by which the organic fraction of combustion particles can directly affect B cells and influence human allergic asthma.

Cultured epithelial cells from atopic asthmatics show a greater response to DPM exposure when compared with cells from nonatopic nonasthmatics. IL-8, GM-CSF, and soluble ICAM-1 increased in response to DPM at a concentration of 10 µg/mL DPM (Bayram et al., 1998a,b). This study suggests that particles could modulate airway disease through their actions on airway epithelial cells. This study also suggests that bronchial epithelial cells from asthmatics are different from those of nonasthmatics in regard to their mediator release in response to DPM.

Sagai and colleagues (1996) repeatedly instilled mice with DPM for up to 16 weeks and found increased numbers of eosinophils, goblet cell hyperplasia, and nonspecific airway hyperresponsiveness, changes which are central features of chronic asthma (National Institutes of Health, 1997). Takano et al. (1997) extended this line of research and examined the effect of repeated instillation of DPM on the antibody response to antigen OVA in mice. They observed that antigen-specific IgE and IgG levels were significantly greater in mice repeatedly instilled with both DPM and OVA. Because this upregulation in antigen-specific immunoglobulin production was not accompanied by an increase in inflammatory cells or cytokines in lavage fluid, it would suggest that, in vivo, DPM may act directly on immune system cells, as described in the work by Tsien et al. (1997). Animal studies have confirmed that the adjuvant activity of

DPM also applies to the sensitization of Brown-Norway rats to timothy grass pollen (Steerenberg et al., 1999).

Diaz-Sanchez and colleagues (1996) have continued to study the mechanism of DPM-induced upregulation of allergic response in the nasal cavity of human subjects. In one study, a 200 µL aerosol bolus containing 0.15 mg of DPM was delivered into each nostril of subjects with or without seasonal allergies. In addition to increases in IgE in nasal lavage fluid (NAL), they found an enhanced production of IL-4, IL-6, and IL-13, cytokines known to be B cell proliferation factors. The levels of several other cytokines also were increased, suggesting a general inflammatory response to a nasal challenge with DPM. In a following study, these investigators delivered ragweed antigen, alone or in combination with DPM, on two occasions, to human subjects with both allergic rhinitis and positive skin tests to ragweed (Diaz-Sanchez et al., 1997). They found that the combined challenge with ragweed antigen and DPM produced significantly greater antigen-specific IgE and IgG4 in NAL. A peak response was seen at 96 h postexposure. The combined treatment also induced expression of IL-4, IL-5, IL-10, and IL-13, with a concomitant decrease in expression of Th1-type cytokines. Although the treatments were not randomized (antigen alone was given first to each subject), the investigators reported that pilot work showed no interactive effect of repeated antigen challenge on cellular and biochemical markers in NAL. Diesel particulate matter also resulted in the nasal influx of eosinophils, granulocytes, monocytes, and lymphocytes, as well as the production of various inflammatory mediators. The combined DPM plus ragweed exposure did not increase the rhinitis symptoms beyond those of ragweed alone. Thus, DPM can produce an enhanced response to antigenic material in the nasal cavity.

Extrapolation of these findings of enhanced allergic response in the nose to extremely high concentrations of DPM to the human lung would suggest that ambient combustion particles containing DPM may have significant effects on allergic asthma. A study by Nordenhall et al. (2001) has addressed the effects of diesel PM on airway hyperresponsiveness, lung function and airway inflammation in a group of atopic asthmatics with stable disease. All were hyperresponsive to methacholine. Each subject was exposed to DE (DPM = 300 $\mu g/m^3$) and air for 1 h on two separate occasions. Lung function was measured before and immediately after the exposures. Sputum induction was performed 6 h, and methacholine inhalation test 24 h, after each exposure. Exposure to DE was associated with a significant increase in the degree of

hyperresponsiveness, as compared to after air, a significant increase in airway resistance and in sputum levels of interleukin (IL)-6 ($p=0.048$). No changes were detected in sputum levels of methyl-histamine, eosinophil cationic protein, myeloperoxidase, and IL-8.

These studies provide biological plausibility support for the exacerbation of allergic asthma likely being associated with episodic exposure to PM. Although DPM may make up only a fraction of the mass of urban PM, because of their small size, DPM may represent a significant fraction of the ultrafine particle mode in urban air, especially in cities and countries that rely heavily on diesel-powered vehicles.

In an examination of the effect of concentrated ambient PM on airway responsiveness in mice, Goldsmith et al. (1999) exposed control and ovalbumin-sensitized mice to an average concentration of 787 $\mu g/m^3$ PM for 6 h/day for 3 days. Although ovalbumin sensitization itself produced an increase in the nonspecific airway responsiveness to inhaled methylcholine, concentrated ambient PM did not change the response to methylcholine in ovalbumin-sensitized or control mice. For comparison, these investigators examined the effect of inhalation of an aerosol of the active soluble fraction of ROFA on control and ovalbumin-sensitized mice and found that ROFA could produce nonspecific airway hyperresponsiveness to methylcholine in both control and ovalbumin-sensitized mice. Similar increases in airway responsiveness have been observed after exposure to ROFA in normal and ovalbumin-sensitized rodents (Gavett et al., 1997, 1999; Hamada et al., 1999, 2000).

Gavett et al. (1999) have investigated the effects of ROFA (intratracheal instillation) in ovalbumin (OVA) sensitized and challenged mice. Instillation of 3 mg/kg (approximately 60 µg) ROFA induced inflammatory and physiological responses in the OVA mice that were related to increases in Th2 cytokines (IL-4, IL-5). Compared to OVA sensitization alone, ROFA induced greater than additive increases in eosinophil numbers and in airway responsiveness to methylcholine.

Hamada et al. (1999, 2000) have examined the effect of a ROFA leachate aerosol in a neonatal mouse model of allergic asthma. In the first study, neonatal mice sensitized by intraperitoneal (ip) injection with OVA developed airway hyperresponsiveness, eosinophilia, and elevated serum anti-ovalbumin IgE after a challenge with inhaled OVA. Exposure to the ROFA leachate aerosol had no marked effect on the airway responsiveness to inhaled methacholine in nonsensitized mice, but did enhance the airway hyperresponsiveness to methylcholine produced

in OVA-sensitized mice. No other interactive effects of ROFA exposure with OVA were observed. In a subsequent study, Hamada et al. clearly demonstrated that, whereas inhaled OVA alone was not sufficient to sensitize mice to a subsequent inhaled OVA challenge, pretreatment with a ROFA leachate aerosol prior to the initial exposure to aerosolized OVA resulted in an allergic response to the inhaled OVA challenge. Thus, exposure to a ROFA leachate aerosol can alter the immune response to inhaled OVA both at the sensitization stage at an early age and at the challenge stage.

Lambert et al. (1999) and Gilmour et al. (2001) also examined the effect of ROFA on a rodent model of pulmonary allergy. Rats were instilled intratracheally with 200 or 1,000 μg ROFA 3 days prior to sensitization with house dust mite (HDM) antigen. HDM sensitization after 1,000 μg ROFA produced increased eosinophils, LDH, BAL protein, and IL-10 relative to HDM alone. Although ROFA treatment did not affect antibody levels, it did enhance pulmonary eosinophil numbers. The immediate bronchoconstrictive and associated antigen-specific IgE response to a subsequent antigen challenge was increased in the ROFA-treated group in comparison with the control group. Together, these studies suggest that components of ROFA can augment the immune response to antigen.

Evidence that metals are responsible for the ROFA-enhancement of an allergic sensitization was demonstrated by Lambert et al. (2000). In this follow-up study, Brown Norway rats were instilled with 1 mg ROFA or the three main metal components of ROFA (iron, vanadium, or nickel) prior to sensitization with instilled house dust mite. The three individual metals were found to augment different aspects of the immune response to house dust mite. Nickel and vanadium produced an enhanced immune response to the antigen as seen by higher house dust mite-specific IgE serum levels after an antigen challenge at 14 days after sensitization. Nickel and vanadium also produced an increase in the lymphocyte proliferative response to antigen in vitro. In addition, the antigen-induced bronchoconstrictive response was greater only in nickel-treated rats. Thus, instillation of metals at concentrations equivalent to those present in the ROFA leachate mimicked the response to ROFA, suggesting that the metal components of ROFA are responsible for the increased allergic sensitization observed in ROFA-treated animals.

Although these studies demonstrate that inhalation or instillation of ROFA augments the immune response in allergic hosts, the applicability of these findings to ambient PM is an

important consideration. Goldsmith et al. (1999) have compared the effect of inhalation of concentrated ambient PM for 6 h/day for 3 days versus the effect of a single exposure to a ROFA leachate aerosol on the airway responsiveness to methylcholine in OVA-sensitized mice. Exposure to ROFA leachate aerosols significantly enhanced the airway hyperresponsiveness in OVA-sensitized mice; whereas exposure to concentrated ambient PM (average concentration of 787 $\mu g/m^3$) had no effect on airway responsiveness in six separate experiments. Thus, the effect of the ROFA leachate aerosols on the induction of airway hyperresponsiveness in allergic mice was significantly different than that of a high concentration of concentrated ambient PM. Although airway responsiveness was examined at only one post-exposure time point, these findings do suggest that a great deal of caution should be used in interpreting the results of studies using ROFA particles or leachates in the attempt to investigate the biologic plausibility of the adverse health effects of PM.

7.5.4 Resistance to Infectious Disease

The development of an infectious disease requires both the presence of the appropriate pathogen, as well as host susceptibility to the pathogen. There are numerous specific and nonspecific host defenses against microbes, and the ability of inhaled particles to modify resistance to bacterial infection could result from a decreased ability to clear or kill microbes. Rodent infectivity models frequently have been used to examine the effect of inhaled particles on host defense and infectivity. Mice or rats are challenged with a bacterial or viral load either before or after exposure to the particles (or gas) of interest; mortality rate, survival time, or bacterial clearance are then examined. A number of studies that used the infectivity model to assess inhaled PM effects were discussed previously (U.S. Environmental Protection Agency, 1982, 1989, 1996a). In general, acute exposure to sulfuric acid aerosols at concentrations up to 5,000 $\mu g/m^3$ were not very effective in enhancing mortality in a bacterially mediated murine model. In rabbits, however, sulfuric acid aerosols altered anti-microbial defenses after exposure for 2 h/day for 4 days to 750 $\mu g/m^3$ (Zelikoff et al., 1994). Acute or short-term repeated exposures to high concentrations of relatively inert particles have produced conflicting results. Carbon black (10,000 $\mu g/m^3$) was found to have no effect on susceptibility to bacterial infection (Jakab, 1993); whereas TiO_2 (20,000 $\mu g/m^3$) decreased the clearance of microbes and the bacterial response of lymphocytes isolated from mediastinal lymph nodes (Gilmour et al.,

1989a,b). In addition, exposure to DPM (2 mg/m^3, 7h/d, 5d/wk for 3 and 6 mo) has been shown to enhance the susceptibility of mice to the lethal effects of some, but not all, microbial agents (Hahon et al., 1985). Thus, the pulmonary response to microbial agents has been shown to be altered at relatively high particle concentrations in animal models. Moreover, these effects appear to be highly dependent on the microbial challenge and the test animal studied. Pritchard et al. (1996) observed in rats exposed to particles with a high concentration of metals (e.g., ROFA), that the increased mortality rate after streptococcus infection was associated with the amount of metal in the PM.

There are few recent studies that have examined mechanisms potentially responsible for the effect of PM on infectivity. In one study, Cohen and colleagues (1997) examined the effect of inhaled vanadium (V) on immunocompetence. Healthy rats were repeatedly exposed to 2 mg/m^3 V, as ammonium metavanadate, and then instilled with polyinosinic-polycytidilic acid (poly I:C), a double-stranded polyribonucleotide that acts as a potent immunomodulator. Induction of increases in lavage fluid protein and neutrophils was greater in animals preexposed to V. Similarly, IL-6 and interferon-gamma were increased in V-exposed animals. Alveolar macrophage function, as determined by zymosan-stimulated superoxide anion production and by phagocytosis of latex particles, was depressed to a greater degree after poly I:C instillation in V-exposed rats as compared to filtered air-exposed rats. These findings provide evidence that inhaled V, a trace metal found in combustion particles and shown to be toxic in vivo in studies using instilled or inhaled ROFA (Dreher et al., 1997; Kodavanti et al., 1997b, 1999), has the potential to inhibit the pulmonary response to microbial agents. However, it must be remembered that these effects were found at very high exposure concentrations of V, and as with most studies, care must be taken in extrapolating the results to the ambient exposure of healthy individuals or those with preexisting cardiopulmonary disease to trace concentrations (~3 orders of magnitude lower concentration) of metals in ambient PM.

7.6 RESPONSES TO PARTICULATE MATTER AND GASEOUS POLLUTANT MIXTURES

Ambient PM itself is a mixture of particles of varying size and composition. Ambient PM co-exists in outdoor and indoor air with a number of co-pollutant gases (e.g., O_3, SO_2, NO_2, CO) and innumerable other non-PM components that are not routinely measured. The following discussion examines effects of mixtures of ambient PM or PM surrogates with gaseous pollutants, as evaluated by studies summarized in Table 7-12. Toxicological interactions between PM and gaseous co-pollutants may be antagonistic, additive, or synergistic (Mauderly, 1993). The presence and nature of any interaction appears to depend on the chemical composition, size, concentration and ratios of pollutants in the mixture, exposure duration, and the endpoint being examined. It may be difficult to predict *a priori* from the presence of certain pollutants whether any interaction will occur and, if there is interaction, whether it will be synergistic, additive, or antagonistic.

Mechanisms responsible for the various forms of interaction are speculative. In terms of potential health effects, the greatest hazard from pollutant interaction is the possibility of synergy between particles and gases, especially if effects occur at concentrations at which no effects occur when individual constituents are inhaled. Various physical and chemical mechanisms may underlie synergism. For example, physical adsorption or absorption of some other material on a particle could result in transport to more sensitive sites or accumulation at sites where this material would not normally be deposited in toxic amounts. This physical process may explain, for example, interactions found in studies of mixtures of carbon black and formaldehyde or of carbon black and acrolein (Jakab, 1992, 1993).

Also, chemical interactions between PM and gases can occur on particle surfaces, thus forming secondary products whose surface layers may be more active toxicologically than the primary materials and that can then be carried to a sensitive site. The hypothesis of such chemical interactions has been examined in gas and particle exposure studies by Amdur and colleagues (Amdur and Chen, 1989; Chen et al., 1992) and Jakab and colleagues (Jakab and Hemenway, 1993; Jakab et al., 1996). These investigators have suggested that synergism occurs as secondary chemical species are produced, especially under conditions of increased temperature and relative humidity.

