CHAPTER 7

TOXICOLOGY OF PARTICULATE MATTER IN HUMANS AND LABORATORY ANIMALS

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7. TOXICOLOGY OF PARTICULATE MATTER IN HUMANS AND LABORATORY ANIMALS

7.1 INTRODUCTION

The 1997 U.S. PM NAAQS revisions (Federal Register, 1997) were based, in large part, on new 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 human or laboratory animal exposure studies were available that provided more direct evidence supporting the plausibility of the observed PM-mortality/morbidity associations being causal at the relatively low ambient PM concentrations studied epidemiologically. The then-limited PM toxicologic data was assessed in Chapter 11 of the 1996 PM Air Quality Criteria Document or PM AQCD (U.S. Environmental Protection Agency, 1996a) that provided scientific assessment inputs supporting the 1997 PM NAAQS decisions.

Since the 1996 PM AQCD, numerous hypotheses have been advanced and extensive new toxicologic evidence generated with regard to possible pathophysiological mechanisms by which PM exposures at ambient or near ambient concentrations might induce increased morbidity and/or mortality. The extensive new PM toxicological research 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; (4) what types of interactive effects of particles and gaseous co-pollutants have been demonstrated; and (5) are there toxicologic findings on PM-related mutagenic/genotoxic effects that support the plausibility of ambient PM-lung cancer relationships observed epidemiologically in U.S. populations?

7.1.1 Methodological Considerations

Various research approaches have been and continue to be used to address the above 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, mouth-only, and nose-only inhalation exposures to concentrated ambient particles (CAPs) or laboratory-generated particles; intratracheal, intrapulmonary, and intranasal instillation; and in vitro exposures 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 those types of 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 quantification of dose-response relationships for experimentally-induced toxic effects.

Reflecting this, most of the toxicology studies assessed here have generally used exposure concentrations or doses that are relatively high compared to concentrations commonly observed in ambient air. An important consideration contributing to the use of relatively high experimental exposure concentrations is the fact that healthy animals have most typically been used in many controlled-exposure toxicology studies, whereas epidemiologic findings often reflect ambient pollutant effects on compromised humans (e.g., those with one or another chronic disease) or other susceptible groups rendered at increased risk due to other factors. Implicit in the use of relatively high concentrations in experimental studies of healthy subjects is the assumption that increasing the dose somehow makes up for compromised tissue/organ functions that may contribute to observed ambient PM effects. However, this may not be the case, unless the increased susceptibility of an “at risk” group is based on enhanced respiratory tract PM deposition/retention per se. In light of this, there exists a great need for expanded development and use of animal models that more closely mimic important characteristics contributing to increased human susceptibility to ambient PM effects; and some notable progress has been made in this regard, as reflected by the growing number of PM toxicologic studies of compromised animal models published since the 1996 PM AQCD and assessed in this chapter.

Given the relatively high concentrations used, much care should therefore be taken when attempting to interpret and extrapolate effects seen in these studies to provide insight into the biological plausibility and mechanisms of action underlying effects seen 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, differences between humans and rodents with regard to the inhalability, deposition, clearance, and retention profiles for PM (see Chapter 6 for details) could conceivably make doses to some specific respiratory tract tissues from experimental exposures relatively similar to doses resulting from human ambient exposures. To help place the toxicologically relevant concentrations/doses into context in relation to ambient conditions, EPA has carried out some illustrative dosimetric/extrapolation modeling 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 human or rat lung tissue from experimental exposures and PM doses to the human lung from exposures during normal activities. These analyses and their interpretation of results (described in Appendix 7-A) provide context for the exposure concentrations used and results obtained in toxicological studies assessed here.

The effects of controlled exposures to ambient PM have been increasingly investigated during recent years since the 1996 PM AQCD by use of particles collected from 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). In the first type of study, particles from ambient air samplers are first collected on filters or other media, then stored, and later resuspended in an aqueous medium for use in inhalation, intratracheal instillation, or in vitro studies. 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). Both ambient PM extracts and concentrated ambient particles (CAPs) have been used to evaluate effects in healthy and compromised laboratory animals and humans. 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 offset somewhat by the inability of CAPs studies to precisely control the mass concentration and day-to-day variability in ambient particle composition, and they often lack detailed characterization of variations in chemical composition from one CAPs exposure to another. Because the composition of concentrated ambient PM varies across both time and location, a thorough physical-chemical characterization is necessary to compare results between studies or even among exposures within studies in order to link particle composition to effects. Another limitation that should be taken into account in interpreting results from CAPs studies is the fact that concentrators in use at the time of many of the studies could not efficiently concentrate ambient particles ≤ 0.1 µm. Thus, it is likely that a large portion of potentially important combustion-generated particles (e.g, from diesel, gasoline vehicle, wood smoke, coal smoke, etc.) were present only at ambient (not higher concentrated) levels in most or all of the CAPs studies assessed here.

Controlled human and laboratory animal exposures to particulate material obtained from emission source bag house filters or other emission source collection devices have also been used extensively in recent years to evaluate the in vitro and in vivo respiratory toxicity of complex combustion-related PM. Residual oil fly ash (ROFA) collected from large industrial sources (e.g., oil-fired power plants) has been extensively used, and, to a lesser extent, domestic oil furnace ash (DOFA) or coal fly ash (CFA). The major disadvantage associated with the use of such emission source materials derives from questions concerning the potential relevance of results obtained for helping to understand and interpret ambient PM exposure effects. There is little doubt that in years past, before the extensive implementation of air pollution controls, ambient U.S. air contained mixtures of PM species analogous to those contained in many of the emission-source samples used in toxicologic studies during the past decade or so. On the other hand, it is rare that high concentrations of certain materials that typify such samples would be found or approached in ambient air PM samples obtained at community monitoring sites in U.S., Canada, and much of Western Europe that provided the aerometric data collected during the past 20 to 30 ys that were analyzed to estimate PM exposures in most of the PM epidemiology studies assessed in this document. For example, very high concentrations of metals typify most ROFA samples (especially extremely high nickel and vanadium levels), and experimental exposures to such materials have generally resulted in exposures/doses that are orders of

magnitude (100s) of times higher than would be associated with exposures to much lower levels of such metals in ambient PM measured routinely since the 1970s at community monitoring sites across the U.S. (except perhaps at times very near some sources without modern emission control devices or during temporary breakdowns of such). Thus, significant issues arise concerning the extent to which ROFA or other high concentration emission source effects can be extrapolated to aid in interpretation of ambient air PM exposure effects on humans.

Analogous issues arise in connection with evaluation of the toxicity of particulate material obtained as emission products from mobile source combustion devices, e.g., diesel and gasoline vehicle engines. The complex combustion-related mixtures in such mobile source emissions include many different types of particles and gaseous compounds in high concentrations which are not necessarily representative of ambient PM derived from such sources after passage through particle traps, catalytic converters, exhaust pipes, etc. For example, ultrafine particles emitted from gasoline and diesel engines are reduced in numbers and concentrations as they agglomerate to form larger, accumulation-mode particles as they cool in passing through exhaust systems and/or as they undergo further physical and chemical transformation as they "age" as ambient air components. Further complicating evaluation of the toxicity of mobile source emission components is: (1) the difficulty in separating out toxic effects attributable to particles versus those of gaseous components in automotive exhausts; and (2) the changing nature of those exhaust mixes as a function of variations in engine operating mode (e.g., cold start versus warm start or "light" versus "heavy" load operation, etc.) and changes in engine technology (e.g., "old diesels" versus "new diesels").

The in vivo studies discussed first and the 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. Collection of these size fractions has been made easier by widespread availability of ambient sampling equipment for PM_{10} and $PM_{2.5}$. However, 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 now available by which to assess effects of these potentially important particle sizes. Similarly, although organic compounds often comprise 20 to 70% of the dry fine particle mass of ambient PM (see Chapter 3), little research has addressed mechanisms by which such compounds may contribute to ambient PM-related effects. One

exception to this has been the evaluation of contributions of diverse organic compounds to mutagenic, genotoxic, and carcinogenic effects discussed later in Section 7.8.

7.1.2 Organization of the Chapter

Ambient particulate matter as noted above, is comprised of myriad physical and chemical species that can vary widely from one geographic location to another or even from one time to another time at a given location. It is not surprising that only a relatively few ambient air mixes from selected urban areas or subsets or combinations of the diverse variety of physical/chemical species have been investigated in controlled human or laboratory animal studies. However, a full discussion of all types of ambient particles that have been identified (see Chapter 2) is beyond the scope of this chapter. 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 of 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 some aspects are discussed to a limited extent 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 also not assessed in detail in this chapter.

Because of the sparsity of toxicological data on ambient PM at the time of the 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 current interest in combustion particles is that these particles, along with materials adsorbed to such particles and secondary aerosols formed from them, are typically among the most dominant components represented in the fine fraction of ambient air PM found in most U.S. urban areas.

This chapter is organized as follows. The cardiovascular and systemic effects of in vivo PM exposure are discussed first in Section 7.2. This is followed by discussion in

Section 7.3 of respiratory effects of ambient PM, specific components of ambient PM, combustion source-related particle mixes, or other laboratory-generated particles delivered by controlled in vivo exposures of humans or laboratory animals (note that the specific components discussion includes summary points drawn from detailed discussion of ambient bioaerosols in Appendix 7B). In vitro exposure studies are then next discussed (Section 7.4) and are valuable in providing information on potential hazardous constituents and mechanisms of PM injury. The next section (Section 7.5) focuses on studies of PM effects in laboratory animal models meant to mimic human disease, as a means for providing information useful in characterizing factors affecting susceptibility to PM cardiovascular and respiratory system effects. Section 7.6 then assesses controlled-exposure studies evaluating health effects of mixtures of ambient PM or specific PM constituents with gaseous pollutants. Section 7.7 discusses exposure/dose-effects relationships for cardiovascular and respiratory effects and comparisons for illustrative health endpoints of experimental exposures/data needed to produce similar effects across species (rats, humans) and/or under ambient conditions (drawing upon extrapolation modeling results presented in Appendix 7A). The following Section (7.8) discusses studies of PM-related mutagenic/genotoxic effects thought to be useful in evaluating the relative carcinogenic potential of ambient PM and its constituents, as well as particulate constituents in emissions from various types of combustion sources. This organization provides the underlying information used for interpretive summarization (in Section 7.9) of the extensive new findings discussed in the earlier sections with regard to PM-related effects, all of which may individually contribute to and/or combine through intricate linkages among them to mediate ambient PM exposure effects.

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

7.2.1 Introduction

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 appear to be induced via direct particle uptake into the blood and/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, possibly due to release of endothelin 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 autonomic 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

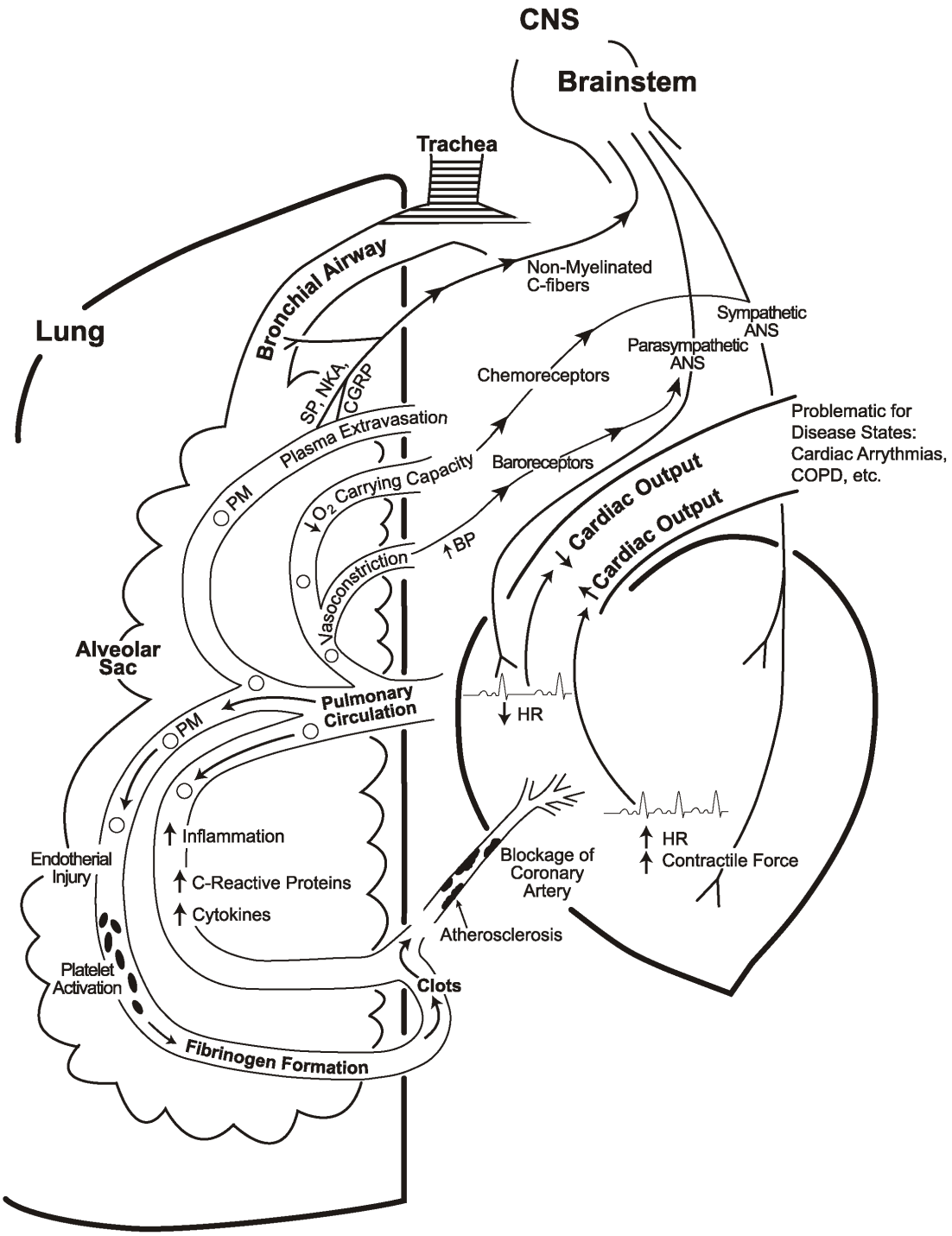

Figure 7-1. Schematic illustration of hypothesized pathways/mechanisms potentially underlying cardiovascular effects of PM.

autonomic modulation of heart rate. Under certain circumstances, HRV provides insight into 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-h period (Kleiger et al., 1991). Despite the inherent variability of short-term HRV measures during routine daily activity, long-term measures (i.e., measures averaged over 24 hs) 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-myocardial infarction (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 La Rovere et al., 2003). However, acute changes in HRV parameters do not necessarily occur immediately prior to sudden fatal ventricular arrhythmias (Waxman et al., 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, including changes in HRV associated with exposure to PM, is simply a marker for enhanced risk.

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 vascular inflammation, which induces release of C-reactive proteins and cytokines. These cause further inflammatory responses that, on a chronic basis, can lead to atherosclerosis. In narrowed coronary arteries, the clots formed in the aforementioned cascade may block blood flow, resulting in acute myocardial infarction.

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; Braunwald, 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 and 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.

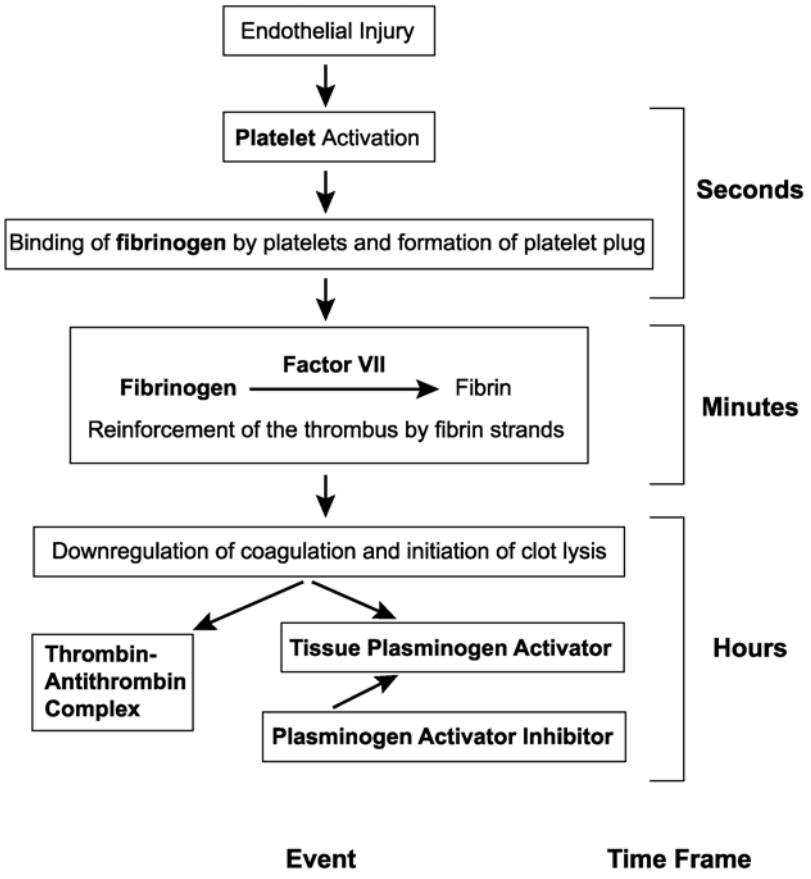

Figure 7-2. Simplified overview of blood coagulation system. The coagulation parameters often measured in the study of PM effects on blood coagulation are indicated by bold type. The relations of these selected parameters with the rest of the coagulation system are outlined.

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 (Piedimonte et al., 1992; Baluk et al., 1992).

There were few studies assessed in the 1996 PM AQCD that evaluated cardiovascular system effects of exposures to particulate matter. Since 1996, numerous studies have now become available that evaluated cardiovascular effects of exposures (via inhalation or instillation) of ambient PM, constituent components, complex mixtures from PM emission sources and/or exposures to single PM substances or binary/ternary combinations of particles of varying chemical composition. Also, whereas earlier studies tended to focus on healthy animals, more recent studies have, in addition, begun to focus on evaluation of PM effects in animal models of disease states thought to mimic aspects of pathophysiologic states experienced by compromised humans at increased risk for PM effects.

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. 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 an apparent "general particle" effect that seemed to be implied by somewhat similar 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.

Muggenburg et al. (2000a) has described several potential animal models of cardiac disease (MCT-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 injury effects include selective pulmonary endothelial damage and progressive pulmonary arterial muscularization. Pulmonary hypertension develops, the blood flow is impeded, and compensatory right ventricular hypertrophy occurs. To produce pulmonary hypertension, animals are injected subcutaneously with 50-60 mg/kg monocrotaline (MCT). Within 2 wks following treatment, experimental animals, primarily rats, develop pulmonary hypertension (Kodavanti et al., 1998a). A growing number of studies have used extracts of collected/stored ambient PM or real-time generated concentrated ambient particles (CAPs) drawn from various airsheds (e.g., Boston, New York City, etc.) to evaluate cardiovascular and other systemic effects of PM. Many other new animal studies have also used metal-laden ROFA as one type of combustion source particle mix and others have used other combustion source materials, e.g., domestic oil fly ash (DOFA), coal fly ash (CFA), or diesel exhaust (DE). The following discussion of the cardiovascular/systemic effects of PM first focuses mainly on the ambient PM studies and then discusses findings from the studies using ROFA and other types of particles.

Tables 7-1 and 7-2 summarize newly-available studies (since the 1996 PM AQCD) that evaluated cardiovascular effects of ambient PM mixtures or other types of PM in response to controlled inhalation exposures of humans or laboratory animals; and intratracheal instillation studies are summarized in Table 7-3. In vitro exposure studies of cardiovascular effects are discussed in Section 7.4.

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

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration, PE[b] Time to Analysis	Cardiovascular Effects	Reference
Humans, healthy nonsmokers, 18-40 y old n = 38	CAPs (Chapel Hill)	Inhalation	23.1 to 311.1 µg/m^3	0.65 µm σ_g = 2.35	2 h, analysis at 18 h	Increased blood fibrinogen with CAPs exposure. PM concentration in chamber varied with ambient air PM level. Estimated total dose of 1200 µg.	Ghio et al. (2000a)
Humans, healthy 18-40 y old n = 4	CAPs (Toronto)	Inhalation (face mask)	24 to 124 µg/m^3	0.1 - 2.5 µm		Trend toward increased fibrinogen levels 2 h post high CAPs (124 µg/m^3) exposure, but stat. sig. not specified. Also, no sig. ECG Holter effects.	Petrovic et al. (2000)
Humans, healthy; 19-41 y old n = 4	CAPs (Los Angeles)	Inhalation	148 to 246 µg/m^3	$PM_{2.5}$	2 h	No significant changes in in arterial O_2 saturation or Holter ECGs observed, nor in lung function or symptoms. The maximum steady state fine particle concentration in the breathing zone was typically seven times the ambient concentration.	Gong et al. (2000)
Humans, healthy (n = 12) and asthmatic (n = 12) 18-45 y old, nonsmoking	CAPs (Los Angeles)	Inhalation whole body chamber	99-224 µg/m^3 (mean 174)	80% 0.1 to 2.5 µm	2 h with alternating exercise/rest. Analysis at 0, 4, and 22 h PE.	CAPs-related decrease in Factor VII blood levels, - but no significant changes in blood fibrinogen or serum amyloid A with CAPs. Both healthy and asthmatic subjects had modest increases in HR variability and significant increases in HR during exercise. Some reported cardiac symptoms (faintness, dizziness, pain related to heart, etc) during CAPs exposure.	Gong et al. (2003)
Dogs, female mongrel, 14 to 17 kg	CAPs (Boston)	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; Balloon-occluded LAD coronary artery in some, n = 14	CAPs (Boston)	Inhalation via tracheostomy	~100-1000 µg/m^3	0.23 to 0.34 µm σ_g = 0.2 to 2.9	6 h/day for 3 days	Decreased heart and respiratory rate and increased lavage fluid neutrophils in normal dogs. Decreased time to ST segment elevation and increased magnitude in compromised dogs. PM concentration varied depending on ambient PM level and concentrator operation. No dose-response relationship evident.	Godleski et al. (2000)

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

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration, PE[b] Time to Analysis	Cardiovascular Effects	Reference
Rats	CAPs (Tuxedo, NY)	Inhalation (nose-only)	110-350 µg/m^3	N/A	3 h	Small but consistent increase in HR; increased peripheral blood neutrophils and decreased lymphocytes. No pulmonary injury found. 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 (Manhattan)	Inhalation	132-919 µg/m^3	0.2-1.2 µm σ_g = 0.2-3.9	Single 3 h or 3 daily 6 h exposures	No increase in cardiac arrhythmias; inconsistent PM-associated increases in HR, blood cell differential counts, and atrial conduction time of rats. No adverse cardiac or pulmonary effects in hamsters.	Gordon et al. (2000)
Rats, male, F344, 250-275 g	CAPs (NYC)	Inhalation (nose-only)	95-341 µg/m^3	< 2.5 µm	0, 12, and 24 h	No consistent exposure-related effects on platelet count fibrinogen level, factor VII activity, thrombin-anti-thrombin complex, tissue plasminogen activator, or plasminogen activator inhibitor.	Nadziejko et al. (2002)
Rats, Wistar	Ott ambient (EHC-93) (ECH-93L) Diesel soot (DPM) Carbon black (CB)	Inhalation (nose only)	48 mg/m^3 49 mg/m^3 5 mg/m^3 5 mg/m^3	36, 56, 80, 100, and 300 µm	4 h, Analyses at 2, 32, 36, 48 h PE	EHC-93 elevated blood pressure on day 2, ET-1 levels at 32 h, and ET-3 levels at 2, 32, and 48 h postexposure. EHC-93 L had no effect on blood pressure, transient effect on ET-1, -2, -3 levels at 2 h but not 32 h postexposure. DPM had no effect on blood pressure, but elevated ET-3 levels at 36 h PE. CB had no effect.	Vincent et al. (2001)
Humans, male, healthy, age 19-24 y	PM_{10} from S.E. Asian Smoke Haze	Inhalation	~125 µg/m^3 (range = 47 to 216)	N/A	4 wks, analysis at 3 and 5 wks PE	Band cell counts were significantly increased during the haze period	Tan et al. (2000)

[a] CAPs = concentrated ambient particles
UAP = urban ambient partciles
DPM = diesel particulate matter
Ott ambient = resuspended UAP from Ottawa, CA

[b] PE = postexposure

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TABLE 7-2. CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INHALED ROFA AND OTHER COMBUSTION-RELATED PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration, PE[b] Time to Analysis	Cardiovascular Effects	Reference
Dogs, beagles, 10.5-y- old, healthy, n = 4	ROFA (Boston)	Oral inhalation	3 mg/m^3	2.22 µm MMAD σ_g = 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. (2000b)
Rats, S-D, MCT-treated, 250 g	ROFA (Boston)	Inhalation	580 ± 110 µg/m^3	2.06 µm MMAD σ_g = 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)
Rats, S-D, SH rats, WKY rats, healthy and MCT-treated	ROFA (location not given)	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 showed 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-wk-old	ROFA (Florida)	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. (2000a)
Rats, male, SH and WKY; 12 to 13 wks old	ROFA (Boston)	Inhalation	15 mg/m^3	1.5 µm σ_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 (2 or 4 wk) exposure caused pulmonary injury but no changes in fibrinogen.	Kodavanti et al. (2002a)
Rats, male, S-D, WKY and SH	ROFA (Boston)	Inhalation (nose only)	2, 5, 10 mg/m^3 10 mg/m^3		6 h/d for 4 consec. days 6 h/d 1 d/wk, 4 or 16 wks	No cardiovascular effects see in SD or SH rats with acute or chronic exposure. Cardiac lesions (active chronic inflammatory, multifocal myocardial degeneration, fibrosis, decreases in number of granulated mast cells) seen for WKY rats with chronic (16 wk) exposures.	Kodavanti et al. (2003)

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TABLE 7-2 (cont'd). CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INHALED ROFA AND OTHER COMBUSTION-RELATED PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Mass Concentration	Particle Size	Exposure Duration, PE[b] Time to Analysis	Cardiovascular Effects	Reference
Rats, male, S-D, healthy and MI	ROFA (Boston) Carbon black	Inhalation	3 mg/m^3	1.81 µm 0.95 µm	1 h	In MI group, with thermocoagulation of left coronary artery, 41% of rats had one or more premature ventricular complexes (PVCs). ROFA but not CB or room air, increased arrhythmia frequency in those with PVCs and decreased heart rate variability.	Wellenius et al. (2002)
Human, healthy, non-smoking males	Ultrafine carbon particles	Mouthpiece exposure	10 µg/m^3	< 100 nm	2 h exposure; assessment before and immediately, 3.5 h, and 21 h after exposure	No effects on blood coagulability, circulating leukocyte activation, leukocyte expression of activation and adhesion molecules.	Frampton (2001)
Rats, SD, 60 days old	VSO_4 $NiSO_4$	Inhalation	0.3 -2.4 mg/m^3	N/A	6h/day x 4 days	No effects with V at all doses. Ni caused delayed bradycardia, hypothermia and arrhythmogenesis at >1.2 mg/m^3. V+Ni produced delayed effects at 0.5 mg/m^3 and potentiated responses at 1.3 mg/m^3, suggesting synergism of effect.	Campen et al. (2001)
Rats, SH	DE	Whole body	30,100, 300, or 1000 µg/m^3	90% < 1 µm	6 h day, 7 day/wk for 1 wk	Elevated daytime HR; concentration-dependent prolongation of PQ interval.	Campen et al. (2003)
Rats, male & female 10-12 wks	DE	Whole body	50,100, 300, or 1000 µg/m^3	90% < 1 µm	6 h day, 7 day/wk, 1 wk or 6 mos	Blood cholesterol decreased at high dose; Gamma-glutamyl transpeptidase increased at 1000; Factor VII decreased in males at 1 wk and in both genders at 6 mos at 1000. TAT decreased in M at 1000 at 1 wk.	Reed et al. (2004)

[a] ROFA = Residual oil fly ash
$NiSO_2$ = Nickel sulfate
$Fe_2(SO_4)_3$ = Iron sulfate
DE = diesel exhaust
VSO_4 = Vanadium sulfate
MCT = monocrotaline
MI = Myocardial infarction

[b] PE = Post Exposure

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TABLE 7-3. CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INSTILLED ROFA AND OTHER PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Dose	Particle Size	PE[b] Time to Analysis	Cardiovascular Effects	Reference
Rats, male, S-D, 60 days old, healthy and MCT-treated	ROFA DOFA CFA	Intratracheal 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 PM (St. Louis Dusseldorf, Ottawa, Wash. DC)		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, healthy and MCT-treated, n = 64	ROFA (location not given)	Intratracheal instillation	0.0, 0.25, 1.0, and 2.5 mg/rat	1.95 µm	Analysis at 96 h postexposure	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 postexposure	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				
Rats, male, S-D; 60 days old	ROFA (Florida) MSH Vol. Ash	Intratracheal instillation	0.3, 1.7, or 8.3 mg/kg	1.95 µm $\sigma_g = 2.19$	Analysis at 24 h	Increased plasma fibrinogen only at 8.3 mg/kg ROFA.	Gardner et al. (2000)

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TABLE 7-3 (cont'd). CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INSTILLED ROFA AND OTHER PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Dose	Particle Size	PE[b] Time to Analysis	Cardiovascular Effects	Reference
Rats, male, SD, 60 days old	ROFA (Boston) classified by soluble metals (As, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn, and sulfate)	Intratracheal instillation	0.83, 3.3 or 8.3 mg/kg	< 3.0 µm MADD	Anaylsis at 24 h postexposure	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)
Rats, male, S-D, MCT-treated	ROFA (Florida)	Intratracheal instillation	0.25, 1.0, or 2.5 mg in 0.3 mL saline	1.95 µm MMAD σ_g = 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)
Rat, SD, 60 d old; 250-300 g healthy or MCT-treated	ROFA (Florida)	Intratracheal instillation	0.83 or 3.33 mg/kg	1.95 µm MMAD, σ_g = 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 wk old	ROFA (Boston)	Intratracheal instillation	1 and 5 mg/kg	1.5 µm σ_g = 1.5	Analysis at 1, 2, and 4 days	ROFA increased plasma fibrinogen and decreased peripheral lymphocytes in both SH and WKY rats at 5.0 mg/kg dose.	Kodavanti et al. (2002)
(1) Rats, S-D healthy and MCT, cold-stressed, and ozone-treated	ROFA (location not given)	Intratracheal instillation	0.0, 0.25, 1.0, or 2.5 mg/rat	1.95 µm σ_g = 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)

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TABLE 7-3 (cont'd). CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INSTILLED ROFA AND OTHER PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Dose	Particle Size	PE[b] Time to Analysis	Cardiovascular Effects	Reference
(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.	
Rats, Wistar, male, 200-250g, healthy and ozone-treated	Ottawa EHC-93	Instillation	0.5, 1.5 or 5 mg/rat in 0.3 mL saline	0.5 µm	Analysis at 2,4, or 7 days after exposure	At high doses, 20% increase in plasma fibrinogen at 2 days PE correlated with increases in ET-1 and iNOS mRNA and decrease in ACE.	Ulrich et al. (2002)
Rabbits, female, New Zealand, 2.2 to 3.0 kg	OTT 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 wk for 4 wks	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)
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 at 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)

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TABLE 7-3 (cont'd). CARDIOVASCULAR AND SYSTEMIC EFFECTS OF INSTILLED ROFA AND OTHER PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle[a]	Exposure Technique	Dose	Particle Size	PE[b] Time to Analysis	Cardiovascular Effects	Reference
Hamsters, 100-150 g	polystyrene particles (unmodified)	Intravenous (IV) administration and intratracheal instillation	5, 500, 5000 µg/kg	60 nm		IV doses of 5,500 or 5,000 µg/kg b.wt. of unmodified polystyrene particles did not affect thrombus formation.	Nemmar et al. (2002a)
	carboxylate-modified polystyrene		50, 100, 500 µg/kg			IV doses of 100 and 500 µg/kg b.wt. carboxylate-modified polystyrene particles decreased ($p < 0.05$) thrombus formation intensity.	
	amine-modified polystyrene		5, 50, 500 µg/kg			IV doses of 100 and 500 µg/kg b.wt. of amine-polystyrene particles increased thrombus formation ($p < 0.01$).	
						Intratracheal instillation of 5,000 µg/kg of amine-polystyrene particles significantly ($p < 0.05$) increased thrombus formation but not 5,000 µg/kg of unmodified- or carboxylate-polystyrene particles.	
						Platelet aggregation (ADP-induced in vitro) was not affected by unmodified-polystyrene up to 100 µg/mL; and was strongly increased by amine-polystyrene particles.	
						Authors attributed observed prothrombic activity of ultrafine paticles, at least, in part, to platelet activation by positively charged amine-modified polystyrene particles.	