TABLE 7-12. RESPIRATORY AND CARDIOVASCULAR EFFECTS OF PM AND GASEOUS POLLUTANT MIXTURES

Species, Gender, Strain Age, or Body Weight	Gases and PM	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiopulmonary Effects of Inhaled PM and Gases	Reference
Humans; healthy 15 M, 10 F, 34.9±10 years of age	CAPs	Inhalation	150 µg/m^3 0.12 ppm	$PM_{2.5}$ O_3	2 h	$PM_{2.5}$ + O_3 exposure increased acute brachial artery vasoconstriction (as determined by vascular ultrasonography performed before and 10 min after exposure), but not endothelial-dependent or -independent nitroglycerine-mediated dilation. No effects on any endpoints seen with other combined exposures of $PM_{2.5}$ + CO or NO_2 or SO_2.	Brook et al. (2002)
Mice, BALB/c, 3 days old	CAPs (Boston) O_3 CAPs + O_3	Inhalation	0-1500 µg/m^3 0.3 ppm 100-500 µg/m^3 + 0.3 ppm	$PM_{2.5}$	5 h	A small increase in pulmonary resistance and airway responsiveness was found in both normal mice and mice with ovalbumin-induced asthma immediately after exposure to CAPs, but not O_3; no evidence of synergy; activity attributed to the AlSi PM component. For every 100 µg/m^3 CAPs, Penh increased 0.86%.	Kobzik et al. (2001)
Rats	Resuspended urban PM and O_3	Inhalation whole-body	5,000 or 50,000 µg/m^3 PM and 0.8 ppm O3		Single 4-h exposure	PM alone caused no change in cell proliferation in bronchioles or parenchyma. Co-exposure at both dose levels with O_3 greatly potentiated the proliferative changes induced by O_3 alone. These changes were greatest in the epithelium of the terminal bronchioles and alveolar ducts.	Vincent et al. (1997)
Rats	Ottawa urban dust and O_3	Inhalation	40,000 µg/m^3 and 0.8 ppm O_3	4.5 µm MMAD	Single 4-h exposure followed by 20 h clean air	Co-exposure to particles potentiated O_3-induced septal cellurity. Enhanced septal thickening associated with elevated production of macrophage inflammatory protein-2 and endothelin 1 by lung lavage cells.	Bouthillier et al. (1998)
Humans, children: healthy (N = 14) asthmatic (N = 26)	H_2SO_4, SO_2, and O_3	Inhalation	100 ± 40 µg/m^3 H_2SO_4, 0.1 ppm SO_2, and 0.1 ppm O_3	0.6 µm H_2SO_4	Single 4-h exposure with intermittent exercise	A positive association between acid concentration and symptoms, but not spirometry, in asthmatic children. No changes in healthy children.	Linn et al. (1997)

TABLE 7-12 (cont'd). RESPIRATORY AND CARDIOVASCULAR EFFECTS OF PM AND GASEOUS POLLUTANT MIXTURES

Species, Gender, Strain Age, or Body Weight	Gases and PM	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiopulmonary Effects of Inhaled PM and Gases	Reference
Rats, S-D, male, 250-300 g	H_2SO_4 and O_3	Inhalation, nose-only	500 µg/m^3 H_2SO_4 aerosol (two different particle sizes), with or without 0.6 ppm O_3	Fine (0.3 µm MMD, σg = 1.7) and ultrafine (0.06 µm, σg = 1.4)	4 h/day for 2 days	The volume percentage of injured alveolar septae was increased only in the combined ultrafine acid/O_3 animals. BrdU labeling in the periacinar region was increased in a synergistic manner in the combined fine acid/O_3 animals.	Kimmel et al. (1997)
Rats, S-D 300 g	H_2SO_4-coated carbon and O_3.	Inhalation, nose-only	0.2 ppm O_3 + 50 µg/m^3 C + 100 µg/m^3 H_2SO_4 0.4 ppm O_3 +250 µg/m^3 C +500 µg/m^3 H_2SO_4	0.26 µm σg = 2.2	4 h/day for 1 day or 5 days	No airway inflammation at low dose. Greater inflammatory response at high dose; greater response at 5 days than 1 day. Contrasts with O_3 alone where inflammation was greatest at 0.40 ppm on Day 1.	Kleinman et al. (1999)
Rats	H_2SO_4 and O_3	Inhalation, whole body	20 to 150 µg/m^3 H_2SO_4 and 0.12 or 0.2 ppm O_3	0.4 to 0.8 µm	Intermittent (12 h/day) or continuous exposure for up to 90 days	No interactive effect of H_2SO_4 and O_3 on biochemical and morphometric endpoints.	Last and Pinkerton (1997)
Mice, Swiss, female, 5 weeks old	Carbon and SO_2	Inhalation, flow-past, nose-only	10,000 µg/m^3 carbon with or without 5 to 20 ppm SO_2 at 10% or 85% RH	0.3 µm MMAD σg = 2.7	Single 4-h exposure	Macrophage phagocytosis was depressed only in animals exposed to the combination of SO_2 and carbon at 85% humidity. This inhibition in macrophage function lasted at least 7 days after exposure.	Jakab et al. (1996) Clarke et al. (2000)
Rats, Fischer NNia, male, 22 to 24 mo old	Carbon, ammonium bisulfate, and O_3	Inhalation	50 µg/m^3 carbon + 70 µg/m^3 ammonium bisulfate + 0.2 ppm O_3 or 100 µg/m^3 carbon +140 µg/m^3 ammonium bisulfate + 0.2 ppm O_3	0.4 µm MMAD σg = 2.0	4 h/day, 3 days/week for 4 weeks	No changes in protein concentration in lavage fluid or in prolyl 4-hydroxylase activity in blood. Slight, but statistically significant decreases in plasma fibronectin in animals exposed to the combined atmospheres compared to animals exposed to O_3 alone.	Bolarin et al. (1997)
Rats	Elemental carbon + O_3 + ammonium bisulfate	Inhalation	0.2 ppm O_3 + elemental carbon 50 µm/m^3 + ammonium bisulfate 70 µg/m^3	0.46 µm 0.3 µm	4 hr/d 3 d/wk 4 wk	Increased macrophage phagocytosis and increased respiratory burst; decreased lung collagen.	Kleinman et al. (2000)

TABLE 7-12 (cont'd). RESPIRATORY AND CARDIOVASCULAR EFFECTS OF PM AND GASEOUS POLLUTANT MIXTURES

Species, Gender, Strain Age, or Body Weight	Gases and PM	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration	Cardiopulmonary Effects of Inhaled PM and Gases	Reference
Rats, F344/N male	O_3 + nitric acid NO_2 + carbon particles + ammonium bisulfate	Inhalation	low: 0.16 ppm + 0.11 ppm + 0.05 mg/m^3 + 0.03 mg/m^3 medium: 0.3 ppm + 0.21 ppm + 0.06 mg/m^3 + 0.1 mg/m^3 high: 0.59 ppm + 0.39 ppm + 0.1 mg/m^3 + 0.22 mg/m^3	0.3 µm	4 h/d 3 d/wk 4 wk	Dose-dependent decrease in macrophage Fc-receptor mediated-phagocytosis (only significant in high dose group), nonsignificant increase in epithelial permeability and proliferation, altered breathing pattern in high dose group.	Mautz et al. (2001)
Rats, F344/N male	O_3 HNO_3 O_3 + HNO_3	Inhalation	0.151 ± 0.003 ppm 51.1 ±5.4 $\mu g/m^3$ 0.152 ± 0.003 ppm + 49.9 ±7.0 $\mu g/m^3$		4 h/d 3 d/wk 40 wk	Increased lung putrescine content in all exposed rats. Synergistic effect.	Sindhu et al. (1998)
Dogs	Ambient particles and gases	Natural 24-h exposure in four urban areas of Mexico City and one rural area			Continuous ambient exposure	No significant differences in AMs or total cell counts in lavage from dogs studied among the five regions. A significant increase in lavage fluid neutrophils and lymphocytes in the southwest region, where the highest O_3 levels were recorded, compared to the two industrial regions with the highest PM levels.	Vanda et al. (1998)
Rats, F344, 9-weeks-old, male and female	Ambient particles and gases	Natural 23 h/day exposure to filtered and unfiltered Mexico City air.	0.018 ppm O_3 3.3 ppb CH_2O 0.068 mg/m^3 TSP 0.032 mg/m^3 PM_{10} 0.016 mg/m^3 $PM_{2.5}$		23 h/day for 7 weeks	Histopathology examination revealed no nasal lesions in exposed or control rats; tracheal and lung tissue from both groups showed similar levels of minor abnormalities.	Moss et al. (2001)

Another potential mechanism of gas-particle interaction may involve a pollutant-induced change in the local microenvironment of the lung, enhancing the effects of the co-pollutant. For example, Last et al. (1984) suggested that the observed synergism between ozone (O_3) and acid sulfates in rats was due to a decrease 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. Likewise, Pinkerton et al. (1989) showed increased retention of the mass and number of asbestos fibers in rats exposed to O_3, suggesting increased lung fiber burden due to co-exposure to this gas.

One newly available controlled exposure study evaluated the effects of a combined inhalation exposure to CAPs and O_3 in human subjects. In a randomized, double-blind crossover study, Brook et al. (2002) exposed 25 healthy male and female subjects, 34.9 ± 10 (SD) years of age, to filtered ambient air containing 1.6 μg/m^3 $PM_{2.5}$ and 0.09 ppb O_3 (control) or to unfiltered air containing 150 μg/m^3 CAPs and 0.12 ppb O_3 while at rest for 2 h. Blood pressure was measured and high-resolution brachial artery ultrasonography was performed prior to and 10 min after exposure. The brachial artery ultrasonography (BAUS) technique was used to measure brachial artery diameter (BAD), endothelium-dependent flow-mediated dilation (FMD), and endothelial-independent nitroglycerine-mediated dilation (NMD). Although no changes in blood pressure or endothelial-dependent or endothelial-independent dilatation were observed, a small (2.6%) but statistically significant ($p = 0.007$) decrease in BAD was observed in CAPs plus O_3 exposures (−0.09 mm) when compared to filtered air exposures (+0.01 mm). Pre-exposure BAD showed no significant day-to-day variation (± 0.03 mm), and no significant exposure differences were found for other gaseous pollutants (CO, NO_x, SO_2) in the ambient air. This finding suggests that combined exposure to a mixture of CAPs plus O_3 (but not several other gaseous pollutants) produces vasoconstriction, potentially via autonomic reflexes or as the result of an increase in circulating endothelin, as has been described in rats exposed to urban PM (Vincent et al., 2001). It is not known, however, whether this effect is caused by CAPs or O_3 alone although it is unlikely CAPS alone did it, given lack of effects for CAPs plus other gases. The likelihood that analogous vasoactive responses could be found at $PM_{2.5}$ and O_3 concentrations typically found in most urban locations in North America is enhanced by the fact that such responses would likely have been seen even at distinctly lower exposure levels had the PM and

O_3 exposures occured during moderate to heavy exercise (which enhances delivery of both PM and O_3 to lower regions of the respiratory tract).

The interaction of PM and O_3 was further examined in a murine model of ovalbumin (OVA)-induced asthma. Kobzik et al. (2001) investigated whether coexposure to inhaled, concentrated PM from Boston, MA and to O_3 could exacerbate asthma-like symptoms. On days 7 and 14 of life, half of the BALB/c mice used in this study were sensitized by ip injection of OVA and then exposed to OVA aerosol on three successive days to create the asthma phenotype. The other half received the ip OVA, but were exposed to a phosphate-buffered saline aerosol (controls). The mice were further subdivided ($n \geq 61$/group) and exposed for 5 h to CAPs, ranging from 63 to 1,569 $\mu g/m^3$, 0.3 ppm O_3, CAPs + O_3, or to filtered air. Pulmonary resistance and airway responsiveness to an aerosolized MCh challenge were measured after exposures. A small, statistically significant increase in pulmonary resistance and airway responsiveness, respectively, was found in both normal and asthmatic mice immediately after exposure to CAPs alone and to CAPs + O_3, but not to O_3 alone or to filtered air. By 24 h after exposure, the responses returned to baseline levels. There were no significant increases in airway inflammation after any of the pollutant exposures. In this well-designed study of a small-animal model of asthma, CAPs and O_3 did not appear to be synergistic. In further analysis of the data using specific elemental groupings of the CAPs, the acutely increased pulmonary resistance was found to be associated with the AlSi fraction of PM. Thus, some components of concentrated $PM_{2.5}$ may affect airway caliber in sensitized animals.

Linn and colleagues (1997) examined the effect of a single exposure to 60 to 140 $\mu g/m^3$ H_2SO_4, 0.1 ppm SO_2, and 0.1 ppm O_3 in healthy (N = 15) and asthmatic children (N = 26). The children performed intermittent exercise during the 4-h exposure to increase the inhaled dose of the pollutants. An overall effect on the combined group of healthy and asthmatic children was not observed. The combined pollutant exposure had no effect on spirometry in asthmatic children, and no changes in symptoms or spirometry were observed in healthy children. A positive association between acid concentration and symptoms was seen, however, in the subgroup of asthmatic children. Thus, the effect of combined exposure to PM and gaseous co-pollutants appeared to have less effect on asthmatic children exposed under controlled laboratory conditions in comparison with field studies of children attending summer camp (Thurston et al., 1997). However, prior exposure to H_2SO_4 aerosol may enhance the subsequent

response to O_3 exposure (Linn et al., 1994; Frampton et al., 1995); and the timing and sequence of the exposures may be important.

Vincent et al. (1997) exposed rats to 5 or 50 mg/m^3 of resuspended urban particles for 4 h in combination with 0.8 ppm O_3. Although PM alone caused no change in cell proliferation(^{3}H-thymidine labeling), co-exposure to either concentration of resuspended PM with O_3 greatly potentiated the proliferative effects of exposure to O_3 alone. These interactive changes occurred in epithelial cells of the terminal bronchioles and the alveolar ducts. These findings using resuspended dusts, although at high concentrations, are consistent with studies demonstrating interaction between sulfuric acid (H_2SO_4) aerosols and O_3.

Kimmel and colleagues (1997) examined the effect of acute co-exposure to O_3 (0.6 ppm) and fine (MMD = 0.3 µm) or ultrafine (MMD = 0.06 µm) H_2SO_4 aerosols (0.5 mg/m^3) on rat lung morphology. They determined morphometrically that alveolar septal volume was increased in animals co-exposed to O_3 and ultrafine, but not fine, H_2SO_4. Interestingly, cell labeling, an index of proliferative cell changes, was increased only in animals co-exposed to fine H_2SO_4 and O_3, as compared to animals exposed to O_3 alone. Importantly, Last and Pinkerton (1997) extended their previous work and found that subchronic exposure to acid aerosols (20 to 150 µg/m^3 H_2SO_4) had no interactive effect on the biochemical and morphometric changes produced by either intermittent or continuous O_3 exposure (0.12 to 0.2 ppm). Thus, the interactive effects of O_3 and acid aerosol co-exposure in the lung disappeared during the long-term exposure.

Kleinman et al. (1999) examined the effects of O_3 (0.2 and 0.4 ppm) plus fine (MMAD = 0.26 µm), H_2SO_4-coated, carbon particles (100, 250, and 500 µg/m^3) for 1 or 5 days. They found the inflammatory response with the O_3-particle mixture was greater after 5 days (4 h/day) than after Day 1. This contrasted with O_3 exposure alone (0.4 ppm), which caused marked inflammation on acute exposure, but no inflammation after 5 consecutive days of exposure.

Kleinman et al. (2000) examined the effects of a mixture of elemental carbon particles (50 µg/m^3), O_3 (0.2 ppm), and ammonium bisulfate (70 µg/m^3) on rat lung collagen content and macrophage activity. Decreases in lung collagen, and increases in macrophage respiratory burst and phagocytosis were observed relative to other pollutant combinations. Mautz et al. (2001) used a similar mixture (i.e., elemental carbon particles, O_3, ammonium bisulfate, but with NO_2

also) and exposure regimen as Kleinman et al. (2000). There were decreases in pulmonary macrophage Fc-receptor binding and phagocytosis and increases in acid phosphatase staining. Bronchoalveolar epithelial permeability and cell proliferation were increased. Altered breathing patterns were also observed, with some adaptations occurring.