[a] ROFA = Residual oil fly ash
$Fe_2(SO_4)_3$ = Iron sulfate
VSO_4 = Vanadium sulfate
CFA = Coal fly ash
DOFA = Domestic oil burning furnace fly ash
OTT = Ottawa urban ambient particles
MSH = Mt. St. Helen's volcanic ash
$NiSO_2$ = Nickel sulfate

[b] PE = Post Exposure

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7.2.2 Ambient Particulate Matter Cardiovascular Effects

The results of epidemiology studies discussed in Chapter 8 suggest that homeostatic changes in the vascular system can occur after episodic exposure to ambient PM. However, very few controlled human exposure studies of ambient PM effects on cardiovascular endpoints have been conducted thus far. In one such study, Ghio et al. (2000a) reported that inhalation of concentrated ambient particles (CAPs) in healthy nonsmokers increased blood fibrinogen levels. They exposed 38 volunteers exercising intermittently at moderate levels of exertion for 2 h to either filtered air (FA) or particles concentrated from the air in Chapel Hill, NC. The CAP exposure concentrations ranged from 23 to 311 $\mu g/m^3$, reflecting variations in particles collected outside the facility. At all exposure levels, CAPs-exposed subjects had significantly more blood fibrinogen than FA-exposed subjects; however, differences between pre- and post-CAPs exposure fibrinogen levels at 18 h postexposure were not significant. Other blood parameters tested in this study (including numbers of RBCs, monocytes, lymphocytes, platelets, or neutrophils) did not change significantly. The observed effects in blood may be associated with mild pulmonary inflammation also found 18 h after exposure to such CAPs (see Section 7.2.3).

Two other human inhalation studies with CAPs are limited by the small numbers of subjects studied. In one, Petrovic et al. (2000) exposed four healthy volunteers (aged 18 to 40) under resting conditions to filtered air and low, mid, and high concentrations (23 to 124 $\mu g/m^3$ of concentrated ambient particles (0.1 to 2.5 μm) from downtown Toronto for 2 hs using a face mask. The low CAP exposures were reflective of typical ambient $PM_{2.5}$ levels and the high ones of maximum $PM_{2.5}$ levels seen in Toronto. On each day prior to exposure, pulmonary function, nasal lavage, nasal acoustic rhinometry, blood collection for plasma fibrinogen and clotting factor VII antigen, and a resting ECG were taken. Pulmonary function measurements were taken every 30 min. and ECG readings recorded continuously during exposure, followed by ECG measures after 30 min. of postexposure exercise and nasal lavage, sputum induction, and blood collection at about 2 h and about 24 h postexposure. Petrovic et al. reported that review of the ECG data by a cardiologist showed no clinically significant cardiac effects during exposure, the ensuing exercise period, or 24 h postexposure. On the other hand, 2 of 4 subjects showed notable 15-20% pre- to postexposure increases in blood fibrinogen levels within 2 h post high CAPs exposure (124 $\mu g/m^3$) versus maximum 5-6% increases after filtered air exposures,

although there were no statistically significant differences for mean fibrinogen between CAP and FA exposures.

In another small pilot study, reported by Gong et al. (2000), four healthy adult volunteers (2 male, 2 female; aged 19 to 40 y) were exposed for 2 h while at rest in whole body chamber to filtered air or to concentrated ambient $PM_{2.5}$ from Los Angeles air. The CAP exposures at mean 2 h concentrations of 148 to 246 $\mu g/m^3$ (the latter approximating likely maximum exposure levels in Los Angeles) resulted in "no meaningful changes" in heart-rate variability, or ECG ST voltages, lung function, or respiratory symptoms, based on data collected during 2-h exposures or 10 or 22 h afterward. These results appear to be suggestive of Los Angeles ambient $PM_{2.5}$ exposures being unlikely to affect cardiorespiratory functions in healthy non-elderly adults. However, such a conclusion must be tempered by several considerations: (a) the small number of subjects tested and only while at rest (versus planned further studies to evaluate larger numbers of both healthy and compromised volunteers with respiratory or vascular disease, presumably to include exposures involving intermittent exercise); and (b) the Harvard model concentrator used did not likely concentrate effectively ambient particles < 0.1 to 0.2 µm MMAD, thus not exposing subjects to concentrated levels of potentially important combustion-derived (from diesel/gasoline vehicles; wood smoke, etc.) ambient particles.

More recently, Gong et al. (2003) exposed 12 healthy and 12 asthmatic subjects (age range 18 to 45) to Los Angeles CAPs ($PM_{2.5}$) that were primarily of motor vehicle origin. An exposure to CAPs averaging 174 $\mu g/m^3$ (range 99-224) in a whole body chamber for 2 h was alternated with a filtered air (FA) exposure at least 14 days apart. Subjects exercised for 15 min of each half hour at a ventilation rate of 15-20 $l/min/m^2$ body surface. Tests were performed just before exposure (pre), just after (immediately post), 3.5 to 4 h after (4 h), and the next day (day 2). No significant CAPS-related changes in routine blood parameters were observed, except for some mediators of blood coagulation and systemic inflammation. These included Factor VII, which declined at immediately postexposure and 4 h later and then rebounded on day 2. This is in agreement with a recent finding by Reed et al. (2004) wherein rats exposed to DE demonstrated lower Factor VII levels. However, the Gong et al. (2003) subjects did not have any accompanying changes in fibrinogen or serum amyloid A with CAPs exposure. Both groups exhibited modest increases in HR variability, and both had significant differences in HR during exposure (FA: 76 at rest and 96 at exercise; CAPs: 72 at rest and 92 at exercise). There were

no significant differences in diastolic BP and HR-systolic product. Systolic BP increased in healthy subjects after CAP exposure at immediate, 4 h, and 2 days postexposure. Systolic BP decreased in asthmatics immediately and at day 2 postexposure. Holter ECG data suggested to the authors a CAP-induced increase in parasympathetic input to the heart, about which they are uncertain as to the health significance. Some subjects also reported a set of 'cardiac symptoms' during CAPs exposure which included faintness, dizziness, irregular heartbeat, and pain related to the heart.

Godleski and colleagues (2000) have performed a series of experiments examining the cardiopulmonary effects of inhaled 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 1000 $\mu g/m^3$). 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 from Boston. 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 CAP 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.

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 $\mu g/m^3$) to maximize possible differences in effects between normal and cardiopulmonary-compromised laboratory animals. No ECG changes were detected in normal Fischer 344 rats or hamsters exposed by inhalation to the New York City CAPs for a single 3-h exposure or for 3 daily 6-h exposures. Similarly, no deaths or ECG changes were seen 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. (2000) in both the normal and MCT rats exposed to New York CAPs, 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 observed increase in heart rate in rats is similar to that observed in some human population CAPs studies.

Gordon and colleagues (1998) have also 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 a 3 h exposure to 150 to 400 $\mu g/m^3$ New York City CAPs. 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 determine statistically 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 particles (CAPs) from New York City air at concentrations in the range of 95 to 341 $\mu g/m^3$ for 6 hs and sampled blood at 0, 12, and 24 hs postexposure. 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.

Studies by Vincent et al. (2001) indicate that very high concentrations (48 mg/m^3) of urban ambient particles from Ottawa ambient air administered by nose-only inhalation for 4 h to laboratory rats can cause a vasopressor response and affect blood levels of endothelin without causing acute lung injury. The authors reported a MMAD of ~4 μm for the EHC93 Ottawa urban ambient particles resuspended from samples from the air purification system of an office building. In this study, they also found that exposure to water-leached Ottawa samples (EHC93L) can modify the potency to influence hemodynamic changes by removal of polar organic compounds and soluble elements from the Ottawa 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 (CB) had no effects. The authors concluded that their results suggest a novel mechanism by which inhaled particles can affect cardiovascular function, i.e., by causing elevated endothelins, which are among the most potent vasoconstrictors in the systemic circulation and which have been shown to correlate with severity of disease in congestive heart failure and to predict cardiac death (Galatius-Jensen, et al., 1996), possibly due to ET-1 being cardiotoxic by promoting infarct size (Omland et al., 1994). However, the very high exposure concentrations used leave it unclear as to the extent that such effects may be pertinent to ambient PM exposure conditions.

Another study (Ulrich et al., 2002) utilized Ottawa EHC93 and an exposure paradigm consisting of saline control, 5 mg EHC93 only, or ozone pretreatment (8 h to 1600 μg/m^3) on the day preceding instillation. Instillations were at concentrations of 0.5, 1.5, or 5 mg/rat (approximately 2.2, 6.7, or 22.2 mg/kg based on reported body weights). At the high doses, both with (170 mg/dl) and without (160 mg/dl) ozone-pretreatment, they observed a 20% increase in plasma fibrinogen at 2 days post exposure compared to saline controls (140 mg/dl). These changes correlated with increases in endothelin (ET)-1 levels and iNOS mRNA and a decrease in angiotensin-converting enzyme (ACE). The authors suggest that the hematological changes seen in this study model heart failure in high-risk groups exposed to PM.

7.2.3 ROFA and Other Combustion Source-Related Particles

Turning to studies evaluating cardiovascular effects of controlled exposures to combustion source-related materials, using the MCT model of cardiorespiratory disease, Killingsworth et al. (1997) examined the effects of a combustion source-related irritant particle mix (residual fuel oil fly ash [ROFA] from the Boston area). They observed 42% mortality in MCT rats exposed to ~580 μg/m^3 ROFA 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 ROFA. The increase in MCT/ROFA 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/ROFA animals. Cardiac immunohistochemical analysis indicated increased MIP-2 in cardiac macrophages. The ROFA-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 ROFA exposure can produce significant lung inflammation and possible increases in proinflammatory signals in the heart.

Using a similar experimental model, Watkinson et al. (1998) examined the effects of intratracheally instilled Florida area power plant 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 ROFA-induced increase in 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. It is also possible that the reported mortalities were simply related to the MCT-induced pulmonary hypertension.

In order to help gauge the biological relevance of intratracheal instillation of ROFA particles, Kodavanti et al. (1999) exposed MCT rats to Florida area 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 caused about 50% mortality. However, very notably, no mortality occurred in MCT rats exposed to ROFA by the inhalation route despite the high exposure concentration (15 mg/m^3); nor was there any mortality 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, were evident.

Watkinson and colleagues further examined whether the effects of instilled ROFA would be exacerbated in rodents already under increased stress by being 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 were similar to that produced with MCT. Bradycardia, arrhythmias, and hypothermic changes were consistently enhanced in the ozone-exposed and cold-stressed animals treated with ROFA (0.25, 1.0, or 2.5 mg/rat); but, unlike for the MCT animals, no deaths occurred. Thus, it appears that preexisting cardiopulmonary disease or increased physiological stress may make rodents more susceptible to cardiovascular changes induced by intratracheal instillation of $\geq$ 0.25 mg of ROFA. 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 responses to these metals in conscious rats by whole-body inhalation exposure. The authors tried to ensure valid dosimetric comparisons with the instillation studies, by using concentrations of V and Ni ranging from 0.3 to 2.4 mg/m^3. The concentrations used 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. The results are suggestive of a possible synergistic relationship between inhaled Ni and V, albeit these studies were performed at metal concentrations orders of magnitude greater than their typical ambient concentrations.

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 ambient PM- and ROFA-treated rats than in the volcanic ash-treated rats. The cardiovascular changes observed with the Ottawa particles were actually greater than with the ROFA particles. These experiments indicate: (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. (2000a) exposed (via nose-only inhalation) spontaneously hypertensive (SH) and normotensive (WKY) rats to 15 mg/m^3 ROFA (Florida area) particles for 6 h/day for 3 days. The high exposure concentration (~1,000 times higher than current U.S. ambient PM levels) was selected to produce a frank but non-lethal injury. 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 may have occurred. Thus, exposure to a very high concentration of ROFA exacerbated an aberrant variation in the electroconductivity pattern of the heart in an animal model of hypertension. However, 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.

Contrary to findings from Godleski's dog study, Muggenburg et al. (2000b) reported that inhalation exposure to high concentrations of Boston area ROFA caused no consistent changes in amplitude of the ST-segment, form of the T wave, or arrhythmias in healthy 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 urban ambient 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 using dogs with pre-existing cardiovascular disease, 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 ECG readings were studied in conscious beagle dogs (selected for having pre-existing cardiovascular disease) that inhaled respirable particles of oxide and sulfate forms of transition metals (manganese, nickel, vanadium, iron, copper oxides, and nickel and vanadium sulfates) at concentrations of 0.05 mg/m^3. Such concentrations are 2 to 4 orders of magnitude higher than typical for ambient U.S. levels (usually $\leq$ 0.1 to 1.0 $\mu g/m^3$ for such metals). No significant effects of exposure to the transition metal aerosols were observed. The discrepancy between the results of Muggenburg 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) 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 while control rats underwent sham surgery. Diazepam-sedated rats were exposed (1 h) to ROFA (Boston area), carbon black, or room air at 12 to 18 h after surgery. Each exposure at a concentration of 3 mg/m^3 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 at 3 mg/m^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 (Florida) 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 PM-induced alterations in the blood fibrinogen or 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 likely 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 Boston ROFA using both inhalation and intratracheal instillation 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 to 4 wk) lung injury. A bolus

intratracheal instillation of ROFA at 5 mg/kg body weight increased plasma fibrinogen in both SH and WKY rats; whereas the increase was evident only in SH rats after acute (1 wk) 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 ROFA from Boston, which contained bioavailable zinc at doses of 2, 5, or 10 mg/m^3 for 6h/day for 4 consecutive days. A second exposure paradigm used exposure to 10 mg/m^3 ROFA for 6 h/day, 1 day/wk, 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 wk exposure group had 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 high concentrations of ROFA.

Perhaps of more direct relevance to evaluation of ambient PM effects, the effects of DE on ECG and HR were evaluated in SH rats, both male and female, exposed to diesel emissions (DE) generated from a 2000 model diesel engine (Campen et al., 2003). Whole body exposures included dilutions at concentrations of 30, 100, 300, and 1000 µg/m^3 for an exposure period of 6 h/day, 7 days/wk, for 1 wk. Exposed rats showed a significantly elevated daytime HR throughout the exposure (290 ± 7 versus 265 ± 5 for control rats). Additionally, a concentration-dependent prolongation of the PQ interval was observed in exposed rats. The authors suggested that these high level exposures to DE may affect the pacemaking system of rats by means of ventricular arrhythmias. However, the design of the study did not include testing of DPM (versus whole DE) so that one cannot clearly attribute the reported effects to DPM versus associated gases or a combination of both.

Also, Reed et al. (2004) exposed both male and female F344 rats to diesel emissions (DE) generated from a 2000 model diesel engine to evaluate the effects of DE on blood parameters. More than 90% of the DE PM mass was < 1 µm in aerodynamic diameter. Whole body exposures included dilutions at concentrations of 30, 100, 300, and 1000 µg/m^3 for exposure periods of 6 h/day, 7 days/wk, for either 1 wk or 6 mos. Blood cholesterol was decreased in both

females and males at 1 wk and in males at 6 mos at the 1000 μg/m^3 concentration. Gamma-glutamyl transpeptidase (GGT) was increased in both genders at 6 mos at the higher doses. This was the first report of effects on GGT in rodents with DE exposure, but the authors did not find liver pathology in these animals to corroborate this finding. Blood chloride, sodium, and calcium levels were increased in 6 mo exposures across DE concentrations. Total WBC counts in females were decreased at the high dose. Two blood parameters involved in coagulation were changed with exposure. Factor VII was decreased in males at 1 wk at the 300 and 1000 μg/m^3 concentration and was also decreased in both genders at the 6 mo exposure at the 1000 μg/m^3 concentration. Thrombin-antithrombin complex (TAT) was decreased in males at the highest dose after 1 wk of exposure only. Plasma fibrinogen levels were unchanged with these exposures. Analogously, lowered Factor VII levels in humans following a 2h CAPs exposure at 174 μg/m^3 were reported recently (Gong et al., 2003). Reed et al. (2004) suggest that the lowered Factor VII may be due to a decrement in clotting potential or to Factor VII being consumed in an ongoing coagulation process. This study did not find significant changes in other blood parameters including hemoglobin, hematocrit, platelet count, alkaline phosphatase, creatinine, total protein, and a host of other clinical chemistry endpoints.

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 saline vehicle for 4 wks, 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 Ottawa, 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.

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.

Frampton (2001) exposed healthy, nonsmoking subjects (18 to 55 ys old) to 10 $\mu g/m^3$ ultrafine carbon while at rest via a mouthpiece for 2 h ½ , 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, and respiratory symptoms, spirometry, blood pressure, pulse-oximetry, blood markers, and exhaled NO were evaluated before, immediately following, and 3.5 and 21 h postexposure. Blood markers focused on parameters related to acute response, i.e., 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 ECG Holter monitoring. Preliminary findings indicated no particle-related effects, including neither cardiovascular nor respiratory-related effects.