Studies have examined interactions between carbon particles and gaseous co-pollutants. Jakab et al. (1996) and Clarke et al. (2000c) challenged mice with a single 4-h exposure to a high concentration of carbon particles (10 mg/m^3) in the presence of 10 ppm SO_2 (~140 µg $cpSO_4^{2-}$) at low and high relative humidities. Macrophage phagocytosis was depressed significantly only in mice exposed to the combined pollutants under high relative humidity (85%) conditions. There was no evidence of an inflammatory response based on total cell counts and differential cell counts from BAL; however, macrophage phagocytosis remained depressed for 7 to 14 days. Intrapulmonary bactericidal activity also was suppressed and remained suppressed for 7 days. This study suggests that fine carbon particles can serve as an effective carrier for acidic sulfates where chemical conversion of adsorbed SO_2 to acid sulfate species occurred. Interestingly, the depression in macrophage function was present as late as 7 days postexposure. Bolarin et al. (1997) exposed rats to 50 or 100 $\mu g/m^3$ carbon particles in combination with ammonium bisulfate and O_3. Despite 4 weeks of exposure, they observed no changes in protein concentration in lavage fluid or blood prolyl 4-hydroxylase, an enzyme involved in collagen metabolism. Slight decreases in plasma fibronectin were present in animals exposed to the combined pollutants versus O_3 alone. Thus as, previously noted, the potential for adverse effects in the lungs of animals challenged with a combined exposure to particles and gaseous pollutants is dependent on numerous factors, including the gaseous co-pollutant, concentration, and time.

The effects of O_3 modifying the biological potency of PM (diesel PM and carbon black) was examined by Madden et al. (2000). Reaction of NIST Standard Reference Material # 2975 diesel PM with 0.1 ppm O_3 for 48 hr increased the potency (compared to unexposed or air-exposed diesel PM) to induce neutrophil influx, total protein, and LDH in lung lavage fluid in response to intratracheal instillation. Exposure of the diesel PM to high, non-ambient O_3 concentration (1.0 ppm) attenuated the increased potency, suggesting destruction of the bioactive reaction products. Unlike the diesel particles, carbon black particles exposed to 0.1 ppm O_3 did not exhibit an increase in biological potency, which suggested that the reaction of organic components of the diesel PM with O_3 were responsible for the increased potency. Reaction of

particle components with O_3 was ascertained by chemical determination of specific classes of organic compounds.

In a complex series of exposures, Oberdörster and colleagues examined the interaction of ultrafine carbon particles (100 μg/m^3) and O_3 (1 ppm) in young and old Fischer 344 rats that were pretreated with aerosolized endotoxin (Elder et al., 2000a,b). In old rats, exposure to singlet ultrafine carbon and O_3 produced an interaction that resulted in a greater influx in neutrophils than that produced by either agent alone. This interaction was not seen in young rats. Oxidant release from lavage fluid cells was also assessed and the combination of endotoxin, carbon particles, and O_3 produced an increase in oxidant release in old rats. This combination produced the opposite response in the cells recovered from the lungs of the young rats, indicating that the lungs of the aged animals underwent greater oxidative stress in response to this complex pollutant mix of particles, O_3, and a biogenic agent.

Wagner et al. (2001) examined the synergistic effect of co-exposure to O_3 and endotoxin on the transition and respiratory epithelium of rats that also was mediated, in part, by neutrophils. Fisher 344 rats (10 to 12 week old) exposed to 0.5 ppm O_3, 8 h per day, for 3 days, developed mucous cell metaplasia in the nasal transitional epithelium, an area normally devoid of mucous cells; whereas, intratracheal instillation of endotoxin (20 μg) caused mucous cell metaplasia rapidly in the respiratory epithelium of the conducting airways. A synergistic increase of intraepithelial mucosubstances and morphological evidence of mucous cell metaplasia were found in rat maxilloturbinates upon exposure to both ozone and endotoxin, compared to each pollutant alone.

The effects of gaseous pollutants on PM-mediated responses also have been examined by in vitro studies, though to a limited extent. Churg et al. (1996) demonstrated increased uptake of asbestos or TiO_2 into rat tracheal explant cultures in response to 10 min O_3 (up to 1.0 ppm) pre-exposure. These data suggest that O_3 may increase the penetration of some types of PM into epithelial cells. Additionally, Madden et al. (2000) demonstrated a greater potency for ozonized diesel PM to induce prostaglandin E_2 production from human epithelial cell cultures, suggesting that O_3 can modify the biological activity of PM derived from diesel exhaust.

Several new studies have examined autopsy materials for indications of possible cardiopulmonary effects of complex air pollution mixtures in Mexico, Spain, and Italy. These studies, taking advantage of differences in pollutant mixtures and concentrations in relatively

"clean" rural areas versus urban environments found morphological changes in the nasopharynx (Calderón-Garcidueñas et al., 2001c), the lower respiratory tract (Gulisano et al., 1997; Lorz and Lopez, 1997; Calderón-Garcidueñas et al., 2001c) and in the heart (Calderón-Garcidueñas et al., 2001c) of lambs, pigeons, and dogs, respectively, experiencing long-term continuous natural exposures to elevated ambient air pollution. Each study provided evidence suggesting that animals living in urban environments with higher air pollution levels have greater pulmonary and cardiac changes than those living in cleaner rural areas. It is not possible, however (a) to attribute specific relative roles to PM versus other gaseous components of the urban air mixtures in producing the observed effects or (b) to extrapolate the findings to U.S. urban situations having typically much lower air pollutant concentrations (e.g. much higher PM and O_3 levels in Mexico City than in U.S. cities). These studies are, therefore, not particularly useful for present purposes.

7.7 INTERPRETIVE SUMMARY OF PM TOXICOLOGY FINDINGS

Toxicological studies can play an integral role in addressing several key important questions regarding ambient PM health effects:

(1) What types of pathophysiological effects are exerted by ambient PM or constituent substances and what are potential mechanisms that likely mediate various PM health effects?

(2) What PM characteristics (size, chemical composition, etc.) cause or contribute to health effects?

(3) What susceptible subgroups are at increased risk for ambient PM health effects and what factors contribute to increased susceptibility?

(4) What types of interactive effects of particles and gaseous copollutants have been demonstrated?

This summary focuses on highlighting salient findings that reflect the notable progress that toxicological studies have made towards addressing these questions. All these questions have important implications bearing on the issue of biological plausibility of epidemiologically-observed ambient PM effects.

One overarching question in the interpretation of toxicology study results is the relevance of findings from experimental human or animal studies using high controlled exposure concentrations or doses within the context of results from epidemiology studies with ambient pollutant concentration exposures. To provide insight on this issue, EPA conducted a series of illustrative analyses using dosimetric modeling of the type discussed in Chapter 6. The results of these analyses, discussed in detail in Appendix 7A, are briefly summarized below.

First, taking into account certain key points regarding dose metrics, one of the publically available dosimetry models (the MMPD model) discussed in Chapter 6 was employed to compare estimates of deposited and/or retained respiratory tract PM doses in the human and rat lung using different dose metrics as described in Table 7A-1. The MPPD model estimates in Table 7A-6 suggest that a rat exposed to 300-400 $\mu g/m^3$ of resuspended PM over a 6-hr period would result in an incremental dose (measured as deposited or retained mass) in the alveolar (A) region comparable to that of a healthy human working for 6 hours near a busy road and exposed to 150 $\mu g/m^3$ ambient PM_{10}. To achieve an incremental dose retained in the rat TB region (averaged over 6 hrs) comparable to that in the human, the rat would need to be exposed for 6 hrs to approximately 2 mg/m^3 resuspended PM. However, because of the more rapid clearance

in the rat, a higher concentration (on the order of 6-7 mg/m^3) would be needed for the rat to achieve a retained TB dose (averaged over 24 hours) comparable to that achieved in the human.

The second approach involves application of the same publically-available model (a) to estimate likely respiratory tract doses (again using various dose metrics) resulting from experimental exposures (via PM inhalation or instillation) of human or laboratory animals (rats) actually employed in representative published PM toxicology studies assessed in this chapter and (b) to estimate likely ambient PM exposure concentrations that would be needed in order to obtain comparable human and rat PM respiratory tract doses. Comparing toxicology and controlled human exposure studies using Utah Valley particles, the ambient exposure concentrations over 24 hours which would be required to achieve the same dose per unit surface area in humans are about 4-fold higher with the rat 250 µg instilled dose compared to the human 500 µg instilled dose (Table 7A-8). Analysis of doses per surface area in rats and humans exposed to CAPs (Table 7A-9) in other examples of published studies indicate that, for the same concentration and exposure times, humans have five times the dose per surface area of the rat.

These results indicate that higher PM concentration exposures in rats are needed and justified to achieve nominally similar doses per unit surface area relative to the human. Given that rats clear PM from the respiratory tract much faster than humans, MPPD modeling indicates that high exposure concentrations and instillation doses in the rat provide a useful and relevant approach to investigate toxicological endpoints which are predictive of health outcomes in humans and to investigate biological mechanisms.

7.7.1 Particulate Matter Health Effects and Potential Mechanisms of Action

Numerous epidemiologic analyses discussed in Chapter 8 have shown associations between ambient PM levels and increased risk for cardiorespiratory effects, as well as for lung cancer. Findings since 1996 have provided evidence supporting many hypotheses regarding induction of PM effects; and this body of evidence has grown substantially. Various toxicologic studies using PM having diverse physicochemical characteristics have shown that such characteristics have a great impact on the specific response that is observed. Thus, there appear to be multiple biological mechanisms that may be responsible for observed morbidity/mortality due to exposure to ambient PM, and these mechanisms appear to be highly dependent on the type and dose of particle in the exposure atmosphere. It also appears that many biological

responses are produced by PM whether it is composed of a single component or a complex mixture.

The following discussion focuses on summarizing key lines of toxicological evidence useful in (a) delineating various types of health effects attributable to PM exposures, and (b) identifying potential pathophysiological mechanisms by which the effects of particle exposure are mediated. Major emphasis is placed on discussions of PM effects on the cardiopulmonary system, and some attention is accorded to PM-related mutagenic/genotoxic effects of relevance to evaluating the carcinogenic potential of ambient PM or constituent substances.

7.7.1.1 Direct Pulmonary Effects

When the 1996 PM AQCD was written, the lung was thought to be the primary organ affected by particulate air pollution. Although the lung still is a primary organ affected by PM inhalation, there is growing toxicological and epidemiologic evidence that the cardiovascular system is also affected and may be a co-primary organ system related to certain health endpoints such as mortality. Nonetheless, understanding how particulate air pollution affects respiratory system functions or exacerbates respiratory disease remains an important goal. The toxicological evidence from controlled exposures to ambient PM or constituents appear to support three hypothesized mechanisms for PM inducing direct pulmonary effects, as discussed below.

Lung Injury and Inflammation

Particularly compelling evidence pointing towards ambient PM causing lung injury and inflammation derives from the study of extracts of ambient PM materials on filters collected from community air monitors before, during and after the temporary closing of a steel mill in Utah Valley. Ghio and Devlin (2001) found that intratracheal instillation of filter extract materials in human volunteers provoked greater lung inflammatory responses for materials obtained before and after the temporary closing versus that collected during the plant closing. The instilled dose of 500 μg of extract material was calculated by Ghio and Devlin to result in focal lung deposition in the lingula roughly equivalent to 5 times more than would be deposited if an active person experienced 24-h inhalation exposure to 100 $\mu g/m^3$ PM_{10} (during wintertime

temperature inversions in Utah Valley 24-h PM_{10} levels can exceed 100 $\mu g/m^3$). Moreover, 100 µg of filter extract collected during the winter before the temporary plant closure similarly instilled into the lungs of human volunteers also increased levels of neutrophils, protein, and inflammatory cytokines. Ghio and Devlin (2001) indicated that these results and calculations suggest that biologic effects found in their study could be experienced during a typical winter inversion in the Utah Valley. Further, the instillation in rats (Dye et al., 2001) of extract materials from before and after the plant closing resulted in a 50% increase in air way hyperresponsiveness to acetylcholine compared to 17 or 25% increases with saline or extract materials for the period when the plant was closed, respectively. Analysis of the extract materials revealed notably greater quantities of metals for when the plant was opened, thus suggesting that such metals (e.g., Cu, Zn, Fe, Pb, As, Mn, Ni) may be important contributors to the pulmonary toxicity observed in the controlled exposure studies, as well as to health effects shown epidemiologically to vary with PM exposures of Utah Valley residents before, during, and after the steel mill closing.

Still other toxicological studies point towards lung injury and inflammation being associated with exposure of lung tissue to complex combustion-related PM materials, with metals again being likely contributors. For example, in the last few years, numerous studies have shown that high doses/concentrations of instilled and inhaled ROFA, a product of fossil fuel combustion, can cause substantial lung injury and inflammation. The toxic effects of ROFA are largely caused by its high content of soluble metals, and some of the pulmonary effects of ROFA can be reproduced by equivalent exposures to soluble metal salts. In contrast, controlled exposures of animals to sulfuric acid aerosols, acid-coated carbon, and sulfate salts cause little lung injury or inflammation, even at high concentrations. Inhalation of concentrated ambient PM (which contains only small amounts of metals) by laboratory animals at concentrations in the range of 100 to 1000 $\mu g/m^3$ have been shown in some (but not all) studies to cause mild pulmonary injury and inflammation. Rats with SO_2-induced bronchitis and monocrotaline-treated rats have been reported to have a greater inflammatory response to concentrated ambient PM than normal rats. These studies suggest that exacerbation of respiratory disease by ambient PM may be caused in part by lung injury and inflammation.

There are also new in vitro data indicating a potential neurogenic basis for the effects of particulate matter (Veronesi et al., 1999a,b; Oortgeison et al., 2000; Varonesi et al., 2002b).

More specifically, these studies indicate that the proton cloud associated with negatively charged colloidal PM particles could activate acid sensitive VR1 receptors found on human airway epithelial cells and sensory terminals; this activation, in turn, results in an immediate influx of calcium and the release of inflammatory neuropeptides and cytokines, which initiate and sustain inflammatory events in the pathophysiology of neurogenic inflammation. This implies that a wide variety of particulate substances, from many different types of sources (both natural and anthropogenic), falling across wide size ranges (from ultrafine through accumulation mode and including small, < 10-15 μm, coarse fraction particles), and of highly diverse chemical composition could possibly exert neurogenically-mediated pathophysiological effects depending on shared physical properties of their surface molecules (i.e., a negatively-charged proton cloud).

Increased Susceptibility to Respiratory Infections

A few newly published studies have provided some evidence for ambient PM potentially affecting lung defense mechanisms and increasing susceptibility to infection. The studies of Zelikoff et al. (2000a,b) showed that brief exposures (3 to 5 h) of Fischer rats to New York City CAPs (~90 to 600 $\mu g/m^3$) either before or after IT-instillation of Streptococcus pneumoniae increased numbers of lavageable PAM cells, and increased bacterial burden over control levels at 24 h postinfection. Similarly, Antonini et al. (2002) found that preexposure to ROFA (0.2 or 1.0 mg/100 g body weight) of Sprague-Dawley rats 3 days before IT instillation of Listeria monocytogenes (a bacterial pathogen) led to notable lung injury, slowed clearance of the bacteria, and reduced AM NO production, although AM numbers were not reduced. Lastly, new studies by Ohtsuka et al. (2000a,b), showing decreased phagocytic activity of alveolar macrophages (AM) in mice after a 4 h inhalation exposure to acid-coated carbon particles (albeit at a high mass concentration of 10 mg/m^3), are suggestive of possible impairment of an important lung defense mechanism even in the absence of lung injury.