Nemmar et al. (2002a) studied effects of ultrafine (60 nm) polystyrene particles on thrombus formation in a hamster model after intravenous administration of unmodified, carboxylate-polystyrene, or amine-polystyrene particles. Unmodified particles did not affect thrombosis at IV doses up to 5000 μg/kg; whereas carboxylate-polystyrene particles significantly inhibited thrombus formation at 100 and 500 μg/kg, but not at 50 μg/kg body weight. Thrombosis was significantly enhanced by amine-polystyrene particles at 50 and 500 μg/kg, but not at 5 μg/kg body weight. Intratracheal instillation of 5 mg/kg of amine-polystyrene particles, but not unmodified or carboxylate-modified particles, also increased

thrombosis formation. Platelet aggregation (ADP-induced in vitro) was also enhanced significantly by amine-modified polystyrene particles, but not by unmodified or carboxylate-modified particles. Thus, only positively charged ultrafine particles resulted in enhancement of 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 charge of the particles, i.e., positive-charged particles induce prothrombotic effects, at least partly via platelet activation.

7.2.4 Summary of Cardiovascular/Systemic Effects

In summary, experimental controlled human exposure studies of cardiovascular-related effects have yielded only very limited evidence for ambient PM effects on cardiac function as indexed by ECG readings or on systemic endpoints (e.g., vasopressor control, blood coagulation control, etc.) linked to more serious cardiovascular events. Probably of most note, the controlled human exposure CAPs study by Ghio et al. (2000a) and another by Petrovic et al. (2000) did find evidence indicating that ambient levels (ranging up to ~125 to 300 $\mu g/m^3$) of inhaled $PM_{2.5}$ can produce some biochemical changes (increased fibrinogen) in blood suggestive of PM-related increased risk for prothrombotic effects. Similarly, Ulrich et al. (2002) found a 20% increase in plasma fibrinogen in rats 2 days after instillation exposure to 6.7 or 22.2 mg/kg of Ottawa EHC93 ambient PM extract. Also, decreased Factor VII levels were observed by Gong et al. (2003) in humans (with 2-h CAPs exposure at ~174 $\mu g/m^3$) and by Reed et al (2004) in rats (with DE exposure 6h/day, 7 day/wk, for 1 wk at 300 and 1000 $\mu g/m^3$). The decreased Factor VII levels may be due to that enzyme being consumed in an ongoing coagulation process. On the other hand, the same and many other human and animal studies did not find changes in other factors (e.g., increased platelets or their aggregation) related to blood coagulation control. Overall, then, some available laboratory studies provide limited evidence suggesting that high concentrations/doses of inhaled or instilled particles can exert cardiovascular-related systemic effects; but many of the studies provide conflicting evidence, especially with regard to heart rate, heart rate variability, or other ECG markers of cardiac function. Thus, although some of the reported changes have been used as clinical "markers" for cardiovascular diseases, the causal relationship between such PM-related changes and potential life-threatening alterations in cardiovascular function remains to be better established.

Among the most salient hypotheses proposed to account for cardiovascular/systemic effects of PM are: alterations in coagulability (Seaton et al., 1995; Sjögren, 1997); cytokine effects on heart tissue (Killingsworth et al., 1997); perturbations in both conductive and hypoxemic arrythmogenic mechanisms (Watkinson et al., 1998; Campen et al., 2000); altered endothelin levels (Vincent et al., 2001); and activation of neural reflexes (Veronesi and Oortgiesen, 2001). Only limited progress has been made in obtaining evidence bearing on such hypotheses (as discussed in later sections of this chapter), and much future research using controlled exposures to PM of laboratory animals and human subjects will clearly be needed to test further such mechanistic hypotheses (as well as others proposed in the future) so as to more fully understand pathways by which low concentrations of inhaled ambient PM may be able to produce life-threatening systemic changes.

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

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 tract 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 (concentration, duration, 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 laboratory derived surrogate particles, e.g., metals or pure sulfuric acid droplets. Toxicological studies of various "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 mixtures. 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 $\mu g/m^3$ (50 mg/m^3) of respirable particles with inherently low toxicity. Although there was no mortality, some mild pulmonary function changes after exposure ranging from 1 h to 24 mos 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 chronic exposure (6 to 7 h/day 5 day/wk for 20 to 24 mo.) of rats to $\geq$ 5,000 $\mu g/m^3$ of coal dust. Changes in macrophage clearance after exposure to > 10,000 $\mu g/m^3$ to a variety of particles over various exposure periods (days to months) 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 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, do provide evidence of likely greater relevance to understanding respiratory effects of ambient PM exposure and underlying mechanisms, as discussed below.

7.3.1 Ambient Particulate Matter

Some new in vivo toxicology studies have employed inhalation exposures to evaluate the respiratory effects of ambient particles in humans and laboratory animals, using either concentrated ambient particles (CAPs) or resuspended urban ambient PM from various U.S. and Canadian locations. Other new in vivo exposure studies have mainly utilized intratracheal instillation techniques. The pros and cons of the latter in comparison to inhalation are discussed 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 instillation studies, ambient PM samples were first collected on filters; then after various storage times, PM materials were extracted from the filters and resuspended in a vehicle (usually saline), followed by a small volume of the suspension medium then being instilled intratracheally into the animals. It is important to note that 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 use in attempting to extrapolate experimentally observed results to humans in ambient exposure scenarios, greater importance should be placed on inhalation study results. Instillation studies have been most valuable in comparing effects of different types of PM and/or for investigating potential mechanisms by which particles may cause inflammation and lung injury.

7.3.1.1 Ambient Particles Inhalation Exposures

Table 7-4 summarizes newly available studies in which various biological endpoints were measured following inhalation exposures to CAPs or, in the case of one study, resuspended urban ambient particles.

With regard to newly available experimental studies that most directly parallel aspects of ambient PM inhalation exposures, Ghio et al. (2000a) exposed 38 non-smoking healthy adult volunteers (aged 18 to 40 ys) exercising intermittently at moderate levels of exertion (breathing rate = 25 L/min) for 2 h to either filtered air or $PM_{2.5}$ concentrated 6- to 10-fold from

TABLE 7-4. RESPIRATORY EFFECTS OF INHALED AMBIENT PARTICULATE MATTER IN CONTROLLED EXPOSURE STUDIES OF HUMAN SUBJECTS AND LABORATORY ANIMALS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Exposure Concentration*	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Humans, healthy nonsmokers; 18 to 40 y old n = 38	CAPs (Chapel Hill)	Inhalation	23.1 to 311.1 µg/m^3	0.65 µm σ_g = 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 18-40 y old n = 4	CAPs (Toronto)	Inhalation (face mask)	24 to 124 µg/m^3	0.1 - 2.5 µm	2 h; analyses pre- & during exposure and about 2 to 24 h postexposure.	Only stat. sig. effect on pulmonary function was mean increase of 6.4% in thoracic gas volume after high CAP exposure versus mean 5.6% after filtered air exposure, but not in respiratory symptoms. Trend towards increased nasal neutrophils, but no respiratory inflammatory response.	Petrovic et al. (2000)
Humans, healthy; 19-41 y old n = 4	CAPs (Los Angeles)	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)
Humans healthy (12) and asthmatic (12) 18-45 y old, nonsmoking	CAPs (Los Angeles)	Inhalation whole body chamber	99-224 µg/m^3 (mean 174)	80% 0.1 to 2.5 µm	2 h with alternating exercise/rest. Analysis at 0, 4, and 22 h PE.	CAPs-related decrease in columnar cells in induced sputum at 0 h PE. No significant changes in S_aO_2,** FVC, or FEV_1.	Gong et al. (2003)
Mongrel dogs, some with balloon occluded LAD coronary artery n = 14	CAPs (Boston)	Inhalation via tracheostomy	~100-1000 µg/m^3 (variable from day-to-day)	0.23 to 0.34 µm σ_g = up 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, healthy-air, bronchitic-air, healthy-CAPs, bronchitic-CAPs n = 48 (12 per group)	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 σ_g = 2.9	5 h/day for 3 days	Bronchitis induced by pre-treatment with 170 ppm SO_2 for 6 wks at 5 hr/day, 5 days/wk. The 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 bronchitic than healthy rats. Bronchitic CAPs-exposed rats showed evidence of inflammation-related epithelial permeability.	Clarke et al. (1999)

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TABLE 7-4 (cont'd). RESPIRATORY EFFECTS OF INHALED AMBIENT PARTICULATE MATTER IN CONTROLLED EXPOSURE STUDIES OF HUMAN SUBJECTS AND LABORATORY ANIMALS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Exposure Concentration*	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Rats, male S-D 200-250 g, healthy-air, bronchitic-air, healthy-CAPs, bronchitic-CAPs n = 259 (6 studies, 40-48 per study, 8-10 per group)	CAPs (Boston)	Inhalation; Harvard/EPA fine particle concentrator; animals restrained in chamber	3-day mean CAPs ranged from 187 to 481 µg/m^3 29 °C, 47% RH	0.27 µm σ_g = 2.3	5 h/day for 3 days	Bronchitis induced by pre-treatment with an average of 276 ppm SO_2 for 5 wks at 5 hr/day, 5 days/wk. Increase in neutrophils in both healthy and bronchitic rats associated with CAPs exposure concentration. Specific CAPs components associated with neutrophil increase. In bronchitic rats, CAPs components also associated with lymphocyte increase. Histologic examination suggested bronchial-alveolar junction as the site of greatest inflammation response.	Saldiva et al. (2002)
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. (2000b)
Rats, male F344 Hamsters, male, 8-mo-old Bi TO-2	CAPs (NYC)	Inhalation	132 to 919 µg/m^3	0.2 to 1.2 µm σ_g = up 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 F344 7-8 mo	CAPs (NYC)	Inhalation	100 to 350 µg/m^3 (mean 225 µg/m^3)	0.4 µm σ_g = 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. Concentrations tested represents a range over the 3 h exposure period.	Zelikoff et al. (2003)
Rats, male, F-344; 200-250 g	Ottawa ambient	Nose-only Inhalation	40 mg/m^3	4 to 5 µm MMAD	4 h	No acute lung injury; however, lung NO production decreased and macrophage inflammatory protein-2 from lung lavage cells increased after exposure. Increased plasma levels of endothelin-1.	Bouthillier et al. (1998)

*Concentration = range of CAP concentrations at inlet of exposure chamber or in breathing zone of exposed subjects.

[b] PE = Post Exposure

**S_aO_2 = arterial oxygen saturation.

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Chapel Hill, NC air at the inlet of the exposure chamber. Neither respiratory symptoms nor decrements in pulmonary function (RAW, FVC, $FEV_{1.0}$, PEF measurements) were found immediately after exposure to CAPs. However, analysis of BAL cells and fluid obtained 18 h after Chapel Hill CAPs exposure (at 23 to 311 $\mu g/m^2$) 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. 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 any evident effect on immune defenses, as determined by lymphocyte or macrophage effects. The increase in BAL neutrophils may represent a normal physiological 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, per se, constitutes a biologically significant injury to the lung is an ongoing controversial issue.

In the small study by Petrovic et al. (2000) described earlier (Section 7.2.2), the four healthy volunteer subjects (aged 18 to 40 y) exposed for 2 h to CAPs (23-124 $\mu g/m^3$ from downtown Toronto were not only evaluated for CAP effects on cardiovascular endpoints, but also on several respiratory endpoints (nasal lavage, nasal acoustic rhinometry, pulmonary function). No cellular signs of inflammation were observed in induced sputum samples collected at 2 or 24 hs after exposure. The authors said there was a trend toward an increase in nasal lavage neutrophils (but level of statistical significance not specified). The only statistically significant ($p < 0.01$) change in pulmonary function was a 6.4% decrease in thoracic gas volume after high CAPs exposure to 124 $\mu g/m^3$ PM versus a 5.6% increase after filtered air exposure, but no increase was seen in respiratory symptoms. These results, overall, suggest that acute exposures (~2 h) to Toronto ambient PM are unlikely to exert untoward respiratory effects in healthy adults at $PM_{2.5}$ levels below about 100 $\mu g/m^3$, but may begin to have some mild effects on pulmonary function as $PM_{2.5}$ levels reach or exceed 125 $\mu g/m^3$. However, further evaluations

of these possibilities with larger numbers of healthy subjects are needed, as well as analogous studies of compromised human subjects.

As also discussed earlier in Section 7.2.2, Gong et al. (2000a) conducted a small pilot study (n = 4 subjects, aged 19-41 y) in which healthy adult volunteers were exposed to Los Angeles CAPs (148 to 246 $\mu g/m^3$) for 2 h and evaluated during or immediately after exposure for possible respiratory effects or ECG changes. No significant effects were observed for various lung function measures, respiratory symptoms, oxygen saturation, or in Holter ECG readings, even at $PM_{2.5}$ levels (~246 $\mu g/m^3$) approximating likely maximum levels for Los Angeles.

As also noted earlier, the effects of Los Angeles ambient air were studied further by Gong et al. (2003), who exposed 12 healthy and 12 asthmatic subjects (age 18 to 45 y) to LA CAPS ($PM_{2.5}$). Exposures averaging 174 $\mu g/m^3$ (range 99-224) CAPs in a whole body chamber for 2 h were alternated with filtered air exposure at least 14 days apart. Subjects exercised for 15 min of each half hour at a ventilation rate of 15-20 $l/min/m^2$ body surface. Tests were performed just before exposure, just after, 3.5 to 4 h after (4 h), and the next day. Ventilation during CAPs exposure was significantly lower in both the healthy and asthmatic groups, and both groups showed a CAPs-related decrease in columnar cells in induced sputum immediately postexposure, but the authors were uncertain as to the health significance of this effect. There were no significant differences in SaO_2, FVC, FEV_1, or other respiratory parameters in this study.

Godleski, et al. (2000), in another study noted previously (Section 7.2.2), exposed mongrel dogs to Boston CAPs (ranging from ~100 to 1000 $\mu g/m^3$) for 6 h/day for 3 days. The only two respiratory effects reported were a decreased respiratory rate over time and a modest increase in neutrophils in lavage fluid from the lungs of the normal dogs, even with exposures to ambient Boston particles concentrated by 30-fold over ambient levels.

Saldiva et al. (2002) studied the effects of CAPs from Boston on rat lung. The study was designed to: (1) determine whether short-term exposures to CAPs cause pulmonary inflammation in normal rats and in compromised rats with chronic bronchitis (CB); (2) identify the site within the lung parenchyma where CAPs-induced inflammation occurs; and (3) characterize the component(s) of CAPs significantly associated with development of the inflammatory reaction. Chronic bronchitis was induced by exposure to high doses of SO_2 for 5 h/day, 5 days/wk during 5 wks prior to experimental exposures to filtered air or to the Boston CAPs. Thus, four groups of animals were studied: (1) air pre-treated, filtered air-exposed

(air-sham); (2) sulfur dioxide pre-treated (CB), filtered air-exposed (CB-sham); (3) air pre-treated, CAPs-exposed (air-CAPs); and (4) sulfur dioxide pre-treated, CAPs-exposed (CB-CAPs). Normal and CB rats were exposed by inhalation either to filtered air or to CAPs during 3 consecutive days (5 hs/day). CAPs concentrations varied considerably (73.5 to 733 $\mu g/m^3$), with 3-day mean CAPs mass = 126 to 481 $\mu g/m^3$. The average MMAD of the CAPs particles was 0.27 μm (σ_g = 2.3). CAPs mass (as a binary exposure term) and CAPs mass (in regression correlations) induced a significant increase in bronchoalveolar lavage (BAL) neutrophils and in both normal and CB animals. Numerical density of neutrophils (Nn) in alveolar walls significantly increased with CAPs in normal animals only, with greater Nn seen in central, compared with peripheral, regions of the lung. Significant dose-dependent associations were observed between various CAPs components and BAL neutrophils or lymphocytes; however, only vanadium and bromine concentrations were significantly associated 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.

In another study of Boston CAPs, Clarke et al. (1999) exposed healthy normal rats and (SO_2-induced) bronchitic rats by inhalation via tracheostomy for 5 h/day for 3 days to filtered air or concentrated Boston ambient $PM_{2.5}$ particles averaging 206, 773, and 607 $\mu g/m^3$ on the three different days. Significantly increased tidal volume (TV) was observed with CAPs exposures for both the normal rats and, even greater, for the chronic bronchitic rats. Bronchiolar lavage performed 24-h after the final day of exposure revealed evidence for significant pulmonary inflammation following CAPs exposures, especially in chronic bronchitic rats, as indexed by significant increases in neutrophils, lymphocytes, and total lavage protein. The authors concluded that these results suggested two distinct mechanistic responses to inhaled particles: (1) a stressor-type pulmonary function reaction (typified by increases in air flow and volume) and (2) acute pulmonary inflammation characterized by cellular influx (especially neutrophils).

Zelikoff et al. (2003) reported effects on pulmonary or systemic immune defense mechanisms in Fischer rats exposed by inhalation to filtered air or to New York City CAPs (90 to 600 $\mu g/m^3$; mean = 345 $\mu g/m^3$) for 3 h prior to the 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 white blood cell (WBC) counts were significantly increased 24 and 72 h postinfection in both groups. CAPs exposure slowed a decline in TNF-α 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. (2003) also evaluated the effects of CAPs exposure (65-150 $\mu g/m^3$; mean = 107 $\mu g/m^3$) in rats following a single 5 h exposure to IT instilled Streptococcus pneumoniae. The New York City CAPs exposure significantly reduced percentages of lavageable PMN 24 h following CAPs exposure and remained well below control levels for up to 3 days, but lavageable PAM was significantly increased in the CAPs exposed animals. CAPs exposure also reduced the levels of TNF-α, 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.

Bouthillier et al. (1998) reported that 4 h exposure of rats by nose-only inhalation to 40 mg/m^3 of resuspended Ottawa ambient PM (MMAD = 4 to 5 μm) produced no evident acute lung injury. However, lung NO production decreased and macrophage inflammatory protein -2 from lung lavage cells increased at 4 h after exposure, as did plasma levels of endothelin -1 (a powerful cardiac cytotoxic agent).

7.3.1.2 Intratracheal and Intrabronchial Instillation of Ambient Particulate Matter

Other newly-available studies (Table 7-5) that evaluated acute effects of intratracheal or intrabronchial instillation of ambient PM extracts from filters obtained from various locations have found evidence indicating that exposures to such ambient PM materials can cause lung inflammation and injury.

Costa and Dreher (1997) showed that instillation in rats of relatively high concentrations of PM samples from four ambient airsheds (St. Louis, MO; Washington, DC; Dusseldorf, Germany; and Ottawa, Canada) and from three combustion sources (two oil and one coal fly ash) resulted in acute inflammatory responses, as indexed by increases in lung polymorphonuclear leukocytes (PMNs) and eosinophils 24 h after instillation. Biomarkers of permeability (total protein and albumin) and cellular injury, lactic dehydrogenase (LDH), were also increased.

TABLE 7-5. 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; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Humans, healthy nonsmokers; 21 M, 3 F; 26.4 ± 2.2 y old	Provo, UT PM_{10} filters (10 ys 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 during period of mill closure.	Ghio and Devlin (2001)
Rats, male S-D 60-day-old	Provo, UT TSP filters (10 ys 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 ys old), soluble and insoluble extracts	Intratracheal instillation	100, 150, 500, and 1000 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, male, S-D 60 day old	Provo, UT TSP. Collected 1982.	Intratracheal Instillation	100, 500, 1500 µg PM in 0.5 mL saline	N/A	24 h BAL	Increased BAL protein and PMN at ≥ 500 µg dose. Also proliferation of bronchiolar epithelium and intraalveolar hemorrhage at 500 µg dose.	Kennedy et al. (1998)
Rats, Wistar (HAN strain)	Edinborough 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)
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, and β-N-acetylglucosaminidase and myeloperoxidase, 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)

[a] LDH = lactic dehydrogenase
PMN = polymorphonuclear leukocytes

[b] PE = Post Exposure

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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. The relative potencies of the combustion-source particles in producing the acute inflammatory effects ranked in the order: DOFA > ROFA >> CFA = saline vehicle, reflecting closely the much higher amounts of bioavailable metals in the oil fly ash than in the coal-derived fly ash. Analogously, the ambient PM extracts exhibited, on a per mass basis, much less potency in inducing inflammatory responses than the oil fly ash extracts (e.g., ROFA), with the Ottawa extract exerting notably stronger effects than ambient PM extracts from the other cities. However, when the exposures were normalized to match metal content, there was little difference between the ambient PM and ROFA effects. Interestingly, the most potent ambient PM (Ottawa) both was the freshest one collected (3 y versus 10 y old) and had the highest bioavailable metal content of the ambient PM. Thus, this study demonstrated, overall, that the lung dose of bioavailable transition metals, not just 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 from Provo, UT in 1982. The particulate mixture was comprised 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. Doses of 0, 150, 500, and 1500 µg were instilled; and effects were seen at $\geq$ 500 µg. 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.

Toxicological studies of ambient PM collected around Provo, UT (Utah Valley) in the late 1980s are also particularly interesting (Ghio and Devlin, 2001; Dye et al., 2001). Epidemiologic studies by Pope (1989, 1991) showed 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. Ambient PM was collected near the steel mill during the winter of 1986 (before closure), throughout 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; pertinent in vitro studies (e.g., Wu et al., 2001; Soukup et al., 2000; Frampton et al., 1999) 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 (500 μg of extract in 10 mL of sterile saline) 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 are suggestive of pulmonary effects of experimental exposure of humans to Utah Valley PM that 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 in laboratory animals. Sprague-Dawley rats were intratracheally instilled with equivalent masses of aqueous extracts (0, 0.83, 3.3, 8.3, or 16 mg extract/kg body weight in 0.3 mL saline) 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. Chemical analysis of extract solutions demonstrated that nearly 70% of the mass in all three extracts appeared to be sodium-based salts derived from the glass filter matrix. However, 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 $\leq$ 1% of the extracts by mass, the greater quantity

detected in the extracts of particles collected when the plant was open suggested that metals may be among the 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 later in Section 7.4).

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 Edinborough, 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 was shown consistently to cause pulmonary inflammation and injury tends to support epidemiologic studies that report increased PM-associated respiratory effects in populations living in some of the same geographical areas (e.g., Utah Valley). On the other hand, the potential exists that lower, more "realistic" doses associated with ambient PM exposures may activate cells and signaling pathways not observed with much higher than ambient experimental doses, such that lower-dose mechanisms may be overwhelmed. Thus, high-dose instillation studies may actually produce different effects on the lung than inhalation exposures at lower concentrations or doses more closely paralleling those seen with ambient PM exposures.

7.3.2 ROFA and Other Combustion Source-Related Particles

Because combustion emission sources contribute to the overall ambient air particulate burden (Spengler and Thurston, 1983), a number of the studies investigating the response of laboratory animals to particle exposures have used combustion source-related particles (see Tables 7-6 and 7-7). For example, the residual oil fly ash (ROFA) samples used in many 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 typically represent only a very small subfraction of the total ambient PM mass of U.S. monitoring samples. 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. Transition metals generate reactive oxygen species (ROS) pertinent to understanding of one proposed mechanism of PM toxicity and of PM components possibly contributing to toxic responses.

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., 1997a). A leachate prepared from ROFA, containing predominantly Fe, Ni, V, Ca, Mg, and sulfate, produced lung injury similar 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.

TABLE 7-6. RESPIRATORY EFFECTS OF INSTILLED ROFA AND OTHER COMBUSTION SOURCE-RELATED PARTICULATE MATTER IN HEALTHY LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
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	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	ROFA (Florida), DOFA (Boston), CFA (RTP, NC) Ambient PM (St. Louis; Wash, DC; Ottawa; Dusseldorf)	Intratracheal instillation	Total mass: 2.5 mg/rat or Total transition metal: 46 µg/rat	Combustion source PM: 1.78-4.17 µm Ambient PM: 3.27-4.09 µm	Analysis at 24 and 96 h following instillation	Increases in eosinophils PMNs, albumin, and LDH following exposure to ambient and combustion source (ROFA, DOFA, CFA) particles; induction of injury by test samples was determined primarily by constituent metals and their bioavailability in both ambient and combustion source PM.	Costa and Dreher (1997)
Rats, male, S-D, 65 days old	ROFA (Florida)	Intratracheal instillation	2.5 mg (8.3 mg/kg)	1.95 µm	Analysis at 24, 96 h PE	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 (Florida)	Intratracheal Instillation	500 µg/rat ROFA 500 µg/rat ROFA plus DMTU	1.95 µm	Analysis at 24 h PE	ROFA-induced increased neutrophilic inflammation was inhibited by DMTU treatment, indicating role to reactive oxygen species.	Dye et al. (1997)

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TABLE 7-6 (cont'd). RESPIRATORY EFFECTS OF INSTILLED ROFA AND OTHER COMBUSTION SOURCE-RELATED PARTICULATE MATTER IN HEALTHY LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Rats, male, S-D, 60 days old	Two ROFA (Florida) samples (R1, R2) also R2s (supernatant)	Intratracheal instillation	2.5 mg (9.4 mg/kg)	R1: 1.88 µm R2: 2.03 µm	Analysis at 4 days PE	Four of 24 animals treated with R2 or R2s died; none of R1s 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 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 wks	ROFA, #6 lo-S (Florida)	Intratracheal instillation	60 µg in saline (dose 3 mg/kg)	< 2.5 µm	Analysis at 1, 3, 8, 15 days postexposure	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 (Florida)	Intratracheal instillation	500 µg	3.6 µm	Analysis at 4 and 96 h postexposure	Ferritin and transferrin were elevated; greatest increase in ferritin, lactoferrin, transferrin occurred 4 h postexposure.	Ghio et al. (1998a)
Mice, normal and Hp, 105 days old	ROFA (Florida)	Intratracheal instillation	50 µg	1.95 µm	Analysis at 24 h PE	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. (2000b)
Rats, male, S-D, 60 days old	ROFA (Florida)	Intratracheal instillation	1.0 mg in 0.5 mL saline	1.95 µm	Analysis at 24 h PE	Increased PMNs, protein.	Kadiiska et al. (1997)
Rats, male, S-D and F-344 (60 days old)	ROFA (Florida)	Intratracheal instillation	8.3 mg/kg	1.95 µm $\sigma_g = 2.14$	Sacrificed at 24 h PE	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)

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TABLE 7-6 (cont'd). RESPIRATORY EFFECTS OF INSTILLED ROFA AND OTHER COMBUSTION SOURCE-RELATED PARTICULATE MATTER IN HEALTHY LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Rats, male, S-D, WISTAR, and F-344 (60 days old)	ROFA (Florida)	Intratracheal instillation	8.3 mg/kg	1.95 µm σ_g = 2.14	Sacrificed at 6, 24, 48, and 72 h; 1, 3, and 12 wks	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. (1997b)
Rats, male, S-D, 60 days old	ROFA (Florida) $Fe_2(SO_4)_3$, VSO_4, $NiSO_4$	Intratracheal instillation	8.3 mg/kg ROFA-equivalent dose of metals	1.95 µm σ_g = 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. (1997a)
Rats, male, S-D, 60 days old	10 compositionally different ROFAs from Boston area power plant	Intratracheal instillation	0.83, 3.3, 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 (Florida)	Intratracheal instillation	0, 0.83, 3.3 mg/kg	1.95 µm σ_g = 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 wks old	ROFA (Florida) VSO_4, $NiSO_4$, or saline	Intratracheal instillation	3.3 mg/mL/kg 1.5 µmol/kg	1.95 µm σ_g = 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)

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TABLE 7-6 (cont'd). RESPIRATORY EFFECTS OF INSTILLED ROFA AND OTHER COMBUSTION SOURCE-RELATED PARTICULATE MATTER IN HEALTHY LABORATORY ANIMALS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Rats, female, Brown Norway 8-10 wks old	ROFA (Florida) 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	ROFA #6 (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)
	DOFA (NC)	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, Fe.	
Rats, male, S-D; 60 days old	ROFA #6 (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 (Cayman Chemical, Ann Arbor, MI)	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	ROFA, #6 LoS (Florida)	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-MAPKS.	Silbajoris et al. (2000)
Rats, S-D	DPM	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] CFA = Coal fly ash
CMP = Copper smelter dust
DOFA = Domestic oil-burning furnace fly ash
ROFA = Residual oil fly ash
TC = Tungsten carbide

$Fe_2(SO_4)$ = Iron sulfate
VSO_4 = Vanadium sulfate
$NiSO_4$ = Nickel sulfate
LoS = low sulfur

MCT = Monocrotaline
OVA = Ovalbumin

[b] PE = Post Exposure

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TABLE 7-7. RESPIRATORY EFFECTS OF INHALED AND INSTILLED ROFA AND OTHER COMBUSTION SOURCE-RELATED PARTICULATE MATTER IN COMPROMISED LABORATORY ANIMAL MODELS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Inhalation							
Rats, male, WISTAR Bor:WISW strain n = 20	Coal fly ash (CFA)	Inhalation (chamber)	0, 11, 32, and 103 mg/m^3	1.9-2.6 µm σ_g = 1.6-1.8	6 h/day, 5days/wk, for 4 wks	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 (Boston)	Inhalation	580 ± 110 µg/m^3	2.06 µm σ_g = 1.57	6 h/day for 3 days	Mortality seen only in MCT rats exposed to ROFA. Neutrophils in lavage fluid increased significantly in MCT rats exposed to ROFA versus filtered air. MIP-2 mRNA expression induced in lavage cells 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 (Florida)	Nose-only inhalation	15 mg/m^3	1.95 µm σ_g = 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 wks old	ROFA (Florida)	Nose-only Inhalation	15 mg/m^3	1.95 µm σ_g = 2.14	6 h/day × 3 days, 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)

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TABLE 7-7 (cont'd). RESPIRATORY EFFECTS OF INHALED AND INSTILLED ROFA AND OTHER COMBUSTION SOURCE-RELATED PARTICULATE MATTER IN COMPROMISED LABORATORY ANIMAL MODELS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Inhalation (cont'd)							
Mice, male, Swiss-Webster, 6-8 wks 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 observed differences in 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 activation of inflammatory cells. 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)
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	MCT-treated animals had fewer microspheres in their macrophages, probably because of impaired chemotaxis.	Madl et al. (1998)

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TABLE 7-7 (cont'd). RESPIRATORY EFFECTS OF INHALED AND INSTILLED ROFA AND OTHER COMBUSTION SOURCE-RELATED PARTICULATE MATTER IN COMPROMISED LABORATORY ANIMAL MODELS[a]

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration; Time to PE[b] Analysis	Particle Effects/Comments	Reference
Instillation							
Rats, male, S-D; 60-day-old; WKY and SH; cold-stressed SH; O_3-exposed SH; MCT-treated SH	ROFA (source not specified), Ottawa dust, MSH Vol. Ash	Intratracheal instillation	0, 0.25, 1.0, and 2.5 mg/rat	1.95 µm	96 h post-IT	ROFA instillation caused acute, dose-related increase in pulmonary inflammation. Data on Ottawa dust and volcanic ash not reported.	Watkinson et al. (2000a,b)
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 wks.	Murphy et al. (1998)

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

[b] PE = Post Exposure

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To further investigate the response to ROFA with differing metal and sulfate composition, Kodavanti et al., (1997a) instilled male Sprague-Dawley rats (60 days old) intratracheally with ROFA (2.5 mg/rat) or metal sulfates (iron –0.54 µmole [105 µg]/rat, vanadium –1.7 µmole [245 µg]/rat, and nickel –1.0 µmole [263 µg]/rat, individually or in combination). 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 parallel work on the potential importance of metals in mediating ambient PM effects, Kodavanti et al. (2002b) studied the role of zinc in PM-induced health effects in several animal models. Male Sprague-Dawley (SD) rats were instilled IT with ROFA in saline (0.0, 0.8, 3.3, or 8.3 mg/kg) from a Boston area power plant. Also, in order to evaluate the potential role of leachable zinc, additional rats were instilled with either saline, whole ROFA suspension, the saline leachable fraction of ROFA, the particulate fraction of ROFA (8.3 mg/kg, soluble Zn = 14.5 μg/mg ROFA), or $ZnSO_4$ (0.0, 33.0, or 66.0 μg/kg Zn). Three rat strains that differ in PM susceptibility, i.e., male SD, normotensive Wistar-Kyoto (WKY), and spontaneously hypertensive (SH) rats, were exposed at age 90 days nose-only to either filtered air or ROFA (2, 5, or 10 mg/m^3 for 6 h/day × 4 days/wk × 1 wk; or 10 mg/m^3 for 6 h/day × 1 day/wk for 1, 4, or 16 wks) and assessed at 2 days postexposure. Intratracheal exposures to whole ROFA suspensions produced a dose-dependent increase in protein/albumin permeability and neutrophilic inflammation. Pulmonary protein/albumin leakage and neutrophilic inflammation caused by the leachable fraction of ROFA and $ZnSO_4$ were comparable to effects of the whole suspension. However, protein/albumin leakage was not associated with the particulate fraction,

although significant neutrophilic inflammation did occur after instillation. With ROFA nose-only inhalation, acute exposures (10 mg/m^3 only) for 4 days resulted in small increases BAL protein and n-acetyl glucosaminidase activities (~50% above control); but, unlike with IT exposures, no neutrophilic influx was detectable in BAL from any of the inhalation groups. The only major effect of acute and long-term ROFA inhalation was a dose- and time-dependent increase in alveolar macrophages (AM), regardless of 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 WBC counts and plasma fibrinogen, but no major ROFA inhalation effect. The authors attributed the differences in pulmonary responsiveness to ROFA between IT and inhalation exposures to the dose of bioavailable zinc; the IT ROFA exposures, but not acute and long-term inhalation of up to 10 mg/m^3, caused neutrophilic inflammation.