Increased Airway Reactivity and Exacerbation of Asthma

The strongest evidence supporting this hypothesis is from studies on diesel particulate matter (DPM). Diesel particulate matter has been shown to increase production of antigen-specific IgE in mice and humans (summarized in Section 7.2.1.2). In vitro studies have suggested that the organic fraction of DPM is involved in the increased IgE production. ROFA

leachate also has been shown to enhance antigen-specific airway reactivity in mice (Goldsmith et al., 1999), indicating that soluble metals can also enhance an allergic response. However, in this same study, exposure of mice to concentrated ambient PM did not affect antigen-specific airway reactivity. It is premature to conclude from the Goldsmith experiment that concentrated ambient PM does not exacerbate allergic airways disease because the chemical composition of the PM (as indicated by studies with DPM and ROFA) may be more important than the mass concentration.

7.7.1.2 Cardiovascular and Other Systemic Effects Secondary to Lung Injury

When the 1996 PM AQCD was written, it was thought that cardiovascular-related morbidity and mortality most likely would be sequalae occurring secondary to impairment of oxygenation or some other consequence of lung injury and inflammation. Newly available toxicologic studies provide evidence regarding such possibilities, as discussed below.

Impairment of Oxygenation and Increased Work of Breathing That Adversely Affects the Heart Secondary to Lung Injury

Results from new toxicology studies in which animals (normal and compromised) were exposed to concentrated ambient PM (at concentrations many times higher than would be encountered in the United States) indicate that ambient PM is unlikely to cause severe disturbances in oxygenation or pulmonary function. However, even a modest decrease in oxygenation can have serious consequences in individuals with ischemic heart disease. For example, Kleinman et al. (1998) has shown that a reduction in arterial blood saturation from 98 to 94% by either mild hypoxia or by exposure to 100 ppm CO significantly reduced the time to onset of angina in exercising volunteers.

One study of PM effects in a severely compromised animal model hints at possible PM pathophysiologic effects mediated via hypoxemia. Specifically, the instillation of ROFA (0, 0.25, 1.0, 2.5 mg) was shown (Watkinson et al., 2000a,b) to increase (to 50%) the mortality rate observed in monocrotaline-treated rats with pulmonary hypertension. Although blood oxygen levels were not measured in this study, there were ECG abnormalities consistent with severe hypoxemia in about half of the rats that subsequently died. Given the severe inflammatory effects of instilled ROFA and the fact that monocrotaline-treated rats have increased lung

permeability as well as pulmonary hypertension, it is plausible that instilled ROFA may cause severe hypoxemia leading to death in this rat model.

More information is needed, however, on the effects of PM on arterial blood gases and pulmonary function to fully address the above hypothesis.

Systemic Hemodynamic Effects Secondary to Lung Inflammation and Increased Cytokine Production

It has been suggested that systemic effects of particulate air pollution may result from activation of cytokine production in the lung (Li et al., 1997). Results from some studies of compromised animal models provide some support for this idea. For example, there was a significant decrease in the time of onset of ischemic ECG changes following coronary artery occlusion in PM-exposed dogs compared to controls (Godleski et al., 2000). Analogously, Wellenius et al. (2002) found, in another animal model (i.e., left ventricular myocardial infarction induced by thermocoagulation), that 41% of the MI rats exhibited one or more premature ventricular complexes (PVCs) during baseline periods 12-18 h after surgery; and exposure to ROFA, but not carbon black or room air, increased arrhythmia frequency in animals with prior PVCs and decreased their heart rate variability (HRV). Also, severely compromised monocrotaline-treated rats exposed to inhaled ROFA (15,000 $\mu g/m^3$, 6 h/day for 3 days) showed increased pulmonary cytokine gene expression, bradycardia, hypothermia, and increased arrhythmias (Watkinson et al., 2000a,b). On the other hand, spontaneously hypertensive rats manifested similar cardiovascular responses to inhaled ROFA (except that they also developed ST segment depression), but without any increase in pulmonary cytokine gene expression.

Other studies of normal dogs exposed to concentrated ambient PM (322 $\mu g/m^3$, MMAD = 0.23-.034 μm) showed minimal pulmonary inflammation and no positive staining for IL-8, IL-1, or TNF in airway biopsies (Godleski et al., 2000). In addition, several other studies (e.g., Muggenberg et al., 2002a,b) of normal dogs and/or rats failed to show changes in ECG consistent with the types observed in the above studies of compromised models. Thus, the link between PM-induced changes in the production of cytokines in the lung and effects on cardiovascular function is not clear-cut, and more basic information on the effects of mild pulmonary injury on cardiovascular function is needed to understand the mechanisms by which inhaled PM may affect the heart.

Increased Blood Coagulability Secondary to Lung Inflammation

There is abundant evidence linking small prothrombotic changes in the blood coagulation system to increased long-term risk of heart attacks and strokes. However, the published toxicological evidence bearing on whether moderate lung inflammation causes increased blood coagulability is very mixed and inconsistent.

Several new studies have investigated possible effects of ambient PM or surrogate particles on blood chemistry constituents that would be indicative of increased blood coagulability. For example, Ghio et al. (2000a) have shown that inhalation of concentrated ambient PM (~20 to 300 $\mu g/m^3$) in healthy nonsmokers causes increased levels of blood fibrinogen. Gardner et al. (2000) have also shown that a high dose (8,300 µg/kg) of instilled ROFA in rats causes increased blood levels of fibrinogen, but no effect was seen at lower doses. Gordon et al. (1998) also reported increased blood platelets and neutrophils in control and monocrotaline-treated rats on some, but not all, days when exposed to concentrated NYC ambient PM (150-400 $\mu g/m^3$).

On the other hand, exposure of normal dogs to concentrated ambient PM from Boston (~100-1000 $\mu g/m^3$) had no effect on fibrinogen levels (Godleski et al., 2000). Nor were any significant effects on blood fibrinogen or other factors (e.g., blood platelets, tissue plasminogen activator, Factor VII, etc.) involved in the coagulation cascade seen with exposure of normal rats to concentrated NYC ambient PM (~130 to 900 $\mu g/m^3$), as reported by Nadzieko et al. (2002). Frampton (2001) also reported finding no effects on fibrinogen or clotting Factor VII in healthy, nonsmoking human adults exposed to 10 $\mu g/m^3$ ultrafine carbon for 2 h via mouthpiece inhalation while at rest.

All these latter results, indicative of little effect of PM exposure on blood coagulation factors in healthy humans or laboratory animals, stand in contrast to the highly suggestive ambient PM-induced increases in fibrinogen seen by Ghio et al. (2000a) in healthy human adult volunteers. The coagulation system is as multifaceted and complex as the immune system; and there are many other sensitive and clinically significant parameters that should, in addition to fibrinogen, show more extensive and consistent patterns of change reflective of PM effects on blood coagulation. Thus, it is premature to draw any strong conclusions about the relationship between PM and blood coagulation.

Hematopoiesis Effects Secondary to PM Interactions With the Lung

Terashima et al. (1997) found that instillation of fine carbon particles (20,000 µg/rabbit) stimulated release of PMNs from bone marrow. In further support of this hypothesis, Gordon and colleagues reported that the percentage of PMNs in the peripheral blood increased in rats exposed to ambient PM in some but not all exposures. On the other hand, Godleski et al. (2000) found no changes in peripheral blood counts of dogs exposed to concentrated ambient PM. Thus, consistent evidence that PM ambient concentrations can affect hematopoiesis remains to be demonstrated.

7.7.1.3 Direct Effects on the Heart

Although the data are still limited, two types of hypothesized direct effects of PM on the heart are noted below.

Effects on the Heart Secondary to Uptake of Particles into the Circulation and/or Release of Soluble Substances into the Circulation

Drugs can be rapidly and efficiently delivered to the systemic circulation by inhalation. This implies that the pulmonary vasculature absorbs inhaled materials, including charged substances such as small proteins and peptides. Such PM materials could conceivably be rapidly transported to the heart, where they might exert effects directly on cardiac vasculature or heart muscle itself. Alternatively, they could also exert very rapid effects on cardiac function through stimulation of nerve ending receptors in lung tissue, resulting in secretion of inflammatory messenger substances and/or activation of neurally-mediated autonomic reflexes. This raises the question of how inhaled particles could affect the autonomic nervous system. Activation of neural receptors in the lung is a logical area to investigate.

Epithelial cells lining lower respiratory tract airways are damaged or denuded in many common health disorders (e.g., asthma, viral infections, etc.), which may allow inhaled PM to directly encounter sensory nerve terminals and their acid-sensitive receptors. In vitro studies by Veronesi and colleagues provide interesting evidence indicating that (a) ROFA-induced inflammation is mediated by acid-sensitive VR1 receptors on sensory nerve fibers that innervate the airways and on surrounding bronchial epithelial cells (Veronesi et al., 1999a, 1999b); (b) negatively-charged but not neutrally-charged (i.e., zeta potential = 0 MV), particles in ROFA, synthetic polymer aerosols, or extracts from urban St. Louis, residential (woodstove),

volcanic (Mt. St. Helens), and industrial (coal and oil fly ash) sources activate the VR1 receptors (Oortgiesen et al., 2000), with their zeta potential being the key physiochemical property correlated with consequent increases in Ca+ and IL-6 release (Veronesi et al., 2002b); and (c) the receptor activation causing release of inflammatory cytokines and neuropeptides initiates and sustains inflammatory effects in the airways (Veronesi and Oortgeisen, 2001).

Inhaled Particulate Matter Effects on Autonomic Control of the Heart and Cardiovascular System

Besides the above studies, it is worth noting that earlier studies in conscious rats previously have shown that inhalation of wood smoke causes marked changes in sympathetic and parasympathetic input to the cardiovascular system that are mediated by neural reflexes (Nakamura and Hayashida, 1992).

In addition, changes in heart rate variability and conductance system function associated with ambient PM exposure have been reported in some animal studies (Godleski et al., 2000; Gordon et al., 2000; Watkinson et al., 2000a,b; Campen et al., 2000), in several human panel studies (described in Chapter 8), and in a reanalysis of data from the MONICA study (Peters et al., 1997). Some of these studies included endpoints related to respiratory effects but few significant adverse respiratory changes were detected. This raises the possibility that ambient PM may have effects on the heart that are independent of adverse changes in the lung. There is certainly precedent for this idea. For example, tobacco smoke (which is a mixture of combustion-generated gases and PM) causes cardiovascular disease by mechanisms that are independent of its effect on the lung. However, not all studies have shown such alterations in HRV, etc. (Muggenberg et al., 2000; Frampton, 2001).

7.7.1.4 Mutagenic/Genotoxic Effects of PM

As discussed in Chapter 8, the Pope et al. (2002) extension of analyses evaluating long-term ambient PM exposure effects on total (non-accidental) and cause-specific mortality (using longer term followup data from the American Cancer Society or "ACS" database) provides additional strong evidence for chronic ambient PM exposure being associated with increased risks for lung cancer.

Newly available in vitro studies of ambient PM genetoxic/tumorogenic effects, by Hornberg and colleagues (Hornberg, et al., 1996, 1998; Seemayer and Hornberg, 1998) in

Germany provide credible evidence for ambient PM from heavily trafficed and industrialized areas inducing mutagenic effects in human bronchial epithelial cells and enhancing the susceptibility of hamster malignant kidney cells to simian virus-induced transformation. Analogously, Hamers (2000) and Alink (1998) report evidence for in vitro effects of ambient PM extracts or ambient PM from diesel exhaust, rubber/metal industries, or biologic sources (e.g., poultry/swine farming, compost) on genetoxic or intracellular gap junction communication, respectively, in cultured liver tumor cells. Other in vitro studies by Bunger (2000) indicated mutagenic activity in the Ames assay due to particle extracts from combustion of high or low sulfur diesel fuel or other "green" diesel biofuel.

The U.S. EPA Diesel Document (U.S. Environmental Protection Agency, 2002) was also cited earlier in this chapter as discussing a number of other studies utilizing genetoxicity assays with diesel emissions; and key information from that document on a number of studies indicative of diesel emission particle-induced gene mutations, chromosome effects, or other genetoxic effects (e.g., altered DNA adduct patterns, increases in mutagenic DNA, adduct-related vulnerability to oxidative damage) was recounted. Additional findings were also noted which show that, although 50 to 90% of the total mutagenicity of diesel exhaust is likely attributable to its gaseous components, nitrated polynuclear aromatic compounds (PAH's) also appear to account for a notable portion of the mutagenicity. Some results (but not others) further appear to implicate sulfur in diesel emissions as contributing to mutagenic effects. Lastly, of much interest are findings by Drischoll et al. (1996, 1997) showing increased hprt mutations in rat alveolar type II cells with inhalation exposure to carbon black particles or with intratracheal instillation of carbon black or two other (quartz, TiO_2) particles. All three types of particles elicited increased inflammatory responses. Overall, the new studies are highly indicative of mutagenic and other genotoxic effects of ambient PM in general and/or of diesel emission PM in particular.

7.7.2 Links Between Specific Particulate Matter Components and Health Effects

The plausibility of epidemiologically-demonstrated associations between ambient PM and increases in morbidity and mortality has been questioned because adverse cardiopulmonary effects have been observed among human populations at very low ambient PM concentrations.

To date, experimental toxicology studies have provided some intriguing, but limited, evidence for ambient PM mixes or specific PM components potentially being responsible for reported health effects of ambient PM. Overall, the new studies suggest that some of particles are more toxic than others. New findings substantiating the occurrence of health effects in response to controlled exposures to ambient PM mixes and/or their constituent substances are useful in demonstrating or clarifying potential contributions of physical/chemical factors of constituent particles are discussed below.

7.7.2.1 Ambient Particle Studies

Concentrated ambient particle (CAPS) studies are probably most useful in helping to substantiate that particles present in "real-world" ambient air mixes are indeed capable of inducing notable pathophysiological effects under controlled exposure conditions and to clarify further factors affecting increased susceptibility of "at risk" groups for PM effects. CAPs studies, on the other hand, tend to be somewhat less helpful than other toxicologic approaches in helping to delineate the specific characteristics of PM producing toxicity and potential underlying mechanisms. Some, but not all, studies with inhaled concentrated ambient particles (CAPs) have found cardiopulmonary changes in rodents and dogs at high concentrations of fine PM. However, no comparative studies to examine the effects of ultrafine and coarse ambient PM have been done.

Studies using collected urban PM for intratracheal administration to healthy and compromised animals have also produced valuable information. Despite the difficulties associated with extrapolating from the bolus delivery used in such studies, they have provided strong evidence that the chemical composition of ambient particles can have a major influence on toxicity. Instillation of rats with filter extracts of ambient air particles collected from Ottawa CN air (Watkinson, et al. 2002a,b) at 2.5 mg, for example, induced pronounced biphasic hypothermia, severe drop in heart rate, and increased arrhythmias; this was in contrast to no cardiac effects seen with comparable instilled dose of Mt. St. Helens volcanic ash. Similarly, dose-dependent increases in polymorphic neutrophils, other markers of lung inflammation, and decreases in alveolar machrophages were seen with intratracheal exposures of hamsters to urban ambient particles from St. Louis or Kuwaiti oil file particles (Brain et al., 1998).

Importantly, it has become evident that, although the concentrated ambient PM (CAPs) studies can provide important dose-response information, identify susceptibility factors in animal models, and permit examination of mechanisms related to PM toxicity, they are not particularly well suited for the identification of specific toxic components in urban PM. Because only a limited number of exposures using CAPs can be reasonably conducted by a given laboratory in a particular urban environment, there may be insufficient information to conduct a factor analysis on an exposure/response matrix. This may also hinder principal component analysis techniques that are useful in identifying particle components responsible for adverse outcomes. New particle concentrator systems now coming on-line at the U.S. EPA and elsewhere that permit selective concentration of ultrafine, fine, and thoracic coarse PM hold promise for enhancing our understanding of PM characteristics producing toxicity.

7.7.2.1 Acid Aerosols

There is relatively little new information on the effects of acid aerosols. The 1996 PM AQCD previously assessed acid aerosol health effects and concluded that acid aerosols cause little or no change in pulmonary function in healthy subjects, but asthmatics may develop small changes in pulmonary function. This conclusion is further supported by the new study of Linn and colleagues (1997) in which children (26 children with allergy or asthma and 15 healthy children) were exposed to sulfuric acid aerosol (100 $\mu g/m^3$) for 4 h. There were no significant effects on symptoms or pulmonary function when data for the entire group were analyzed, but the allergy group had a significant increase in symptoms after the acid aerosol exposure. Thus, acid aerosol health effects may represent a possible causal physical property for some PM-related respiratory symptom effects. However, it is unlikely that particle acidity alone could account for the pulmonary function effects (Dreher, 2000).

7.7.2.2 Metals

The 1996 PM AQCD (U.S. Environmental Protection Agency, 1996a) mainly relied on data related to occupational exposures to evaluate the potential toxicity of metals in contributing to health effects associated with ambient PM exposures. Since that time, numerous newly published in vivo and in vitro studies using ROFA or soluble transition metals have contributed substantial further information on the health effects of particle-associated soluble metals.

Although there are some uncertainties about differential effects of one transition metal versus another, water soluble metals leached from ambient filter extracts or ROFA have been shown consistently (albeit at high concentrations) to cause cell injury and inflammatory changes in vitro and in vivo.

Perhaps most notable in this argument are the Utah Valley studies that have linked the toxicology (in vitro cell culture as well as human and rodent instillation) with published epidemiological findings. In these studies, filter extracts of Utah Valley PM corresponding to the state/federal sampling sites that had been used to ascribe the impact of PM on hospital admissions and population mortality rates showed remarkable qualitative coherence with toxicological and clinical end points (BAL fluid markers, lung dysfunction) in the human and rodent test subjects. Moreover, the data were themselves consistent with the hypothesized underlying mode of action for metal-associated PM (oxidant generation, inflammation) (Frampton et al., 1999; Dye et al., 2001; Ghio and Devlin, 2001; Soukup et al., 2000; Wu et al., 2001; Pagan et al. 2003). Hence, this rich data set alone provides a key linkage across study disciplines and species without substantive influence of the doses used in the human and animal toxicology as well as in the in vitro studies.

Since the Utah studies were completed, an analogous study has addressed differential exacerbation of allergic asthma-related responses by PM from two German cities (Hettstedt and Zerbst) of contrasting industrial activity. An allergic mouse model (representing an allergic asthma population) was intratracheally instilled with filter extracts from each city and the appropriate allergen to activate the model. The respective responses of the model corresponded to the prevalence of allergy and respiratory disease in the cities (Gavett et al., 2003a). Hence, the data base is growing for studies linking animal and human responses. Some of these linkages are in the laboratory while others are with epidemiology. Why these collective data show coherence despite dose and exposure discrepancies, not to mention species and other differences, is unclear, but the data stand on their merits and attest to the legitimacy of the approach and the value of the animal data in establishing biologic plausibility and insight into potential mechanisms.

Even though it is clear that combustion particles that have a high content of soluble metals can cause lung injury in compromised animals and correlate well with epidemiological findings in some cases (e.g., Utah Valley Studies), it has not been fully established that the small

quantities of metals associated with ambient PM are sufficient to cause health effects. Moreover, it cannot be assumed that metals are the primary toxic component of ambient PM, nor that there is a single primary toxic component. Rather there may be many such components. In studies in which various ambient and emission source particulates were instilled into rats, the soluble metal content did appear to be an important determinant of lung injury (Costa and Dreher, 1997). However, one published study (Kodavanti et al., 2000a) has compared the effects of inhaled ROFA (at 1 mg/m^3) to concentrated ambient PM (four experiments, at mean concentrations of 475 to 900 $\mu g/m^3$) in normal and SO_2-induced bronchitic rats. A statistically significant increase in at least one lung injury marker was seen in bronchitic rats with one out of four of the concentrated ambient exposures; whereas inhaled ROFA had no effect, even though the content of soluble iron, vanadium, and nickel was much higher in the ROFA sample than in the concentrated ambient PM.

Nevertheless, other particularly interesting new findings do point toward ambient PM exacerbation of allergic airway hyperresponsiveness and/or antigen-induced immune responses. Both metal and diesel particles have been implicated, with an expanding array of new studies showing DPM in particular as being effective in exacerbating allergic asthmatic responses.

7.7.2.3 Diesel Exhaust Particles

As described in Section 7.5.3, there is growing toxicological evidence that diesel PM may exacerbate allergic responses to inhaled antigens. The organic fraction of diesel exhaust has been linked to eosinophil degranulation and induction of cytokine production, suggesting that the organic constituents of diesel PM are the responsible part for the immune effects. It is not known whether the adjuvant-like activity of diesel PM is unique or whether other combustion particles have similar effects. It is important to compare the immune effects of other source-specific emissions, as well as concentrated ambient PM, to diesel PM to determine the extent to which exposure to diesel exhaust PM may contribute to the incidence and severity of allergic rhinitis and asthma. It is also notable that rather direct evidence has been obtained which demonstrates adherence of allergen-laden pollen cytoplasm fragments to diesel particles, providing a likely mechanism by which diesel PM acts to concentrate bioaerosol materials and to increase their focal accumulation in lower regions of the respiratory tract. Other evidence substantiates mutagenic/genotoxic effects of diesel emission particles (e.g., PAH's), consistent

with qualitative findings in several studies of increased lung cancer effects being associated with long-term, occupational exposure to diesel emissions.

7.7.2.4 Organic Compounds

Published research on the acute effects of particle-associated organic carbon constituents is conspicuous by its relative absence, except for diesel exhaust particles. Like metals, organics are common constituents of combustion-generated particles and have been found in ambient PM samples over a wide geographical range. Organic carbon constituents comprise a substantial portion of the mass of ambient PM (10 to 60% of the total dry mass [Turpin, 1999]). The organic fraction of ambient PM has been evaluated for its mutagenic effects. Although the organic fraction of ambient PM is a poorly characterized heterogeneous mixture of an unknown number of different compounds, organic compounds remain a potential causal property for PM health effects due to the contribution of diesel exhaust particles to the fine PM fraction (Dreher, 2000). Strategies have been proposed for examining the health effects of this potentially important constituent (Turpin, 1999).

7.7.2.5 Ultrafine Particles

When this subject was reviewed in the 1996 PM AQCD (U. S. Environmental Protection Agency, 1996a), it was not known whether the pulmonary toxicity of freshly generated ultrafine polytetraflouethylene (PTFE; teflon) particles was due to particle size or a result of adsorbed fumes. Subsequent studies with other ultrafine particles have demonstrated a significantly greater inflammatory response than that seen with fine particles of the same chemical composition at similar mass doses (Oberdorster et al., 1992; Li et al., 1996, 1997, 1999).

In other more limited studies, ultrafines also have generated greater oxidative stress in experimental animals. Inhalation exposure of normal rats to ultrafine carbon particles generated by electric arc discharge (100 $\mu g/m^3$ for 6 h) caused minimal lung inflammation per unit mass (Elder et al., 2000a,b), compared to ultrafine PTFE or metal particles. On the other hand, instillation of 125 µg of ultrafine carbon black (20 nm) caused substantially more inflammation per unit mass than did the same dose of fine particles of carbon black (200 to 250 nm), suggesting that ultrafine particles may cause more inflammation per unit mass than larger particles (Li et al., 1997). However, the chemical constituents of the two sizes of carbon black

used in this study were not analyzed, and it cannot be assumed that the chemical composition was the same. Further, when the particle surface area is used as a dosimetric, the inflammatory response to both fine and ultrafine particles may be basically the same (Oberdörster, 1996b, 2000; Li et al., 1996).

With regard to acid aerosols, studies of low concentrations of ultrafine sulfuric acid and metal oxide particles have demonstrated effects in the lung. However, it is possible that inhaled ultrafine particles may have systemic effects that are independent of effects on the lung. Thus, there is still insufficient toxicological evidence to elucidate clearly the extent to which ambient concentrations or high number counts of ultrafine particles may differentially contribute to increased health effects risks associated with ambient PM air pollution.

7.7.2.6 Bioaerosols

Bioaerosols are airborne particles consisting of large molecules or volatile compounds that are living, contain living organisms or have been released from living organisms. Such particles are suspended and/or transported in air as distinct separate entities or adhered to other organic and non-organic particles or in water droplets. Major types of bioaerosol particles encountered in ambient (outdoor) air, indoor air, and/or in contaminated indoor or outdoor dusts that can be resuspended into air include: (1) intact pollen and pollen fragments; (2) fungi, their spores, and other fungal byproducts; (3) humus-like substances (HULIS) and other plant debris; (4) certain animals or associated debris (such as dust mites or their excreta, shed mammalian or avian skin cells, etc.); (5) bacteria or fragments thereof (e.g., endotoxins consisting of proteins and lipopolysaccharides or LPS that comprise portions of cell walls of Gram- bacteria); (6) (1→3)-β-D-glucan (a polyglucose compound in the cell walls of Gram+ bacteria, fungi, and plants); and (7) viruses. Such biological particles can range in size from 0.01 μm (viruses) to > 20 μm (some pollen) with the smaller ones < 10.0 μm being inhalable and, upon inhalation, being capable of penetrating into tracheobronchial and alveolar regions of the lower respiratory tract.

The relationship between bioaerosol exposure and illness is complex. Numerous studies published since the 1996 PM AQCD have produced extensive new information which has greatly enhanced our knowledge regarding environmental occurrence of such biological aerosols, their health effects, and possible combined influences of their being copresent along with other biological and/or non-biological particles in ambient air. In particular, there is

growing recognition that bioaerosols may contribute to health effects related to ambient PM exposures partly through their own direct toxic effects and/or in combination with other PM that carries biologically-derived materials which may elicit untoward effects.

A large number of studies show a relationship between exposure to bioaerosols and airways inflammation and other signs/symptoms of allergic/asthmatic responses. Generally these exposures are most often associated with certain occupational settings (cotton milling, grain workers, feed mill employees, farmers), humid and poorly ventilated indoor environments where moisture/dampness can harbor these organisms, and households with pets (Wheatley and Platts-Mills, 1996).

Grass pollens and their allergens are the most important contributors to pollen-induced hay fever and allergic asthma. Intact pollens grains, because of their generally large aerodynamic diameters (often > 10-20 μm) typically do not reach the lower respiratory tract. However, pollen allergens are associated with respirable size pollen fragments. Taylor et al. (2002) found that grass pollen, when moistened with water, ruptures to release allergen-laden fragmented pollen cytoplasm in the size range of 0.12 to 4.67 μm. Pollen allergens released during rainfall and other moisture conditions (Bellomo et al., 1992; Celenza et al., 1996; Taylor et al., 2002), upon drying, are suspended in ambient air and can be transported over long distances. On dry days, pollen allergen concentrations have been reported to approach 10,000 starch granules/m^3. The starch granules may increase up to 10-fold on days with rainfall (Schäppi et al., 1999).

Pollen, in addition to containing cytoplasmic allergens, has also been shown to be a carrier of other allergenic materials. Several different types of immunoactive, allergenic materials (e.g., Gram– and Gram+ bacteria; endotoxin, fungi) have been shown to be associated with grass and tree pollens in Poland (Spiewak, 1996a,b). Also of much importance, as noted earlier, newly available evidence implicates diesel emission PM as being a carrier for pollen-related allergens. The possibility exists for diesel and/or perhaps other airborne particles to essentially serve as concentrators of allergens from ambient air and conveyors of them to lower regions of the lung.

Several epidemiologic studies during the past five years have demonstrated associations between airborne pollen levels and increased hospital admissions or emergency room visits for asthma (Rosas et al., 1996; Calenza et al., 1996); asthma incidence and medication use (Delfino et al. 1996, 1997); and increased mortality (Brunekreefe et al. (2000).

In the absence of significant sources, fungi and bacteria concentrations in a typical suburban area have been reported to range from 0 to 1,200 colony forming units (cfu)/m^3 mesophilic fungi, 0 to 300 cfu/m^3 thermophilic fungi, 0 to 81 (mean 1) cfu/m^3 Aspergillus fumigatus, 100 to 1,200 cfu/m^3 total bacteria and 10 cfu/m^3 gram negative bacteria, and 60 cfu/m^3 thermophilic bacteria and astinomycetes (Lai et al., 2003). Takahashi (1997) reported total outdoor total fungal colony forming units of < 13 to 2,750 cfu/m^3.

Indoor levels of bioaerosols are generally lower than outside levels (Shelton et al., 2002) except when there is an indoor source (Meklin et al., 2002a). Indoor bacteria concentrations have been reported to range from 35 to 22,000 cfu/m^3 (Lai et al., 2003), while indoor fungal concentrations have been reported as high as 3,750 cfu/m^3. Fungi and bacteria spores in the size range of 1.1 to 2.1 µm and 0.65 to 7.0 µm were found in school buildings in Finland. The highest concentrations of fungi spores in this size range were found in the moisture damaged buildings. Bacteria size range appeared to be dependent on the building construction material (Meklin et al., 2002a). Indoor endotoxin levels were reported to be generally higher than outdoor levels although not consistently depending on the season. That is, during warmer outdoor weather from June to August, outdoor levels were higher (mean 0.92 EU/m^3). From September to April, indoor endotoxin levels were higher than outdoor levels. Also, on average, urban endotoxin levels (0.51 EU/m^3) were also higher than suburban concentrations (0.39 EU/m^3) (Park et al., 2000).

Heinrich et al. (2003) analyzed ambient $PM_{2.5}$ and $PM_{2.5-10}$ collected for Hettstedt and Zerbst, Germany for soluble endotoxin concentrations, to see if differences in levels of such would account for differences in prevalence and incidence of allergic hypersensitivity between the two towns. The PM fractions were collected simultaneously at weekly intervals in both towns during January to June. The $PM_{2.5}$ and $PM_{2.5-10}$ concentrations were 10.2 and 12.4 µg/m^3 $PM_{2.5}$ and 6.1 and 6.8 µg/m^3 $PM_{2.5-10}$ for Hettstedt and Zerbst, respectively. Airborne endotoxin concentrations showed a strong seasonality for both the fine and coarse fractions; generally lower during the winter and early spring and increased in May and June. The corresponding endotoxin concentrations were 0.008 and 0.011 EU (endotoxin units)/mg $PM_{2.5}$ and 0.082 and 0.083 EU/mg $PM_{2.5-10}$ for samples from Hettstedt and Zerbst, respectively; and did not seem to account for the allergy differences between the two towns.

Dose-response studies in healthy volunteers exposed to 0.55 and 50 µg endotoxin, by the inhalation route, suggested a threshold for pulmonary and systemic effects for endotoxin between 0.5 and 5.0 µg (Michel et al., 1997). Monn and Becker (1999) examined effects of size fractionated outdoor PM on human monocytes and found cytokine induction characteristic of endotoxin activity in the coarse-size fraction but not in the fine fraction.

Available information suggests that ambient concentrations of endotoxin are typically very low, rarely exceeding 0.5 ng/m^3. However, there are numerous other bioaerosols present in the ambient air, including pollen and fungal allergens. Of much importance are the seasonal variations in ambient air concentrations of all types of airborne allergens (both plant- and animal-derived) typically observed in temperate climate areas. Typically, (given that warmer, humid conditions tend to facilitate pollen, fungal and bacterial growth) outdoor levels of pollen fragments, fungal materials, endotoxins, and glucans all tend to increase in the spring/summer months and decrease to low ambient levels in late fall/winter months in most U.S. and other temperate areas. Also of much importance are increased levels of cellulose and other plant debris in respirable size fractions of ambient aerosols during spring/summer months — plant materials that can act as carriers for allergenic materials (bacterial, fungal, etc.). The copresence in ambient air of other biological particles capable of acting as carriers of such allergens would probably enhance the risk of allergic/asthmatic reactions to them. Pertinent to this, it is of interest to note, that endotoxin concentrations tend to be higher in coarse fraction ambient PM samples than in fine (< 2.5 µm) ambient PM samples.