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 TNF-α by alveolar phagocytes were studied by Broeckaert et al. (1997). Female NMRI mice were instilled with different particles normalized for arsenic content (20 µg/kg body weight [i.e., 600 ng arsenic/mouse]) and particle load (100 mg/kg body weight [i.e., 3 mg/mouse]). Mice received tungsten carbide (TC) alone, coal fly ash (CFA) alone, copper smelter dust (CMP) mixed with TC, and $Ca_3(AsO_4)_2$ mixed with TC (see Table 7-6 for concentration details). Copper smelter dust caused a severe but transient inflammatory reaction; whereas a persisting alveolitis (30 days postexposure) was seen after treatment with coal fly ash. Also, TNF-α production in response to lipopolysaccharide (LPS) by alveolar phagocytes was 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 As administered) in the lung of CMP- and CFA-treated mice at day 30 postexposure. Hence, suppression of TNF-α production may be dependent on the slow elimination of particles and their metal content from the lung.

Antonini et al. (2002) investigated effects 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 for BAL cells from the right lungs. 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 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, changes that could lead to increased susceptibility to lung infection in exposed populations.

In summary and as listed in Table 7-6, 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 to help understand ambient PM exposure effects in humans remains to be more fully established.

7.3.3 Metals

Results from occupational and laboratory animal studies reviewed in the 1996 PM AQCD indicated that acute exposures to very high levels (hundreds of $\mu g/m^3$ or more) or chronic exposures to lower levels (as low as 15 $\mu g/m^3$) 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 $\mu g/m^3$) 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 $\mu g/m^3$ were not reviewed in the 1996 PM AQCD.

More recently published data from controlled experimental exposure studies, however, are suggestive of particle-associated metals possibly being among PM components contributing to

health effects attributed to ambient PM. Included among such studies are a number of the "ambient PM," ROFA, and other "combustion-source" studies assessed in the preceding two sections which included analyses of potential contributions of metals to observed effects. Other new studies on effects of laboratory-generated metals/metal compounds are summarized in Table 7-8.

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 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 (~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 $\sigma_g = 2.1$) 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 hs 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

TABLE 7-8. RESPIRATORY EFFECTS OF INHALED AND INSTILLED METAL PARTICLES IN HUMAN SUBJECTS AND LABORATORY ANIMALS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration/ Time to Analysis	Particle Effects/Comments	Reference
Inhalation							
Humans, healthy nonsmokers; 8 M, 8 F; 18-34 ys old	Fe_2O_3	Inhalation	12.7 mg/m^3	1.5 µm σ_g = 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)
Instillation							
Humans, healthy nonsmokers; 12 M, 4 F; 18-35 ys 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 y 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, 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)

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TABLE 7-8 (cont'd). RESPIRATORY EFFECTS OF INHALED AND INSTILLED METAL PARTICLES IN HUMAN SUBJECTS AND LABORATORY ANIMALS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration/ Time to Analysis	Particle Effects/Comments	Reference
Instillation (cont'd)							
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)
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, 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)

CdO = Cadmium oxide
Fe_2O_3 = Iron oxide
MgO = Magnesium oxide
MnO_2 = Manganese oxide
$NaVO_3$ = Sodium metavanadate
TiO_2 = Titanium oxide
$VOSO_4$ = Vanadyl sulfate
V_2O_5 = Vanadium pentoxide
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

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produced diffuse alveolar damage, difficulty in breathing, and symptoms of angina. Elemental analyses revealed high metal content (Fe, V, etc.) in fly ash samples; and other evaluations suggested that the high metal content of oil fly ash altered the epithelial cell barrier in the alveolar region.

Several of the other studies summarized in Table 7-8 provide evidence that several different metal salts (when instilled intratracheally in rats or mice at relatively high doses) can produce inflammatory responses in the lung as indicated by various markers (e.g., increased BAL PMNs or other inflammatory cells, induction of cytokines, etc). Two of the studies (by Osier and Oberdörster, 1997; Osier et al., 1997) further indicate that (a) ultrafine metal particles are more effective than fine particles in producing the inflammation and (b) intratracheal inhalation is less effective than instillation in producing the inflammation. Analogously, Campen et al. (2001) did not observe any significant changes in BAL markers with 6 h/day inhalation exposure of rats for 4 days to $VOSO_4$ at concentrations ranging up to 2.1 mg/m^3. The results of these metal studies and their potential significance are elaborated on further in later sections (e.g., Section 7.4.4.1) of this chapter.

7.3.4 Acid Aerosols

Extensive earlier studies (conducted up to the early 1990s) 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. 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 μg/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 7-9. For example, Frampton et al. (1992) found that acid aerosol exposure in humans (1000 μg/m^3 H_2SO_4 for 2 h) 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 for 1 h via facemask to 500 μg/m^3 of simulated acid fog containing H_2SO_4 or ammonium sulfate aerosol.

Zelikoff et al. (1997) compared the responses of rabbits and humans exposed for 3 h to similar concentrations (i.e., 1000 μg/m^3) of H_2SO_4 aerosol. For both rabbits and humans, there was no evidence of PMA 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. Superoxide production by macrophages was somewhat depressed in both species. Macrophage phagocytic activity was also slightly reduced in rabbits but not in humans.

Ohtsuka et al. (2000a,b) also showed 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 caused decreased phagocytic activity of alveolar macrophages, even in the absence of lung injury. However, in another study, Lee et al. (1999) found little effect on female rats or guinea pigs of an inhalation exposure for 4 h to very high concentrations (43 or 94 mg/m^3) of H_2SO_4 aerosol.

In another study, Heyder et al. (1999) exposed healthy beagle dogs by inhalation to 1.5 mg/m^3 of acidic neutral sulfate aerosol for 16.5 h/day for 13 mos or to acidic sulfate aerosol at 5.7 mg/m^3 for 6 h/day for 13 mos. Interestingly, such chronic exposure to particle-associated hydrogen and sulfur ions at very high concentrations resulted in only some subtle respiratory responses, but no evident lung pathology.

Cassee and colleagues, in reports for the National Institute of Public Health and the Environment, Bilthoven, The Netherlands (Cassee et al., 1998a,b,c), have examined the effects of sulfate and nitrate aerosols in several compromised animal models. They used mice sensitized to OA as a model of allergic asthma and compared them to normal mice. One study (Cassee et al., 1998a) used exposures consisting of either fine ammonium bisulfate at 78 μg/m^3

TABLE 7-9. RESPIRATORY EFFECTS OF ACID AEROSOLS IN HUMANS AND LABORATORY ANIMALS [a]

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 ys old	H_2SO_4 aerosol NaCl (control)	Inhalation	1000 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)
Rabbits, New Zealand white Humans, healthy nonsmokers; 10 M, 21-37 ys old	H_2SO_4	Inhalation	1000 µg/m^3	0.8 µm σ_g 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)
Rats, female, F-344; Guinea Pigs, female, Hartley	H_2SO_4 aerosol	Inhalation	94 mg/m^3 43 mg/m^3	0.80 σ_g 1.89 0.93 σ_g 2.11	4 h	Acid aerosol increased surfactant film compressibility in guinea pigs.	Lee et al. (1999)
Dogs, beagle, healthy; n = 16	Neutral sulfite aerosol	Inhalation	1.5 mg/m^3	1.0 µm MMAD σ_g = 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 σ_g = 2.0	6 h/day for 13 mo		

[a] H_2SO_4 = Sulfuric acid
BAL = Bronchoalveolar lavage
LDH = Lactate dehydrogenase
MMAD = Mass median aerodynamic diameter
MMD = Mass median diameter
σ_g = Geometric standard deviation

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(0.53 μm MMD) or 972 μg/m^3 (0.45 μm MMD), or ultra fine particles at 235 μg/m^3 (0.085 μm MMD), for exposure periods of 4 h/day for 3 consecutive days. Animals were analyzed at 1 or 4 days PE for various cellular, biochemical, and immunological endpoints. No changes were seen in BALF NAF, LDH, or protein with any of the particles. Only small changes were seen in the cytokines IL-4, IL-6 and TNF-α, which were not considered relevant. Additionally there were no treatment related effects on lung histopathology or serum IgE levels. The authors conclude that ammonium bisulfate exerts only marginal responses in this compromised model, and suggest that the finding of increases in asthma in epidemiological studies is not due to this component of PM.

A second study by this group (Cassee et al, 1998b) using the same exposure regimen, but with ammonium ferrosulfate at a concentration of 250 μg/m^3 (0.459 μm MMD), found only marginal changes in TNF-α and cell differential, which were not significant. A third study (Cassee et al., 1998c), again using the asthmatic mouse model, assessed exposures of 140 μg/m^3 (0.584 μm MMD) or 250 μg/m^3 (0.219 μm MMD) ammonium nitrate aerosols. This particulate differed from the sulfates in that it caused increases in BAL NAG with exposure to the smaller particle, and increased neutrophils with exposure to the larger particle. Other parameters showed no exposure-related effects. The authors state that, as the effects were mostly seen after exposure to the fine, rather than ultrafine, particle, both mass concentration and specific size of the particles determine adverse effects.

Subsequent studies (Cassee et al., 2002) utilized an animal model of pulmonary hypertension, MCT treatment, to study the effects of ammonium bisulfate exposure. Exposures lasting 4 h/day for 3 consecutive days used concentrations of 70 μg/m^3 (0.070 μm MMD), 275 μg/m^3 (0.565 μm MMD), 344 μg/m^3 (0.107 μm MMD), or 407 μg/m^3 (0.633 μm MMD) and animals were examined at 1 d PE. The same endpoints were assayed in the three previous studies in addition to phagocytic activity. As with the asthma model, no significant exposure-related effects were seen with any of the particles.

Schlesinger and Cassee (2003) have reviewed the literature on nitrate and sulfate secondary inorganic particles. They concluded that in healthy humans and animals, and in the limited number of compromised animal models studied, exposure to environmentally relevant levels of these particles has little biological potency. They also discuss the chemical basis of toxicity of these secondary inorganic particles. They state that acidic particles, upon contact

with epithelial lining fluid (ELF), can be neutralized by the endogenous ammonia present. Additionally, the mucus lining the airway buffers the acidic particles. This neutralization and buffering modulate the effect of the particles, but the capacity of these systems may be reduced in compromised individuals (Holma, 1989). Sarangapani and Wexler (1996) have modeled this defense system and predict greater neutralization for small particles (< 0.1 μm) than for larger particles (> 1.0 μm).

Schlesinger and Cassee (2003) state that the available data indicate an acute exposure of > 1000 $\mu g/m^3$ is necessary to affect pulmonary function in healthy humans. The dosage for adverse effects in asthmatics is 68-100 $\mu g/m^3$, though the data are inconsistent. Transient effects on mucociliary clearance are seen at sulfuric acid aerosol concentrations of 100 $\mu g/m^3$, with no differences observed between asthmatics and normal individuals. Their evaluation of chronic exposure studies shows that concentrations of 100 to 250 $\mu g/m^3$ elicit changes in secretory cell function, mucociliary clearance, and nonspecific airway hyperresponsiveness. The review also states that with acute exposures, effects seen are a function of C and T, and that a threshold appears to exist for both concentration and duration.

Thus, the relatively recent new studies provide little evidence indicative of acute or chronic exposures to aqueous acid aerosols contributing to acute respiratory effects or chronic lung pathology, except at much higher than current ambient concentrations.

7.3.5 Diesel Particulate Matter

Studies of controlled exposures to diesel exhaust (DE) and/or diesel particles (DPM) were previously evaluated in detail in two prior assessment documents, one by the Health Effects Institute (1995) and the other by the U.S. Environmental Protection Agency (2002). As noted in these documents, in addition to carcinogenic effects of exposure to diesel exhaust (DE), there are significant non-cancer health effects.

Acute (short-term exposure) effects in both humans and laboratory animals include eye, throat, and bronchial irritation; neurophysiological symptoms include lightheadedness and nausea; respiratory effects include cough and phlegm; and immunologic effects such as exacerbation of allergenic responses to allergens. Chronic (long-term exposure) effects, as determined mainly from animal studies, include a spectrum of dose-dependent inflammation and histopathological changes in lung.

The most salient findings of the EPA 2002 Health Assessment Document for Diesel Engine Exhaust noncancer health effects are first briefly recapitulated (at times verbatim) below. Then some of these findings are elaborated upon further and the results of additional new studies are discussed.

7.3.5.1 Salient Findings from U.S. EPA 2002 Diesel Document

The EPA 2002 Diesel Document (U.S. Environmental Protection Agency, 2002) indicated that acute human exposure to DE elicits subjective complaints of eye, throat, and bronchial irritation and neurophysiological symptoms including headache, lightheadedness, nausea, vomiting, and numbness and tingling of limbs. With increasing concentrations of DE, the odor is detected more rapidly and the severity of symptoms increase. Studies of occupationally-exposed workers demonstrated that there are minimal, generally not statistically significant, increases in respiratory symptoms and decreases in lung function (FVC, FEV_1, PEFR, and FEF_{25-75}) during the course of the work shift. Smokers showed greater decrements in respiratory functions and increased incidence of respiratory symptoms with DE exposure compared to non-smokers. Taken as a whole, both experimental and epidemiologic studies were not found to show any consistent pattern of acute DE exposure effects on human pulmonary function or respiratory symptoms.

On the other hand, controlled human exposure studies were found to have shown that acute exposures to DE induce airway inflammation (Rudell et al., 1990, 1994) and to cause changes in peripheral blood (Salvi et al., 1999) in healthy humans, as further elaborated on below.

As for chronic exposure effects, epidemiologic studies of chronic DE exposures which occur in occupationally-exposed workers such as bus garage workers, miners, and railroad yard workers were found to indicate an absence of excess risk of chronic respiratory disease associated with exposure. Some respiratory symptoms, (primarily cough, phlegm, or chronic bronchitis) were seen in a few studies; and two studies found statistically significant decrements in baseline pulmonary functions, though most studies did not find changes in these parameters. There was little evidence detected for adverse effects of DE on other organ systems, including the cardiovascular system. The 2002 Diesel Document cautioned that interpretation of these epidemiologic studies is difficult because of some methodological problems that include incomplete information regarding effects of potentially confounding variables (smoking and

exposure to other toxicants concurrently) and the short durations and low intensity of the exposures.

The 2002 EPA Diesel Document further noted that acute exposure of laboratory animals to DE had been shown to cause mild functional effects, but only at high concentrations ($\geq$ 6 mg/m^3 DPM) and durations (20 h/day; Pepelko et al., 1980a). However, short-term exposures to even low levels of DE were found to elicit pathophysiological effects such as accumulation of DPM in lung tissue, inflammation, AM aggregation and accumulation near the terminal bronchioles, Type II cell proliferation, and thickening of the alveolar walls adjacent to AM.

Chronic DPM exposures were found to have little effects on survival in rodents. Some evidence of reduced body weight in rats was seen with exposure concentrations of $\geq$ 1.5 mg/m^3 DPM and durations of 16 to 20 h/day, 5 days/wk for 104 to 130 wks (Heinrich et al., 1995; Nikula et al, 1995). Species-specific changes in organ weights were reported with cats having decreased lung and kidney weights with exposure and rodents having increased lung weights, lung to body-weight ratios, and heart to body-weight ratios. The LOEL for these effects in rats was 1 to 2 mg/m^3 DPM for 7h/day, 5 days/wk for 104 wks (Brightwell et al., 1986; Heinrich et al., 1986a,b).

Chronic exposures were also found to impair pulmonary function in rodents, cats, and monkeys. Parameters affected by DE exposure included lung compliance, resistance, diffusing capacity, volume and ventilatory performance. The exposure levels at which pulmonary function was affected differed among species: 1.5 and 3.5 mg/m^3 DPM in rats (Gross, 1981; Mauderly et al., 1988; McClellan et al 1986), 4.24 and 6 mg/m^3 PDM in hamsters (Vinegar et al, 1980, 1981a,b), 11.7 mg/m^3 in cats (Pepelko et al, 1980b, 1981), and 2 mg/m^3 in cynomolgus monkeys (only level tested in this species; Lewis et al, 1989). Exposures were typically 7 to 8 h/day, 5 days/wk for 104 to 130 wks and resulted in restrictive lung disease in all species except monkeys. Gross (1981) estimated that observed changes in expiratory flow rates in rats indicated a LOEL of 1.5 mg/m^3 for chronic exposures. Obstructive airway disease was evidenced in monkeys exposed chronically to 2 mg/m^3 DE. This disparity with other species tested is probably due to differences in anatomy and physiology, dose delivered, dose retained, site of deposition, and effectiveness of clearance and repair mechanisms.