7.7.3 PM Interactions with Gaseous Co-Pollutants

Some evidence suggestive of possible combined effects of long-term exposures to complex ambient air mixes (containing PM and other gaseous copollutants) comes from the examination of autopsy materials from animals living in “dirty air” rural areas of Mexico, Spain, and Italy. Morphological changes were reported in the nasopharynx (Calderon-Garciduenas et al., 2001c), the lower respiratory tract (Gulisano et al., 1997; Lorz and Lopez, 1997; Calderon-Garciduenas, et al., 2001c); and the heart (Calderon-Garciduenas, et al., 2001c) of lambs, pigeons, and dogs, respectively, naturally chronically-exposed to high levels of air pollution. However, it is not possible from such studies to attribute, clearly, observed morphologic effects to ambient PM,

specific ambient PM components, one or another gaseous pollutant (e.g., O_3, NO_2, etc.) present or combination(s) of PM and specific gases.

On the other hand, several new well-conducted controlled exposure toxicology studies do provide somewhat more readily interpretable results. In one, a randomized double-blind crossover study, Brook et al. (2002) observed increased brachial artery constriction in adult human males and females (mean age = 34.9 yr ± 10 SD), exposed for 2 hr to filtered ambient air containing 150 $\mu g/m^3$ CAPS and 0.125 ppm O_3 while at rest. Another study, by Linn et al. (1997), found a positive association between acid concentration and respiratory symptoms (but not spirometry) among asthmatic children following a single 4-hr exposure to 60 to 140 $\mu g/m^3$ H_2SO_4, 0.1 ppm SO_2, and 0.1 ppm O_3 while undergoing intermittent exercise. No changes were seen among healthy children.

7.7.4 Susceptibility

Progress has been made in understanding the role of individual susceptibility to ambient PM effects. Studies have consistently shown that older animals or animals with certain types of compromised health, either genetic or induced, are more susceptible to instilled or inhaled particles, although the increased animal-to-animal variability in these models has created greater uncertainty for the interpretation of the findings (Clarke et al., 1999, 2000; Kodavanti et al., 1998, 2000b, 2001; Gordon et al., 2000; Ohtsuka et al., 2000c; Wesselkamper et al., 2000; Leikauf et al., 2000; Saldiva et al., 2002). Moreover, because PM seems to affect broad categories of disease states, ranging from cardiac arrhythmias to pulmonary infection, it can be difficult to know what disease models to use in evaluating the biological plausibility of adverse health effects of PM.

Nevertheless, particularly interesting new findings point toward ambient PM exacerbation of allergic airway hyperresponsiveness and/or antigen-induced immune responses. Both metals and diesel particles have been implicated, with an expanding array of new studies showing DPM as one particle that is effective in exacerbating allergic asthma responses (Takano et al., 1997; Nel et al., 2001; Van Zijverden et al., 2000, 2001; Walters et al., 2001; Nordenhall et al., 2001; Hamada et al., 1999, 2000; Lambert et al., 1999; Gilmour et al., 2001).

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Appendix 7A

Rat-to-Human Dose Extrapolation

Introduction

As noted at the outset of this chapter, the 1997 revisions to the PM NAAQS (Federal Register, 1997) were based, in large part, on newly emerging epidemiologic evidence showing associations between (a) ambient PM measured at community monitoring stations) and (b) increased risks for mortality and morbidity (especially cardiorespiratory-related) among human populations exposed to contemporary U.S. ambient concentrations. There was no evidence for a threshold. However, very little experimental toxicology data from controlled laboratory animal or human exposure studies were then available that provided more direct evidence supporting the plausibility of the PM-mortality/morbidity relationships observed at the relatively low ambient PM concentrations.

Since completion of the 1996 PM AQCD supporting the 1997 PM NAAQS decisions, numerous hypotheses have been advanced and extensive new toxicologic evidence generated with regard to possible pathophysiological mechanisms by which PM exposures (even at low ambient concentrations) might induce increased morbidity and/or mortality. Much of the new toxicologic data (as addressed in preceding sections of this chapter) has involved either (a) experimental in vivo exposures of human subjects and/or laboratory animals via inhalation exposures and/or intratracheal instillation of PM materials or (b) in vitro exposures of various (mostly respiratory tract) cells or tissues to diverse types of PM. Concerns have been raised, that the animal exposure concentrations, on the order of mg/m^3, were so large as to produce doses in animals that would be unrealistically large and not particularly relevant to doses to human lungs resulting from ambient PM exposures under normal atmospheric concentrations. Thus, the relevance and utility of such experimental toxicology studies in providing evidence linking PM exposures to various health outcomes consistent with the epidemiologic observations has come into question.

In response to these concerns, EPA has conducted an analysis of the relationship between the PM dose to a rat's lung from inhalation studies and the PM dose to a human lung from exposures during normal activities. Two main approaches, discussed below, were pursued. First, taking into account certain general key points regarding dose metrics briefly summarized below, one of the publically available dosimetry models discussed in Chapter 6 was employed to compare estimates of deposited and/or retained respiratory tract PM doses in the human and rat lung using different dose metrics as described in Table 7A-1. The second approach involves

TABLE 7A-1. POTENTIAL COMBINATIONS OF DOSE METRICS AND NORMALIZING PARAMETERS USED IN DOSIMETRIC MODELING

Dose Metrics			
Respiratory Region	**PM Indicator**	**Dose**	**Normalizing Parameters**
thoracic	mass	total or average	lung mass
tracheobronical (TB)	surface area	deposited or retained	TB or A surface area
alveolar (A)	number	incremental dose only or incremental plus accumulated dose	per alveolus or per macrophage

application of the same publically available model (a) to estimate likely respiratory tract doses (again using various dose metrics) resulting from experimental exposures (via PM inhalation or instillation) of human or laboratory animals (rats) actually employed in representative published PM toxicology studies assessed in this chapter and (b) to estimate likely ambient PM exposure concentrations that would be needed in order to obtain comparable human and rat PM respiratory tract doses.

Dose Metrics

Much of our information on the toxicity of PM comes from studies in which laboratory rats were exposed to PM by inhalation or instillation. For ingested toxicants, the mass of material ingested is the usual dose metric and body mass is the normalizing parameter, thus the equivalent human dose = $\frac{\text{human body mass}}{\text{rat body mass}} \times$ rat dose. For inhalation toxicology, however, there are many possible combinations of dose metrics and normalizing factors, as shown in Table 7A-1. It is not possible to be certain which combination would be most relevant; and so, several combinations are considered in the dosimetric comparisons that follow.

It is customary in risk assessment to use a dosimetric adjustment factor (DAF) to determine the human equivalent exposure concentration (HEC) from the rat exposure concentration (RC), where

$$HEC = RC \times DAF \quad (1)$$

DAF is generally related to breathing parameters and target site deposition fractions as shown below:

$$DAF = \frac{(\dot{V}_E)_R}{(\dot{V}_E)_H} \times \frac{DF_R}{DF_H} \times \frac{NF_R}{NF_H} \quad (2)$$

where $\dot{V}_E$ is the minute ventilation, DF is the deposition fraction in the target site or region, and NF is the normalizing factor in humans (H) and rats (R). The normalizing factor for respiratory effects is often assumed to be per unit lung weight or per unit surface area of the target respiratory tract region (e.g., the tracheobronchial or alveolar). Differences in exposure duration can be added to the above equation to account for differences in human and animal inhalation scenarios. The equation may also be modified to give a DAF for retained dose as well as for deposited dose.

Rat and Human Dosimetry

The publicly available Multiple Pass Particle Dosimetry (MPPD) model (described in Chapter 6) permits calculations for humans and rats based on the same particle deposition principles, taking into account information or various dose metrics listed in Table 7A-1. The MPPD model (Asgharian, et al., 1999; Freijer, et al. 1999) can be used to estimate the fraction of inhaled PM that would be deposited in a human or rat for various particle sizes and breathing patterns (see also Winter-Sorkina and Cassee, 2002). The model also allows for calculation of estimates of the retained dose for various lengths of time. Before providing illustrative examples of how a dosimetric model, the MPPD, may be used in rat-to-human extrapolation, it is useful to discuss some of the many differences between rat and human exposure and dosimetry, as summarized in Table 7A-2.

Anatomy. The structure and function of the respiratory tract differs in rats and humans in ways that affect the deposition of particles in the lung. Rats are obligate nose breathers whereas humans are oral-nasal breathers who breathe increasingly through the mouth with activity. This distinction is important because the nose is a more efficient filter than the mouth for preventing the penetration of particles into the lung. Thus, by breathing through the mouth, humans effectively increase the amount of inhaled aerosols reaching the lung. Due to the lower

TABLE 7A-2. DIFFERENCES BETWEEN RAT AND HUMAN EXPOSURE SITUATIONS

Differences In:	Rats	Humans
Anatomy	Nasal breathers Monopodial branching structure	Oral-nasal Dichotomous branching structure
Exertion Level	Usually resting during exposure	Exposure occurs over a range from sleep to heavy exercise or work.
Clearance	Fast	Slow (must consider retained dose as well as deposited dose.)
Prior Exposure	Usually kept in clean or relatively clean air in laboratory setting. Only a few months of low exposure prior to test exposure.	Mature or elderly humans likely will have accumulated larger burdens of PM from prior exposures than will have laboratory rats, on a normalized basis.
PM Burden	Retained dose reaches equilibrium after several months, and at a lower fraction of deposited dose than for a human.	On the order of 10 years required for the retained dose to reach equilibrium.
PM Size Distribution	Experimental challenge exposures mostly to resuspended dust; representative size distribution: MMD = 1.2 - 2.5 μm, σ_g = 1.5 -2.5	Exposed to all three atmospheric modes: Aitken (.01-.1 μm), σ_g = 1.6-1.7; Accumulation (.1-1 μm), σ_g = 1.6-2.2; Coarse (1-100 μm), σ_g = 1.8-2.4

inhalability of the rat for particles larger than 3 μm and the more torturous nasal passages of rats, the human has a larger TB and A region deposition fraction for coarse particles than a rat even when breathing through the nose. The structure of the intrathoracic airways also differs between rats and humans in ways that affect the regional deposition pattern in the lung. The branching structure of the lung is monopodial in rats and symmetrically dichotomous in humans. A monopodial structure has the potential to allow increased penetration of large particles into the A region. Rats also lack respiratory bronchioles, a site of early airway disease in humans. The rat homologue to the respiratory bronchioles is the bronchoalveolar transition zone.

Exertion Level. Laboratory rats are usually at rest while they are exposed to PM by inhalation. In contrast, humans typically experience a range of breathing patterns during exposure to ambient PM, including those experienced during light and heavy exertion as well as at rest and during sleep. Chapter 6 discusses how increasing exertion leads to greater deposition of PM in the human lung due to changes in the mode of breathing (nasal to oral-nasal to oral) as well as to inhalation of greater quantities of PM per unit time due to an increase in minute ventilation (Figure 6-18).

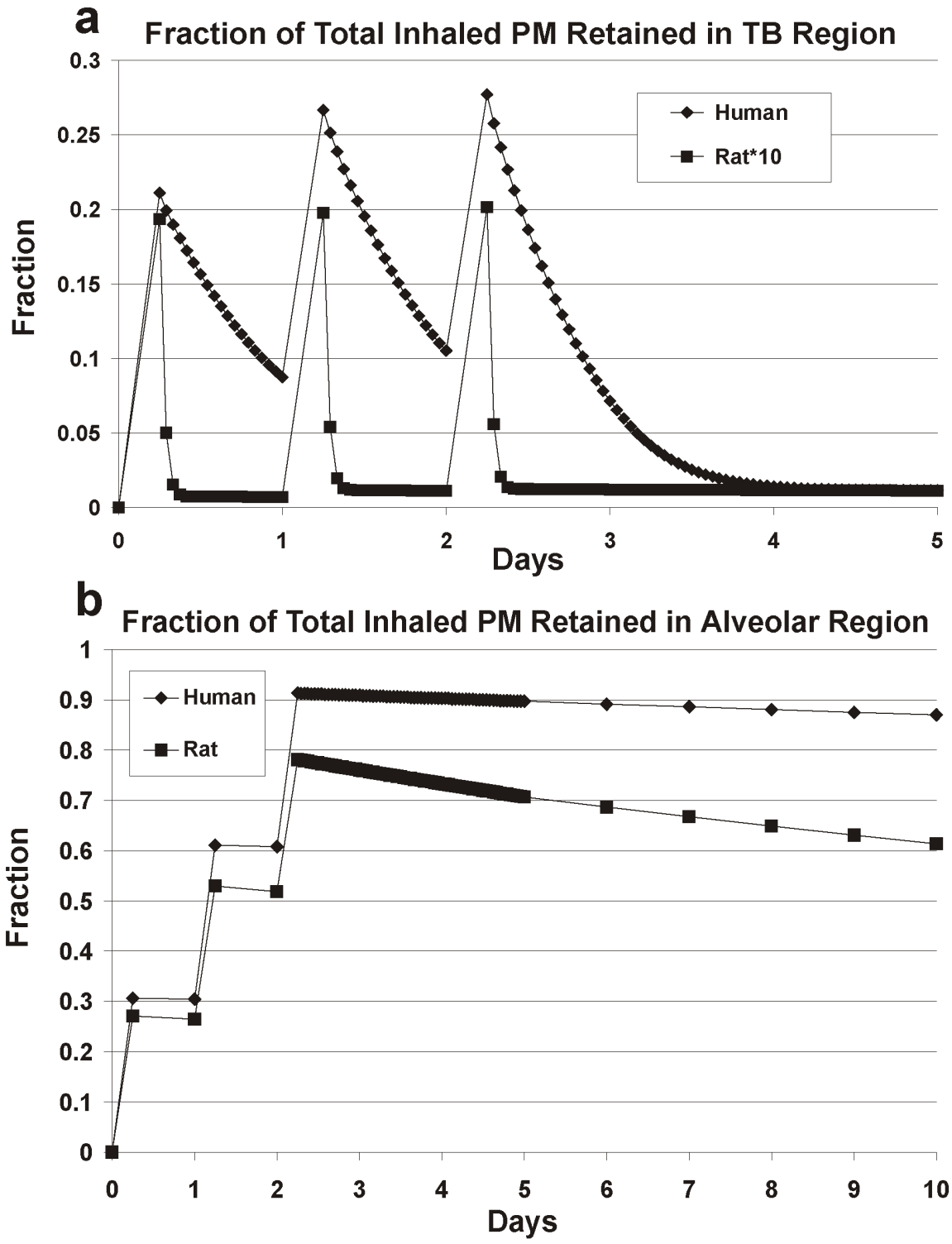

Figure 7A-1a,b. Fraction of total inhaled PM retained in the lung after a six-hour exposure in each of three days: a, TB region; b, A region.