Histopathological effects were also reported to have been observed with chronic DE exposures. These typically included alveolar histiocytosis, AM aggregation, tissue

inflammation, increase in PMNs, hyperplasia of bronchiolar and alveolar Type II cells, thickened alveolar septa, edema, fibrosis, emphysema, and lesions of the trachea and bronchi. These were accompanied by histochemical changes in lung including increases in lung DNA, total protein, alkaline and acid phosphatase, and glucose-6-phosphate dehydrogenase. Additionally, increased synthesis of collagen and release of inflammatory mediators have also been observed with chronic exposures. There appears to be a threshold of exposure to DPM below which these histopathologic effects are not observed. Reported no observed effect levels (NOELs) include: 0.11 to 0.35 mg/m^3 for rats (Ishinishi et al., 1986, 1988); 0.25 mg/m^3 for guinea pigs (Barnhart et al., 1981, 1982); and 2 mg/m^3 for cynomolgus monkeys (only level tested in this species; Lewis et al., 1989) for exposures of 7 to 20 h/day, 5 to 5.5 days/wk for 104 to 130 wks.

Chronic exposures to DPM were further found to have an effect on airway clearance, which in large part determines the pathological effects. Alveolar macrophages phagocytose DPM as part of a multiphasic process of clearance. Exposures of $\geq$1 mg/m^3 DPM were shown to have a detrimental effect on clearance (Wolff et al, 1987; Wolff and Gray, 1980), the net effect being focal aggregations of particle-laden AMs in the peribronchiolar and alveolar regions and also the hilar and mediastinal lymph nodes. As mentioned above, species differences exist in anatomy, physiology, rate of uptake, deposition, clearance, size of AM population, rate of influx of AM and leukocytes, and the relative efficiencies for removal of particles by the mucociliary escalator and lymphatic transport system. Any decrease in AM function of will cause a reduction in clearance. It is mostly particles that are persistently retained in the lungs that impair clearance and this occurs in F344 rats at a PM burden of 0.1 to 1 mg/g lung tissue (Health Effects Institute, 1995). Morrow (1988) estimated that AM loading of $\geq$60 μm^3 PM impairs clearance and $\geq$ 600 μm^3 causes clearance to cease. This results in agglomerated particle-laden AMs remaining in the alveolar region and particles translocating to the pulmonary interstitium.

Consistent with impairment of AM function and clearance, reduction of an animal's resistance to respiratory infection was found with exposure to DPM. This effect was seen after an acute exposure of 5 to 8 mg/m^3 for as little as 2 or 6 h. The effect is thought not to be due to direct impairment of the lymphoid or splenic immune systems. Both animal and human acute exposure data also suggest that DPM is a factor in the increasing incidence of allergic hypersensitivity. Both the nonextractable carbon core and the organic fraction of DPM were

implicated in the effect. It was noted that synergies with DPM may increase the potency of known airborne allergens, and DPM was posited to act as an adjuvant in immune responses.

Chronic DE exposures in rats lasting from birth to 28 days of age were also shown to have behavioral effects on spontaneous locomotor activity and decrements in learning in adulthood (Laurie et al, 1980). These findings were corroborated by physiological evidence of delayed neuronal maturation (Laurie and Boyes, 1980, 1981). These studies, published in the early 1980s, used exposures of 6 mg/m^3 DPM for 8 h/day, 7 days/wk. No recent studies have added to this literature. Also, based on the weight of evidence of a number of studies, essentially no effects were noted for reproductive and teratogenic effects in mice, rats, rabbits, and monkeys; in clinical chemistry and hematology in rat, cat, hamster, and monkeys; and in enzyme induction in the rat and mouse.

Key conclusions arrived at, based on the studies assessed in the U.S. EPA 2002 Health Assessment Document for Diesel Engine Exhaust, included: (1) short-term exposure to the DPM component of DE can result in allergenic inflammatory disorders of the airway; (2) acute occupational exposures to DE can cause respiratory symptoms of cough, phlegm, chest tightness and wheezing (all suggestive of an irritant mechanism) but do not generally cause pulmonary function decrements; and (3) pulmonary histopathology (principally fibrosis) and chronic inflammation are non-cancer effects seen in laboratory animals, but non-cancer effects in humans from long-term chronic exposures to DPM are not evident. Also, current knowledge indicates that the carbonaceous core of DPM is probably the causative agent of lung effects. Further, progressive impairment of AM is a factor in the extent of lung injury. Lung effects occur in response to DE exposures in several species and occur in rats at doses lower than those inducing particle overload and a tumorigenic response.

It is important to note that several DE toxicity studies cited in the EPA 2002 Diesel Document 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 clearly contributors to 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 above past assessment findings, on the whole, tend to suggest the potential importance of DPM contributing to at least some ambient PM-related toxic effects, particularly in urban micro-environments with heavy diesel traffic. The findings of some DE- or DPM-related controlled human exposure studies are elaborated on below and then are further interrelated to pertinent laboratory animal studies discussed later 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 one controlled human exposure 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 ~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 performed 18 hs after exposure was compared with a control BAL performed 3 wks 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, along with a small increase in neutrophils. These findings suggest that DE may induce mild airway inflammation in the absence of spirometric changes. Although this study generated

some potentially important information on the effect of DE exposure in humans, only one exposure level was used, the number of subjects was low, a limited range of endpoints was reported, and no comparisons to clean control exposures were provided. 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 exposures to clean air, DE, or DE with particle numbers reduced 46% by a particle filter. The engine used was a new Volvo model 1990, a six-cylinder direct-injection turbocharged diesel with an intercooler, run at a steady speed of 900 rpm during the exposures. It is hard to compare this study with others, because neither exhaust dilution ratios nor particle concentrations were reported. Concentrations of 27 to 30 ppm CO and of 2.6 to 2.7 ppm NO, however, estimated DPM concentrations likely 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 filter, effects on symptoms and lung function were not significantly reduced. A follow-up study on the usefulness of a particle filter 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 hs 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 filter 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.

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 of 300 $\mu g/m^3$ 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 ontogeny-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. Whether these effects were due to DPM or associated DE gaseous components (or both) could not be disentangled with the study design used.

Nightingale et al. (2000) reported inflammatory changes in healthy volunteers exposed to 200 $\mu g/m^3$ resuspended DPM for 2 h 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 hs 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 h 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 concern about inhalation of ambient particles by sensitive subpopulations, (Nordenhäll et al., 2001) also studied the effect of a 1 h 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 hs 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 hs after exposure, there was a significant increase in the nonspecific airway responsiveness to inhaled methacholine. Although the DPM 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 IL-6 increase seen here 6 hs after DE exposure in asthmatic subjects parallels similar significant IL-6 increases in sputum 6 hs 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 (see Section 7.4).

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 DE (containing 300 mg/m^3 DPM) for 1 h on two separate occasions at least 3 wks apart. Nasal lavage fluid and blood samples were collected prior to, immediately after, and 5.5 h postexposure. Bronchoscopy was performed 6 h after the end of DE exposure. Nasal lavage ascorbic acid concentration increased 10-fold during DE exposure, but returned to basal levels 5.5 h postexposure. 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 DE may help prevent further oxidant stress in the upper respiratory tract of healthy individuals.

Seagrave et al. (2002) evaluated the inflammation and cytotoxicity created by exposure to exhaust from a number of vehicles including automobiles, SUVs and pickup trucks from 1976 to 2000. Both PM and semivolatile organic compound (SVOC) fractions were collected, both at room temperature and in a cold environment. The PM and SVOC fractions were recombined and tested for toxicity in male F344/CrlBR rats at age ~11 wks. The emission samples were intratracheally instilled at doses of 0.1 to 3 mg/rat. BAL was collected at 4 h for cytokine endpoints and collected at 24 h for examination of histopathology and lavage parameters. Three different assays, histopathology, LDH and protein, were used to determine the cytotoxicity of the emission samples. Total protein in BAL and LDH similarly ranked cytotoxicity of the samples, and histology results created similar rankings, except for gasoline, which was ranked least toxic by LDH and protein and fourth by histopathology. The authors uniformly scaled the potencies and ranked the cytotoxicity as: gasoline engine emitting white smoke > gasoline engine emitting black smoke > high emitter diesel > normal diesel 72 °F > current diesel at 30 °F > normal

gasoline 30 °F > normal gasoline 72 °F. Inflammatory endpoints examined were total leukocytes, macrophages, PMNs/mL BALF , MIP-2, TNF-α, and histopathology. There was good agreement among data for total leukocytes, PMNs, and macrophages for which the three highest emissions were ranked: gasoline engine emitting white smoke > gasoline engine emitting black smoke = high emitter diesel. These three exhausts as had equally high inflammatory effects (as indicated by increases in MIP-2, but were less consistent for effects on TNF-α, it being suppressed in some samples and slightly increased in others. Uniformly scaled potencies for inflammation using total leukocytes, PMA, macrophages, histopathology, and MIP-2 endpoints were: gasoline engine emitting white smoke > gasoline engine emitting black smoke > high emitter diesel > current diesel at 30 °F > normal gasoline 72 °F > normal gasoline 30 °F > normal diesel 72 °F.

7.3.6 Ambient 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. 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, e.g., 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 (LPS) that comprise portions of cell walls of Gram-negative bacteria; (6) (1→3)-β-D-glucan, a polyglucose compound in the cell walls of Gram-positive bacteria, fungi, and plants; and (7) viruses.

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. 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 — thus creating potentially serious health problems for sensitive human populations.

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.

Appendix 7B recapitulates a number of key points regarding ambient bioaerosols derived from the 1996 PM AQCD and goes on to update and integrate information derived from newer studies, as well. This includes background information on types and sources of ambient bioaerosols, factors affecting their dispersal and airborne concentrations, and both epidemiologic and toxicologic evaluations of health effects associated with different classes of them. As such some of the materials discussed may have been touched on in other chapters, but are brought together in Appendix 7B and summarized here to provide a coherent overall picture related to bioaerosols as potentially important contributors to ambient PM-related health effects.

A large number of studies show relationships 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 having domestic animals/ pets (Wheatley and Platts-Mills, 1996).

Bioaerosols mainly tend to be in the coarser fraction of ambient PM, but some (e.g., fungal spores, pollen fragments) are in the fine fraction as well. Flowering plants, trees, and grasses produce pollen, the species and quantity being determined by region, season, and meteorological factors (especially humidity/moisture levels). For example, increased levels of grass pollen allergens following thunderstorms have been linked to increased levels of asthma attacks i.e., "thunderstorm asthma" (Bellomo et al., 1992; Ong, 1994; Rosas et al., 1998; Schäppi et al., 1999). Wind-pollinated plants produce large grains >10-20 μm, which when intact, deposit in upper airways, inducing allergic rhinitis. However, rupture of these grains following rain events generates allergen-containing cytoplasmic pollen fragments that constitute respirable particles (~0.1 to 5.0 μm) associated with exacerbation of asthma.

Very importantly, it is now known that interactions between aerosolized allergen-laden pollen debris and other types of ambient airborne particles occur. 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−negative and Gram-positive bacteria; endotoxin, fungi) have been shown to be associated with grass and tree pollens in Poland (Spiewak, 1996a,b). Also, Taylor et al. (2002) suggest that the polycyclic hydrocarbon component of diesel exhaust may interact with allergen-laden pollen debris in a synergistic combination to explain, in part, the notable increase in the prevalence of pollen-induced asthma during the past 50 ys. Ormstad et al. (1998) and Knox et al. (1997) demonstrated that DPM (especially < 2.5 μm) can act as a carrier for plant (and animal) allergens and, further, may act as a mechanism whereby plant allergens can become concentrated in air and trigger asthma attacks. Additionally, evidence from Behrendt et al. (1992, 1995, 1997, 2001) show that pollen grains may incorporate other atmospheric pollutants that alter the pollen surface, leading to exocytosis of proteinaceous material and increased allergen release. As for health-related studies of pollen effects (see Table 7B-2), Hastie and Peters (2001) evaluated in vivo ragweed allergen exposure (via bronchoscopic segmented ragweed challenge) effects on ciliary activity of bronchial epithelial cells harvested 24 h after challenge in nonallergic human adults and in allergic subjects with severe inflammatory response. Allergic subjects with mild inflammatory changes showed slight increases in albumin and doubling of bronchoalveolar cell levels whereas allergic subjects with severe inflammatory changes showed a 12-fold increase in albumin and a 9-fold increase in bronchoalveolar cell levels.

In another study of mice, a mixture of DPM and Japanese cedar pollen caused increased IgE and IL-4 production compared to pollen alone (Fujimaki et al., 1994). Synergistic relationships were also observed with DPM and ragweed allergen in the production of specific cytokines (Diaz-Sanchez et al., 1997).

In an epidemiologic study in The Netherlands, Brunekreef et al. (2000) found a positive correlation between mortality rates and pollen concentration, suggesting that pollen-associated acute exacerbation of allergic inflammation may cause death among some compromised individuals. Increases in hospitalizations for asthma have also been correlated with pollen exposure in Mexico City (Rosas et al., 1998) and London (Celenza et al., 1996), as have increased asthma incidence and medication use (Delfino et al., 1996, 1997).

In summary, newly available information indicates release of allergen-laden material from pollen-spores in respirable-sized aerosols; 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; and indicates that pollen itself may act as a carrier for other allergenic materials. Thus, new information about the synergistic relationship between plant allergens and other forms of PM suggest a possible mechanism which may explain, in part, the increased morbidity (especially asthma) and mortality associated with increased pollen levels.

In addition to pollen, other plant-related bioaerosols are generated by human activities such as the storage, handling and transport of plant material, and they, too cause adverse health effects. A growing database suggests that plant debris is a significant contributor to organic aerosols at continental sites. This debris has a considerable component that is insoluble. Humic-like substances (HULIS), originating from biomass fires and secondary atmospheric reactions, comprise up to 24% of organic carbon in some aerosol samples. In many areas in the western U.S. there are episodic or seasonal increases in plant-derived bioaerosol material from biomass burning emissions. These controlled agricultural burns, forest fires and domestic wood burning all contribute to ambient PM in these regions.

Fungi, growing on dead organic matter, are ubiquitous and produce huge quantities of aerosols including spores, body fragments, and fragments of decomposed substrate material. Fungal spores, ranging in size from 1.5 μm to > 100 μm, form the largest and most consistently present component of outdoor bioaerosols. These cause allergic rhinitis and asthma, while allergic fungal sinusitis and allergic bronchopulmonary mycoses are caused by fungi colonizing thick mucous in the sinuses or lungs of allergic individuals. Yang and Johanning (2002) have shown that, once an individual is sensitized to the fungi, small concentrations can trigger an asthma attack or other allergic response.

Several studies have found relationships between exposure to fungi and their byproducts in respiratory illnesses and immune pathology (Hodgson et al., 1998; Tuomi et al., 2000; Yang and Johanning, 2002). Larsen et al. (1996) showed non-immunological histamine release from leukocytes exposed to a suspension of fungal spores and hyphal fragments. Some fungal byproducts have also 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 (Piecková and Kunová, 2002; Yang and Johanning, 2002).

Fungal concentrations in most parts of the world have a pattern of peak levels in the summer and early fall, but low levels in the winter months. Outdoor air fungal composition affects culturable fungal propagules indoors, but it appears that the levels of fungi inside do not just reflect the outdoor levels. Analogous to pollen exposures, fungal spore exposures have been positively correlated with asthma hospital admissions of children in Mexico City (Rosas et al., 1998) and with asthma deaths in Chicago (Targonski et al., 1995), airborne fungal concentrations of $\geq$1000 spores/m^3 were reportedly associated with asthma deaths among 5 to 34 y olds in Chicago between 1985 and 1989 (Targonski et al., 1995). The odds of death occurring on days with airborne fungal concentrations of $\geq$1000 spores/m^3 were 2.16 times higher than other days.

All classes of animals including humans, house pets, wild and domesticated birds, and insects produce bioaerosols capable of producing hypersensitivity diseases. Dust mites and cockroaches are prolific insects from which fecal material and shed body parts create allergens that are a major causes of sensitization in children (Burge, 1995).

Bacteria and viruses are infectious agents that are released from hosts in droplets exhaled from the respiratory tract. The antigenic component of bacteria can be the whole living bacteria or enzymes or cell wall components of the bacteria. Viruses, composed of either DNA or RNA surrounded by a protein coat, utilize living cells for reproduction. Virus are extremely small ($<<$1 µm), but the infectious droplets are usually larger (1 to 10 µm).