Clearance/Retention. Poorly soluble particles deposited in the lung are cleared more rapidly from the TB region than the A region in both humans and rats. However, the clearance rates for rats are much higher than for humans for both the TB and A regions. Clearance half-times in the TB region are highly dependent on the site of deposition, but generally range from 1-2 hours in rats and 4-10 hours in healthy humans. However, for the A region, clearance half-times are on the order of months in rats but years in humans. Figure 7A-1 shows an example of the retained mass, as a fraction of the total inhaled mass, estimated by the MPPD model, for a 6-hour exposure each day for three days (TB region, 7A-1a; A region, 7A-1b). Note that the retained fraction for the TB region in the rat has been multiplied by 10. Because of the large fraction of particles removed in the nose of the rat and the rapid removal of deposited particles from the TB region, the maximum retained dose in the rat TB region is never greater than 0.02 of the total inhaled dose; whereas, in the case of the human, the maximum retained TB dose reaches as high as 0.275 of the total inhaled dose. If the retained dose were averaged over three days, the discrepancy between rats and humans would be even greater. As shown in Figure 7A-1b, clearance is slower in the A region that the TB region for both rats and humans, but clearance in the rat is faster than that in the human. (Clearance calculations used MPPD default values of 12 breaths/min at a tidal volume of 625 mL for humans and 102 breaths/min at a tidal volume of 2.1 mL for the rat. For both rats and humans the size distribution was MMAD = 2, σ_g = 2, and density = 1 g/cm^3).

Prior Exposure. Rats are usually kept in a laboratory setting and breathe air that has been filtered and conditioned. Thus, they are exposed to relatively clean air for the months prior to the experimental exposure. On the other hand, people are exposed to ambient and nonambient PM all their lives. Therefore, mature or elderly humans will have accumulated, normalized to lung mass or area, a much larger burden of PM in their lungs than a laboratory rat.

Burden. Because of the more rapid clearance, a rat will reach an equilibrium retained dose in the A region in a few months while it will take more than 10 years for a human to do so. Thus, while a 6 month old rat and a 60 year old human will both have reached equilibrium for the A region burden, that of the human will be much greater (in terms of fraction of inhaled PM mass retained) than the rat.

Size Distribution. Rats are frequently experimentally exposed to particles produced by resuspension of bulk material or resuspension of particles previously collected from specific

sources or ambient air. Resuspension produces particles with a nominal MMAD between 1.0 and 2 μm. However, humans are exposed to a mix of particles in the Aitken, accumulation, and coarse modes. The particle size distribution is important because the deposition fraction varies with particle size. In addition, some studies suggest that particle surface area or possibly particle number may be as important or more so than mass in determining the extent of health effects. Figure 7A-2a shows the mass size distribution of a representative resuspended dust (MMAD = 2 μm, σ_g = 2) overlaid on an atmospheric mass size distribution. Figure 7A-2b shows the distribution of particle surface area. Note that the coarse mode and the resuspended dust mode contribute little to the particle surface area. Figure 7A-2c shows the particle number distribution with the number concentration on a logarithmic scale. The coarse and resuspended mode comprise only a tiny contribution to the number concentration.

Dosimetric Calculations Comparing Rats to Humans: General Exposure Scenarios

Extrapolation Modeling. Dosimetric calculations, using the MPPD model (Freijer et al., 1999; Winter-Sorkina and Casse, 2002), were performed for one human and one rat exposure scenario. Input parameters for the dosimetric calculations are shown in Tables 7A-3 to 7A-5; results are given in Table 7A-6 . The MPPD model incorporates features of the Yeh et al. (1979) asymmetric, multipass model for the rat lung and the Yeh and Schum (1980) symmetric model for the human lung. The activity and exertion level scenarios chosen are (a) for a rat (nose breathing) at rest experimentally exposed to resuspended PM and (b) for a human (mouth breathing) working along a busy road and exposed to an ambient atmospheric size distribution at a concentration equivalent to the 150 $\mu g/m^3$ PM_{10} 24-hour NAAQS. Breathing parameters for the human and rat exposure scenarios are provided in Table 7A-3. The particle size characteristics, exposure concentrations, and resulting regional (TB, A, Thoracic = TB + A) particle deposition fractions estimated by the MPPD for particles in each of the three atmospheric modes for the human exposure scenario and in the resuspended PM mode for the rat are shown in Table 7A-4. The particle characteristics indicated for the resuspended PM mode are typical of those used in experimental inhalation studies using rats. Exposure concentrations necessary to obtain TB and A region doses in the rat equivalent to the human doses comparable in regions of the respiratory tract were then calculated for a variety of dose metrics and normalizing parameters. Table 7A-5 shows the values used as normalizing factors. The lung

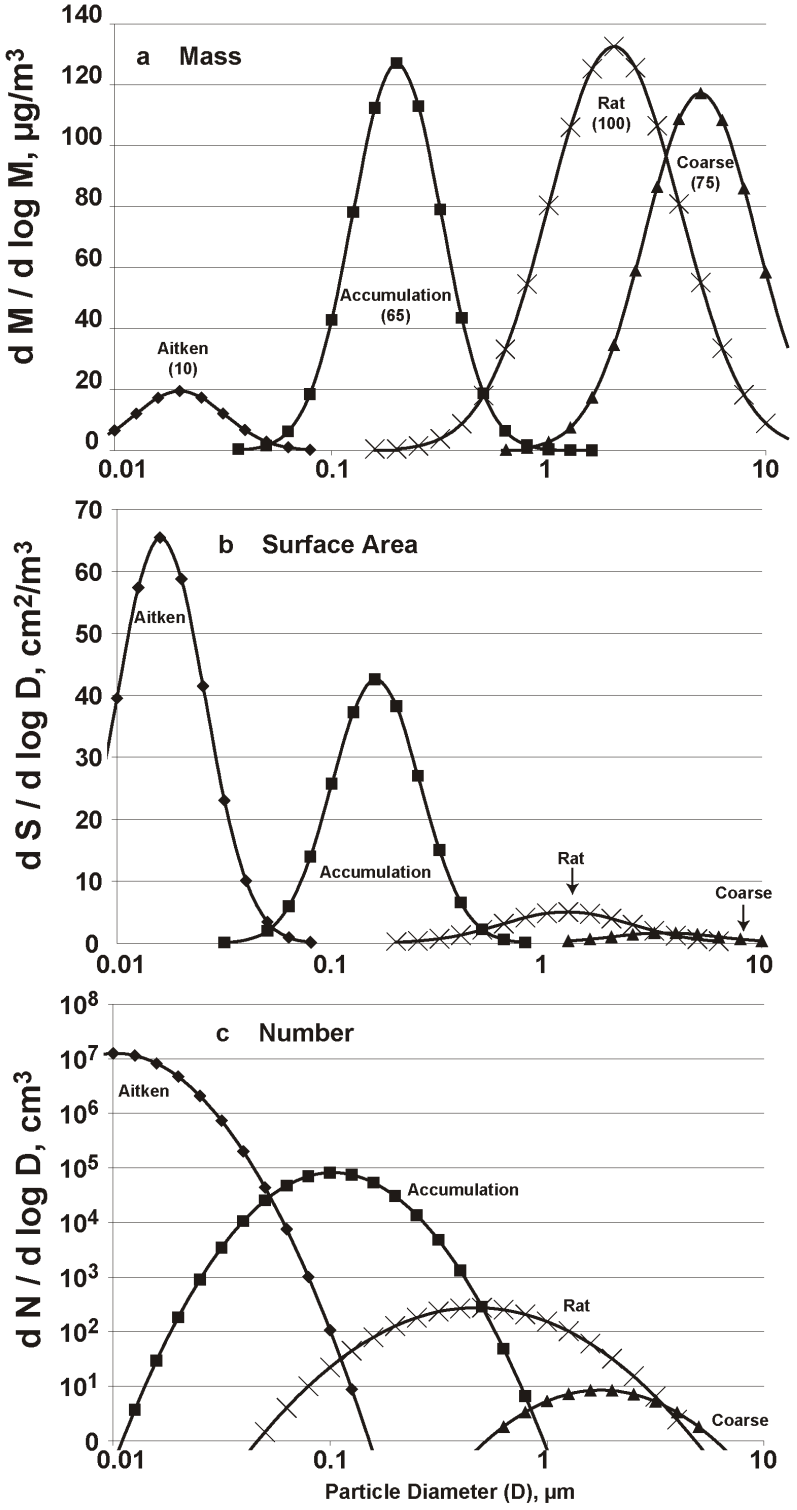

Figure 7A-2a,b,c. Size distributions of the Aitken, accumulation and coarse modes of the average urban aerosol (as reported by Whitby, [1978]) and a resuspended PM mode: a, mass distribution; b, surface area distribution; c, number distribution. Concentrations, in µg/cm^3, are shown for each mode in a.

TABLE 7A-3. BREATHING PARAMETERS SCENARIOS USED TO PRODUCE TABLE 7A-6 FOR HUMAN AND RAT

Scenarios	6 Hour Exposure Increment, No Prior Retention	
	Different Size Distributions for Human and Rat	
	Human Working by Busy Road	Rat at Rest
	Human	**Rat**
Breaths/min	28	102
Tidal Volume, mL	1429	2.1
Minute ventilation, $\dot{V}_E$, mL/min	40000	214
Functional Reserve Capacity, FRC, mL	3300	4
$\dot{V}_E$ / FRC	12	54

TABLE 7A-4. PARTICLE CHARACTERISTICS, CONCENTRATIONS AND REGIONAL DEPOSITION FRACTIONS, USED TO PRODUCE TABLE 7A-6

	Human			**Rat**
Size Distributions	Aitken	Accumulation	Coarse	Resuspended
Diameter, µm	0.031	0.31	5.7	2
Sigma, σ	1.7	2.03	2.15	2
Density, g/ml	1	1	1	1
Conc., µg/m^3	10	65	75	X = ?
TB, Fraction Deposited	0.1664	0.0655	0.1414	0.0397
A, Fraction Deposited	0.3609	0.0893	0.1244	0.0585
Thoracic, Fraction Deposited	0.5273	0.1548	0.2658	0.0982

TABLE 7A-5. NORMALIZING PARAMETERS USED TO PRODUCE TABLE 7A-6

	Normalizing Parameters	
	Human	Rat
Lung Mass, g	1100	4.54
TB Area, m2	0.4419	0.002346
A Area, m2	57.22	0.2972

areas are based on the size of the lung at the functional reserve capacity (FRC) used in the model.

Incremental Dose. Table 7A-6 shows modeled estimates of exposure concentrations ($\mu g/m^3$) required to give a rat an incremental dose equivalent to the incremental dose received by the human for the specified scenario. It also shows the ratio of the rat exposure concentration to the human exposure concentration. The ratio is the dosimetric adjustment factor (DAF) from Equations 1 and 2. Rat exposure concentrations and ratios are given for a variety of dose metrics (mass, surface area, and number; deposited over a six-hour exposure; retained dose averaged over 6 and 24 hours; to the A, TB, and thoracic [A + TB] regions) and are normalized to lung mass, TB area, or A area. As shown in Table 7A-6, for the PM mass dose metrics, the DAF values for deposited mass vary from just under 2 to almost 3 for deposited mass per lung mass or surface area, to about 7 per macrophage, and to about 60 per alveolus. The DAF values range from 2 to 2.5 for retained mass in the A region and from just over 10 to almost 50 for retained mass in the TB region. The DAF values for surface area are higher than those for mass by about a factor of 10 and the DAF values for number are still much higher.

Burden. It may also be useful to compare rat and human exposures in terms of both the incremental dose due to a six-hour exposure plus the total retained burden built up over the time it takes to reach an equilibrium dose, about ten years for a human but less than six months for a rat. Table 7A-7 gives results of a simulation which included the six-hour acute dose plus the accumulated burden of 10 years exposure to 2 $\mu g/m^3$ of Aitken particles and 15 $\mu g/m^3$ each of accumulation and coarse mode particles for a human with an average tidal volume of 900 mL and breathing rate of 17 breaths per minute (minute ventilation of 15.3 L/min). The rat burden was based on a six-hour acute exposure to resuspended dust plus the retained burden due to a 6-month exposure to 10 $\mu g/m^3$ each of accumulation and coarse mode PM at the resting breathing parameters. The additional six hours exposure concentration to give an accumulated dose or PM burden equivalent to the corresponding dose for the six-hour work exposure was estimated using the MPPD model. It was assumed that 50% of the PM from the long-term exposure could be treated as soluble and therefore did not contribute to the long-term burden. This simulation indicates that rat exposure concentrations of the order of 3 mg/m^3 (six-hour average) and 5 mg/m^3 (24-hour average) can give a PM mass burden in the TB region equivalent

TABLE 7A-6. RAT EXPOSURE CONCENTRATION (μg/m^3) TO GIVE A DOSE EQUIVALENT TO THE HUMAN DOSE RECEIVED AT A HUMAN EXPOSURE CONCENTRATION OF 150 μg/m^3 FOR PARTICLE MASS, SURFACE AREA, AND NUMBER

	Mass Dose		Surface Area Dose		Number Dose	
Deposited PM	[a]**μg/m^3**	**DAF**	[a]**μg/m^3**	**DAF**	[a]**μg/m^3**	**DAF**
Thoracic Deposition per Lung Mass	277	1.85	3170	21	5.8×10^6	3.9×10^4
Thoracic Deposition per Lung Area	349	2.32	3990	27	7.5×10^6	4.9×10^4
Tracheal-Bronchial (TB) Deposition per TB Area	413	2.75	4070	27	1.2×10^7	8.0×10^4
Alveolar (A) Deposition per A Area	311	2.07	4000	27	5.9×10^6	3.9×10^4
Deposited PM						
Deposition per Alveolus	8830	58.9	114000	757	1.7×10^8	1.1×10^6
Deposition per Macrophage	1100	7.3	14100	94	2.1×10^7	1.4×10^5
Retained PM in TB						
6-hour Average per lung mass	1980	13.2	36400	242	1.2×10^8	7.8×10^5
24-hour Average per lung mass	5700	38.0	121000	808	3.7×10^8	2.5×10^6
6-hour Average in TB per TB area	2500	16.9	46800	312	1.5×10^8	1.0×10^6
24-hour Average in TB per TB area	7330	48.9	156000	1040	4.8×10^8	3.2×10^6
Retained PM in A						
6-hour Average Retained in A per lung mass	294	1.96	3750	25	5.5×10^6	3.7×10^4
24-hour Average Retained in A per lung mass	297	1.98	3790	25	5.6×10^6	3.7×10^4
6-hour Average A Retained in A per A area	370	2.47	4720	31	7.0×10^6	4.6×10^4
24-hour Average A Retained in A per A area	374	2.49	4780	32	7.0×10^6	4.7×10^4

[1]Equivalent Exposure Concentration.

TABLE 7A-7. SIX-HOUR INCREMENTAL RAT EXPOSURE CONCENTRATION REQUIRED TO GIVE A PM BURDEN IN THE RAT LUNG EQUIVALENT TO THE HUMAN BURDEN FOLLOWING SIX HOURS OF WORK NEAR A BUSY ROAD

Mass Burden in the TB Region	DAF	mg/m3
TB burden per lung mass	18	3
TB burden per TB area	36	5
Mass Burden in the A Region		
A burden per lung mass	241	36
A burden per A area	373	56

to that for a human. However, for other dose metrics, including burden in the A region, extremely high rat exposure concentrations are required.

Caveats. The simulations are based on a model. While the model uses similar deposition calculations for humans and rats, the results of the simulations are only considered as estimates. The particles were assumed to have a density of 1 g/cm^3 so the physical, aerodynamic, diffusion and thermodynamic diameters would be the same. The calculations for the number dose used a single size, 0.013 µm, rather than a distribution since the MPPD model does not go below 0.01 µm diameter. No consideration was given to the difference between human PM exposures and ambient PM concentrations nor to exposures to indoor-generated or occupational PM. Thus, while the results may not be quantitatively accurate, the general relationships between human and rat exposure may provide useful information in the attempt to understand rat to human PM dose extrapolation.

Health Status: a Non-Dosimetric Consideration

Most people appear to be able to resist or otherwise compensate for the effects of inhaled PM most of the time. However, at some times some people show observable effects related to inhaled PM. The epidemiology data base provides strong evidence that certain people or subpopulations exhibit enhanced risk to PM. Clearly, many host factors may come into play when considering response to PM. While the mechanistic reasons for enhanced responsiveness are not understood, some specific host attributes or health conditions seem to be contributory.

Chronic conditions such as diabetes, chronic heart or vascular disease, or chronic lung disease generally have been shown be increased susceptibility. It appears that existent lung conditions which may increase or alter the deposition or retention of PM provided one means by which risk is augmented (i.e., dose). The very old and the very young may also be susceptible due to underlying disease, impaired or immature defenses, or perhaps exacerbated or associated with other factors such as poor nutrition. Rats normally have higher concentrations of some of the major endogenous antioxidants than people (e.g., ascorbate), and, thereby, may be better able to resist the effects of reactive oxygen species thought to be generated by or in response to PM. It may be that people show adverse responses to PM only when their resistance to PM is sufficiently lowered or impaired by disease or chronic exposures to PM or other toxicants. Thus, in order to observe adverse responses to PM in rats, it may be necessary to move the rat into a susceptible condition by: (a) exposing the rat to sufficient quantities of PM to provide a deposited/retained dose of PM that will overcome the rat's resistance to PM; (b) reducing the rats resistance by using rat models of human disease; or (c) reducing the rat's resistance by providing poor nutrition for the rat. Understanding the interplay of dose and responsiveness in animal models as well as in the human will substantially advance our ability to predict adverse health outcomes in the human population.

COMPARATIVE DOSIMETRY FOR SPECIFIC PUBLISHED STUDY EXAMPLES

In an effort to better understand the dosimetry questions surrounding this issue - across species and between inhalation and intratracheal instillation modes of exposure - two discussions follow. The framework for the discussion is based on dosimetry estimates derived from the application of a published dosimetry model as it was used to estimate human exposures to World Trade Center dust from instillation toxicology studies (Gavett et al., 2003). The first discussion focuses on the ***Utah Valley*** experience. Table 7A-8 provides estimated exposure and dose values for the published Utah Valley epidemiology studies by Pope (1989) in the context of instillation studies conducted in humans by Ghio and Devlin (2001) and in rats by Dye et al. (2001). The MPPD model (version 1.0, released 2002) served as the primary means of estimating regional deposition fractions, and from which inhalation exposures could be "back-calculated" when instillation was the means of exposure.

TABLE 7A-8. UTAH VALLEY: EXPOSURE-DOSE MODELING

General Assumptions:	
– Walking or resting human / rat	
Assumed characteristics of Utah Valley Dust (UVD)	
– MMAD = 1 μm; σ_g = 2.5; density = 1 g/cm^3	
Respiratory tract regions	
– Nasal, tracheobronchial (TB), alveolar (A); $\dot{V}_H$ = 15000 ml/min, $\dot{V}_R$ = 214 ml/min	
Exposures	
– Utah Valley concentrations - Dec 86 / Jan 87	
– 300 μg/m^3 PM_{10} ($PM_{2.5}$ assumed to be 65% PM_{10})	
– 195 μg/m^3 $PM_{2.5}$ (24-hour mean)	
– Total dose distal to the 3rd generation to a human for 24-hours, 670 μg	
Human Instillation	*Rat Instillation*
– 500 μg to lingular lobe (10% lung)	– 2 0 μg to entire lung
Dose Calculations	
For human breathing 195 μg/m^3:	Human equivalent 24 h exposure to achieve intratracheal rat dose per lung surface area (SA) (229 μg/m^2):
– 7.5 days to achieve 500 μg	– 5900 μg/m^3 (~6 mg/m^3)
Human equivalent 24h exposure to achieve 500 μg:	Rat equivalent 24h exposure to achieve intratracheal rat dose per lung SA (229 μg/m^2):
– 1500 μg/m^3 (~1.5 mg/m^3)	– 7600 μg/m^3 (~8 mg/m^3)

Secondly, data from ***Concentrated Ambient Air Particle (CAPs)*** inhalation studies in humans (Ghio et al., 2000) and rats (Kodavanti et al., 2000) are compared dosimetrically using the input parameters defining the exposures and test subjects (Table 7A-8). Where possible the health outcomes for each are compared in the context of lung dose. Analogously, inhalation dosimetry in rats for emission PM with a MMAD of 1.96 μm and a σ_g of 2.5 is compared to these findings and those of the Utah Valley studies.

Utah Valley Studies: The Ghio and Devlin (2001) Utah Valley paper provided an estimate of the human lung dose of Utah Valley dust filter extract (UVD) assuming a hypothetical ambient exposure level of PM_{10} (100 μg/m^3). The computations described in their discussion were based on a total lung deposition fraction of 0.42, from which they concluded that the dose instilled (500 μg) into the lingula lobe of human volunteers was roughly comparable to the PM deposited as the result of living about 5 days in the Utah Valley with that ambient PM_{10} concentration (Table 7A-8). However, their assumed total deposition fraction may have resulted in an overestimate of the hypothetical dose from inhalation since their deposition value included the nasal, tracheobronchial (TB) and pulmonary (A) regions.

To establish a more consistent set of calculations to compare the human and rat data from the Utah Valley studies, a standardized deposition model, developed for use with humans and rats which estimates deposition in different compartments of the respiratory tract, was employed. An earlier version of the MPPD model has been used to estimate PM doses resulting from experimental exposures to dispersed dust from the World Trade Center collapse (Gavett et al., 2003). The selection of a 1 μm MMAD (σ_g 2.5) particle was based on the view that the instilled particles were respirable bypassing the nose of both species and should represent a 'typical' ambient PM size range. The hypothetical exposure of a person residing in the Utah Valley region described in the Pope study was reanalyzed using an estimate of exposure concentration from an 'Open-Plant" period (December 1986 - January 1987). On 13 occasions during those 2 months, the 24 hr average PM values exceeded 300 μg/m^3. Assuming that ~65% of PM_{10} is $PM_{2.5}$ yields a 24 hr $PM_{2.5}$ average of 195 μg/m^3, which gives a modeled dose to the pulmonary region of the human lung in one day of 484 μg (Table 7A-8).

The PM dosimetry in the study of Ghio and Devlin (2001) was reanalyzed using the MPPD model with the assumed UVD characteristics and human parameters noted (Table 7A-8). For nasal breathing, the MPPD model estimates the human deposition fraction for UVD at 0.158 for the lung distal to and including the 4^{th} airway generation which is where the instillation occurred. The calculation of the inhalation concentration corresponding to the lingula dose of instilled UVD (500 μg) led to the determination that about 7.5 exposure days at 195 μg/m^3 per day (as per the Pope [1989] exposure data) would be required to achieve the instilled dose (Table 7A-8), as opposed to the 5 exposure days calculated by Ghio and Devlin (2001).

Considered from the perspective of a single exposure day, the corresponding estimated 24h average ambient PM exposure based on the instilled dose was computed to be 1500 μg/m^3.[1]

The rat Utah Valley paper (Dye et al., 2001) involved the intratracheal instillation of 3 doses of UVD - 2500, 1000, and 250 μg of PM extract. While the majority of the toxicological data reported in the paper corresponded to the 2500 μg dose studies, the targeted assessments at 250 μg also yielded considerable inflammation (e.g., ~25-fold increase in neutrophil numbers over control) - consistent with the injury and response pattern of the higher doses. Using this instillation dose to the TB and A regions of the rat (since the instillation was at the tracheal bifurcation), a surface area dose-equivalent was computed for comparison in the human. Inhalation concentration exposure estimates could be computed for the human (as well as the rat). One finds that the rat instillation of 250 μg corresponded to a single 24 hr human exposure concentration of 5900 μg/m^3 to achieve the same dose per unit surface area (Table 7A-8). Thus, it appears that the corresponding "theoretical" rat to human exposure ratio is about 4 (5900 vs. 1500 μg/m^3).

Interestingly, despite the 10-fold range in instilled doses in the rat, the BAL marker responses remained qualitatively consistent with those of the human and exhibited a linear dose-response relationship (see figure 4 in Dye et al., 2001). This would suggest that the doses examined in the rat were not overwhelming to the lung and remained homologous to the human response pattern. The question might be at this point, "What exposure concentration must the rat be exposed to in order to achieve the 250 μg lung dose in the rat?" Using the deposition model, one finds that the 24 hr exposure challenge to UVD to achieve the instilled dose of 250 μg computed to be 7600 μg/m^3, only a 25% difference from the projected human equivalent exposure (Table 7A-8) to achieve the same surface area dose. One might then conclude that to achieve a dose equivalent to the human, the inhalation concentration should be ~25% higher for the rat.

Thus, in conclusion, the dosimetric difference between the rat and human UVD instillation studies was not as substantial as might appear at first glance: only ~4-fold higher for a 250 μg instillation into a rat. Both the controlled studies were significantly above that estimated for a

[1]Because the material actually instilled was an extract of the PM filters, the exact solubility of the deposited PM was difficult to determine with accuracy. As such, this estimated ambient concentration is likely an underestimate, but any error holds constant for both the human and rodent studies and would therefore be moot in this argument.

person exposed on Plant-Open day in the Pope (1989) study. Yet, despite these differences the parallel among the biological findings and coherence with the epidemiology holds tightly.

CAPs Studies. An analogous exposure / dosimetry comparison is made between healthy young adult human subjects exposed to 120 μg/m^3 CAPs for 2 hr (Ghio et al., 2000) and bronchitic rats exposed to 600 μg/m^3 CAPs for a sum total of 18 hr (Kodavanti et al., 2000). The dosimetry calculations using MPPD revealed that the doses per surface area were about 9-fold higher in both the TB and A regions of the rat relative to the human (Table 7A-9). If the CAPs concentration and exposure times were equal in the two studies, the model predicts that humans would have 5 times the dose per surface area of the rat Both studies exhibited increases in BAL neutrophils – about 3 to 5 fold in both humans and rats. The study of Clarke et al. (1999) showed similar dosimetry (and outcomes) to that of Kodavanti et al. (2000) since the concentration of CAPs was about the same (~600 μg) over the three days of 6 hr exposures. Hence, the relative responsiveness of humans and rats to CAPs exposure appears to be within an order of magnitude, with the rat being less sensitive.

TABLE 7A-9. CAPS / EPM: EXPOSURE-DOSE

	Human CAPs Ghio et al. (2000a)	Rat CAPs Kodavanti et al. (2000)	Rat EPM Kodavanti et al. (2003)
PM size MMAD (σ_g)	0.65 (2.35)	0.8 (1.7)	1.96 (2.5)
Conc. (μg/m^3)	120	600	10000
Dose/SA [a,b] (TB - μg/m^2)	68	560	3500
Dose/SA [a,b] (A - μg/m^2)	0.9	8.3	35

[a] $\dot{V}_H$ = 30000 ml/min, $\dot{V}_r$ = 214 ml/min.
[b] Surface areas from Jones and Longworth (1992) and Overton et al. (2001).

To complete the comparisons of respective doses for varied exposures, an estimate of the dose of a PM surrogate (emission particulate material, EPM) was computed for the rat at ~10 mg/m^3. EPM contains poorly soluble zinc and resembles ambient PM; it is not the well-known and studied residual oil fly ash (ROFA). The single 6 hr exposure resulted in a ~52-fold difference in rat EPM dose / human CAPs dose per TB area, and ~39-fold A difference (Table 7A-9). Yet compared to the UVD dose in the rat as discussed above, the EPM dose per surface area in the A region is only about 15% of the instilled dose of UVD. Hence, coherence of the Utah Valley data obtained at the much higher lung doses than in this study and the general consistency of the findings across metal-based doses between emission and ambient PM samples (Costa and Dreher, 1997) lend credence that instillation and inhalation studies provide complimentary data and consistent conclusions.

Summary and Conclusions

Complementary approaches were used to analyze the relationship between PM doses resulting from high concentration inhalation exposures or intratracheal instillation in rats and PM doses in humans resulting from exposures during normal activities. The MPPD model was used to calculate concentrations of resuspended PM which would be necessary to achieve doses in the rat comparable to those in humans breathing ambient PM_{10}, as measured by a variety of dose metrics. The same model was then used to estimate the differences in doses in rats and humans exposed to comparable types of ambient or emission PM in salient published studies.

The MPPD model estimates in Table 7A-6 suggest that a rat exposed to 300-400 μg/m^3 resuspended PM over six hours would receive an incremental dose in the A region (measured as deposited or retained mass) comparable to that of a healthy human working for six hours near a busy road and exposed to 150 μg/m^3 ambient PM_{10}. To achieve an incremental dose retained in the rat TB region (averaged over 6 hours) comparable to that in the human, the rat would need to be exposed to approximately 2 mg/m^3 resuspended PM for 6 hours. However, because of the more rapid clearance in the rat, the higher exposure concentration of 6-7 mg/m^3 would be required for the rat to achieve a retained TB dose (averaged over 24 hours) comparable to that in the human.

If one attempts to simulate not just the incremental dose from an acute single exposure, but the total cumulative burden of PM in the human lung after a decade of exposure, the six-hour

laboratory exposure concentrations required to produce a burden in the rat lung comparable to that in the human lung following six hours of work would be considerably greater. Due to the more rapid clearance of particles from the A region of rats, much higher exposure concentrations would be required to simulate the A dose in humans for either a six-hour or a 24-hour average dose (see Table 7A-7).

In daily life, humans are exposed to PM in the atmosphere inhale a complex profile of ultrafine, accumulation mode, and coarse mode particles covering a size range from below 0.1 to over 10 μm diameter. On the other hand, laboratory exposures of rats to resuspended dusts typically comprise a size range between the accumulation and coarse modes, and cannot fully simulate the human particle number or particle surface area doses with reasonable exposure concentrations. Laboratory exposures of rats to resuspended dust can simulate the dose of particle mass to the alveolar region but cannot simulate other dose metrics unless very high concentrations are used. Other dosimetric differences between humans and rats, summarized in Table 7A-2, also make it difficult to extrapolate from rats to humans.

Instillation studies in either animals or humans (as limited as the latter of these are) have be critiqued at various levels for lack of relevance related to dose and means of administration. Studies performed to address coherence of the biological outcomes of directly administered ambient-derived PM to the lungs of animals and humans have been compared dosimetrically to each other and to a complementary human exposure scenario. In the human study of Ghio and Devlin (2001) the 500 μg dose of Utah Valley dust filter (UVD) extract instilled into the lingula lobe of human volunteers was estimated to be comparable to 7 to 8 days of exposure during peak PM_{10} concentration days in the winter of 1986-1987 (derived from Pope, 1989; Table 7A-8). This dose elicited a robust inflammatory response associated with UVD composition. In the complementary animal study, intratracheal instillation of rats with 250 μg UVD similarly caused a significant degree of inflammation (Dye et al., 2001). Comparing these studies with human UVD exposure estimates, the ambient exposure concentrations over 24 hours which would be required to achieve the same dose per unit surface area in humans are about 4-fold higher with the rat 250 μg instilled dose compared to the human 500 μg instilled dose (Table 7A-8). Analysis of doses per surface area in rats and humans exposed to CAPs (Table 7A-9) indicate that with the same concentration and exposure times, humans have 5 times the dose per surface area of the rat.

These results indicate that higher PM concentration exposures in rats would be justified to achieve nominally similar doses per surface area relative to the human. Given the MPPD model results which show that rats clear PM much faster than humans, higher exposure concentrations and instillation doses in the rat provide a useful and relevant approach to investigate toxicological endpoints which are predictive of health outcomes in humans and to investigate biological mechanisms.

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