Endotoxins are present in the outer cell membrane of gram-negative (Gram -) bacteria. Heederik et al. (2000) noted 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.

Endotoxins act on cells in the respiratory system by binding to receptors and triggering production of cytokines, which initiates a cascade of inflammation, smooth muscle constriction, and vasodilation (Young et al., 1998). Table 7B-3 summarizes studies of the respiratory effects of inhaled endotoxin-laden ambient bioaerosols. Some new occupational exposure studies

suggest declines in lung function due to exposure to endotoxins in pig farm waste (Vogelzang et al., 1998) and potato processing (Zock et al., 1998). Also, increases in BAL lymphocytes were observed in life guards exposed to endotoxins at a swimming pool (Rose et al., 1998). However, these studies do not rule out the effects of other agents in the complex airborne organic aerosols that may contribute to the functional and cellular effects observed. Still, 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.

Two German cities 80 km apart with a differing prevalence of hay fever and allergic sensitization in children were studied for the possible effects of endotoxin (Heinrich et al., 2002a,b), but the researchers could not attribute observed differences between the towns in respiratory disease prevalence to endotoxin levels. Later work by this group showed higher concentration and absolute mass of endotoxin in coarse-mode particles versus fine particles. Levels of endotoxin were also seasonal, demonstrating increased levels in late spring/summer and lower levels in winter.

Dose-response studies in healthy human adults exposed to doses ranging up to 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). Inhalation of 5 or 50 µg of LPS, but not 0.0 or 0.5 µg increased PMNs in blood and sputum. Another controlled human exposure study of endotoxin, involving inhalation of lipopolysaccharide (LPS: the purified derivative of endotoxin) by known smokers showed increases in myeloperoxidase and eosinophilic cationic protein, decreases in FEV_1 and FVC, and irritation, dry cough, breathlessness, and tiredness at a LPS dose of 40 µg (Thorn and Rylander, 1998a).

Lastly, Monn and Becker (1999) also 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.

Certain gram-positive bacteria, fungi, and plants contain the polyglucose compound (1→3)-β-D-glucan in their cells walls, which has been shown to induce stimulation of the reticulo-endothelial system, activation of PMNs, AMs, and complement. Heederik et al. (2000) found T-lymphocyte activation and proliferation with glucan exposure of experimental animals. In homes in Sweden where (1→3)-β-D-glucan levels ranged between 0 and 19 ng/m^3, Thorn and

Rylander (1998b) found that there was a significantly larger number of atopic subjects in the > 65 y old group exposed to > 3 ng/m^3 (1→3)-β-D-glucan in their homes. Rylander (1996) also found that an acute exposure to (1 → 3)-β-D-glucan can produce symptoms of airway inflammation in normal human 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.

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. As indicated by linear regression analysis, 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. The association was strongest for atopic children with asthma.

Thus, new research is focusing on the bioaerosol component of ambient PM. Findings include a greater-than-realized impact of pollen on asthma, and synergistic associations between pollen and other PM, with the potential for increased risk of adverse health effects. Fungi, and especially the spores, are the largest component of outdoor bioaerosols and have been linked to allergic rhinitis, asthma, sinusitis, and allergic bronchopulmonary mycoses. Much research is being directed at characterizing the mechanism by which bacterial endotoxins and (1→3)-β-D-glucan cause adverse health effects, especially in compromised individuals.

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 the spring and 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; but endotoxin concentrations are typically very low, rarely exceeding 0.5 mg/m^3.

7.3.7 Summary of Respiratory Effects

The respiratory effects of PM having varying physical and chemical characteristics have been extensively studied for more than 30 years using a wide range of techniques and with exposure durations ranging from brief periods to months. The most extensively studied materials have been sulfates and acid aerosols formed as secondary pollutants in the atmosphere. Fly ash from coal-fired power plants or other coal-combustion sources has been less extensively studied. The toxicological data available today provide little basis for concluding that these specific PM constituents have substantial respiratory effects at current ambient levels of exposure. Recently, ROFA, a very specific kind of PM, has been studied extensively and found to produce a range of respiratory effects, especially lung inflammation.

Recent studies evaluating controlled human exposures to concentrated ambient particles (CAPs) from diverse locations (e.g., Boston, New York City , Los Angeles, Toronto, and Chapel Hill, NC) have found little or no effects on pulmonary function or respiratory symptoms in healthy human adults acutely exposed (for 2 h) to CAPs concentrations that ranged from about 25 up to about 300 µg/m^3. Some indications of mild lung inflammation were reported with such exposures in some of the studies, but not others. Analogous controlled exposures to CAPs of rats, hamsters, and dogs at concentrations varying across a range of ~100 to 1000 µg/m^3 for 1-6 h/day for 1 to 3 days yielded similar minimal effects on respiratory functions, but some signs of mild inflammation in normal healthy animals and somewhat enhanced indications of lung inflammation in at least one compromised animal model of chronic bronchitis. Another study found some indications of mild impairment of lung immune defense functions and exacerbation of bacterial infection with an acute (3 h) exposure of rats to New York City CAPs (at 100-350 µg/m^3). There is also new evidence for the transition metal components of ROFA and ambient PM from diverse locations having a mediating role in producing injury.

There still remains, however, a critical need for the systematic conduct of studies of the potential respiratory effects of major components of PM from different regions of the U.S., in recognition that PM of different composition and from different sources can vary markedly in its potency for producing different respiratory effects.

7.4 CARDIOVASCULAR AND RESPIRATORY PATHOPHYSIOLOGY AND TOXICITY: IN VITRO PM EXPOSURES

7.4.1 Introduction

Toxicological studies play an important 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 associations between morbidity and mortality and ambient airborne PM observed in human populations studies. One of the difficulties in trying to sort out possible mechanisms is the nature of ambient PM mixes. Ambient PM has diverse physicochemical properties, 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 difficult to duplicate actual properties of ambient PM in a controlled experiment. As a result, controlled exposure studies have as yet neither clearly identified those particle properties nor specific mechanisms by which ambient PM may affect biological systems. However, new in vitro toxicologic studies that have become available since the 1996 PM AQCD have provided additional information useful to help explain how ambient particles may exert toxic effects on the respiratory and cardiovascular systems. Such studies are summarized in Tables 7-10 (ambient PM) and 7-11 (ROFA and other combustion source PM) and are discussed in the next several subsections.

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. For example, 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. Also, alveolar macrophage cells from humans, rats, or other species have been employed in vitro to evaluate effects on phayocytosis and various other aspects of lung defense mechanisms. Limitations of in vitro studies include possible alterations in physiochemical characteristics of PM because of 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 cells in vivo. Also, doses delivered in vitro, like intratracheal administration, can be

TABLE 7-10. IN VITRO EFFECTS OF AMBIENT 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
BEAS-2B Primary culture human tracheal and bronchial epithelial cells	TSP (Provo, Utah)	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 100 - 500 µg/well	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 human tracheal and bronchial epithelial 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)
BEAS-2B	PM_{10} extract (Provo, UT)		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)
BEAS-2B	(Provo, UT) TSP soluble and insoluble extract		500 µg/mL	TSP	24 h	Water soluble fraction caused greater release of IL-8 than insoluble fraction. The effect was blocked by deferoxamine and presumably because of metals (Fe, Cu, Zn, Pb).	Ghio 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 AMs	PM_{10} extract (Provo, UT)	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)

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TABLE 7-10 (cont'd). IN VITRO EFFECTS OF AMBIENT 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 and rat AM	Four urban air particles (UAP): St. Louis; Wash, DC; Ottawa ERC–93; Dusseldorf). ROFA (Florida) MSH Vol. Ash DPM 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 MSH Vol. 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 UAP 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 y	Urban air particles (UAP): St. Louis SRM 1648; Washington, DC, SRM 1649; Ottawa, Canada, EHC-93 ROFA (Florida #6) MSH Vol. Ash	2×10^5 cells/mL	33 or 100 µg/mL	0.2 to 0.7 µm	3, 6, or 18-20 h	AM and MO phagocytosis 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)
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)
Rat AM	UAP (St. Louis) DPM	1×10^6 for TNF-α secretion; 3×10^6 cells/mL for gene egression	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 but 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)

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TABLE 7-10 (cont'd). IN VITRO EFFECTS OF AMBIENT 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 AM	PM_{10} (from Mexico City 1993); MSH Vol. Ash		10 µg/cm^2	< 10 µm	24 h	PM_{10} stimulated AMs to induce up-regulation of PDGF ∝ receptor on myofibroblasts. 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)
Human erythrocytes; mouse monocyte-macrophage cell RAW 264.7	$PM_{10\text{-}2.5}$; $PM_{2.5}$ (Rome, Italy)	1×10^6 cells/mL Raw cells	5 doses across range of 0 to 80 µg/mL saline solution	$PM_{2.5}$ $PM_{10\text{-}2.5}$	1 h for hemolysis 24 h for oxidative stress	Increased hemolysis of erythrocytes linearly related to $PM_{2.5}$ doses across 0 to 80 µg/mL range, but not to $PM_{10\text{-}25}$ below 50 µg/mL. However, little difference seen between $PM_{2.5}$ and $PM_{10\text{-}2.5}$ effects based on surface per volume unit of suspension, suggesting that oxidative stress on cell membranes is related to PM surface area. $PM_{2.5}$ also caused dose-dependent decrease in viability and increased markers of inflammation in RAW 264.7 cells, but significance levels not reported..	Diociaiuti et al. (2001)
Peripheral blood monocytes	Organic extract of TSP, Italy	1×10^4 cells/mL	5, 10, 21, 42, 85, 340 µg	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)
ØX174 RF1 DNA	PM_{10} (Edinborough, 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)
Supercoiled DNA	PM_{10} from Edinborough, 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)

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TABLE 7-10 (cont'd). IN VITRO EFFECTS OF AMBIENT 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 from smokers (mean age 68) and non-smokers (mean age 72), male and female	EHC-93 (Ottawa) ROFA latex beads carbon particles	0.5×10^6 cells/mL	0.01-0.1 mg/mL	< 10 µm 0.1,1, and 10 µm	2, 4, 8, 12, and 24 h (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 increased 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 (Ottawa)	0.5×10^6 cells/mL	0.01-0.1 mg/mL	4-5 µm mass median diameter	2, 4, 8, 12, and 24 h (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	$PM_{2.5}$ (Boston) Indoor and outdoor	1×10^6 cells/mL	100 µg/mL	< 2.5 µm	20 h	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	Boston CAPS, separated in soluble/ insoluble fractions SRM1649, iron oxide, carbon black, diesel dust	2.4×10^6 cells/mL	100 µg/mL	Fe, CV and DD all < 1 µm, UAP was 30% larger	20 h	Priming enhanced AM release of TNF and MIP-2 in response to UAP and some CAPs samples. Other CAPs and CB, DD, Fe 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)
Mouse AM	Boston CAPs	1×10^6 cells/mL	~5-120 µg/mL	≤ 2.5 µm	5 h	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 40%.	Ning et al. (2000)
Rat AM	Switzerland PM collected during the four seasons.	4×10^5/mL		< 10 µm	40 h	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)

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TABLE 7-10 (cont'd). IN VITRO EFFECTS OF AMBIENT 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 PMN	Aqueous and organic extracts of TSP (from Dusseldorf and Duisburg, Germany)	1×10^6 cells/mL	0.51 and 0.78 mg/mL (aqueous extracts) 0.42 – 0.65 mg/mL (organic extracts)	Collected by high volume sampler, 90% < 5 µm, 50% < 1µm, max at 0.3-0.45 µm Water and dichloromethane used to yield aqueous and organic extracts	Up to 35 min	PM aqueous extract significantly stimulated the production and release of ROS at 0.42 mg/mL 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 not affected.	Hitzfeld et al. (1997)
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)
Human AMs	Chapel Hill PM extract; both H_20 soluble(s) and insoluble(is)	2×10^6 cells/mL	100 µg/mL	$PM_{2.5}$ $PM_{10\text{-}2.5}$	24 h	Increased cytokine production (IL-6, TNF-α, MCP-1); isPM_{10} > sPM_{10} > is$PM_{2.5}$; s$PM_{2.5}$ was inactive; endotoxin was partially responsible.	Soukup and Becker (2001)
Human AM from healthy males and females, age 20-35 CHO expressing CD14 and TLR2 or TLR4	EHC-93 (Ott) MSH Vol. Ash ROFA (Niagra, NY) silica PM bacteria from Chapel Hill, NC ambient air	$2\text{-}3 \times 10^5$ cells/mL	PM - 30 µg/mL; bacteria - 10^3 - 2×10^6/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 $PM_{10\text{-}2.5}$ and gram- induced activation. TLR2 activation was induced by both gram + and - bacteria and by PM.	Becker et al. (2002)
Human AM	Urban PM (Netherlands)	3×10^5 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 $PM_{2.5\text{-}10}$ were 10x higher than $PM_{0.1\text{-}2.5}$. Levels induced by $PM_{0.1\text{-}2.5}$ were 2-3× higher than $PM_{<0.1}$. Induction was inhibited by antibody to CD14Phagaocytosis of osonized yeast and yeast-induced oxidative burst inhibited by larger PM. Larger PM decreased CD11b expression more.	Becker et al. (2003)

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TABLE 7-10 (cont'd). IN VITRO EFFECTS OF AMBIENT 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 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	24 h	S.E. Mexico City PM_{10} had most endotoxins and induced the most TNF-α and IL-6 at all doses. Cytokine release was reduced 50-75% by rENPNorthern PMs most cytotoxic.	Osornio-Vargas, et al. (2003)
Mouse monocyte-macrophage cell line RAW 264.7	PM from Taiwan		40 µg/mL	< 2.5 µm 2.5-10 µm	16 h	$PM_{2.5-10}$ had greater endotoxin content and TNF-α 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 cells; A549 = Human lung epithelial cell line; BEAS - 2B = human airway epithelial cell line; AM = Alveolar macrophage.

[b] DEF = Deferoxamine
CAP = Concentrated air particles
UAP = Urban ambient PM
TSP = Total suspended particles
ROFA = Residual oil fly ash
DOFA = Domestic oil fly ash
CFA = Coal fly ash
PFA = Pulverized fuel ash
DPM = Diesel particulate matter
VO = Vanadate oxide
TiO_2 = Titanium oxide
DPM = Diesel particulate matter

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TABLE 7-11. IN VITRO EFFECTS OF ROFA AND OTHER COMBUSTION-SOURCE PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc. [a]	Particle or Constituent [b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Rat AM	ROFA (Florida), 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 (Florida)		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 (Florida)		100 µg/mL	3.6 µm	5 min – 1 h	Lactoferrin binding with PM metal occurred within 5 min and $Fe^{(III)}$, but not Ni, increased the concentration of lactoferrin receptor.	Ghio et al. (1999b)
NHBE cells	ROFA (Florida)		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)
Primary cultures of RTE	ROFA; (Florida) 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)
Hamster AM	ROFA or CAPs (Boston)	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)

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TABLE 7-11 (cont'd). IN VITRO EFFECTS OF ROFA AND OTHER COMBUSTION-SOURCE PARTICULATE MATTER CONSTITUENTS

Species, Cell type, etc. [a]	Particle or Constituent [b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Hamster AM Mouse AM	ROFA, CAPs, and their water-soluble and particulate fractions (Boston)	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)
Human AM	ROFA (Florida) UAP (#1648, 1649) MSH Vol. Ash	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 MSH: 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; ROFA and UAP also affect AM phenotype: increased immune stimulatory, whereas decreased immune suppressor phenotype.	Holian et al. (1998)
BEAS-2B	ROFA (Florida, #6 LoS)		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 (Florida)	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)
Primary GPTE cells	ROFA (Florida) DOFA (Durham) STL (St. Louis) WDC (Wash., DC) OT (Ottawa) MSH Vol. Ash	$2 – 5 \times 10^5$ cells/cm^2	6.25, 12.5, 25, and 50 µg/cm^2	N/A	4, 8, and 24 h	ROFA the most toxic (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 50 µg/cm^2 for 24 h.	Jiang et al. (2000)
Human blood monocytes and neutrophils (PMN)	ROFA (Florida); CFA (Linden, NJ; Niagra, NY; Western U.S.). SRM 1649 (Dusseldorf, Eliz. City, NJ; Charlottesville); MSH Vol. 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, did not affect LCL in PMN, suggesting that metal ions are not related to induction of LCL. Effects were generally dose-dependent, with effects seen at lowest dose.	Prahalad et al. (1999)

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TABLE 7-11 (cont'd). IN VITRO EFFECTS OF ROFA AND OTHER COMBUSTION-SOURCE PARTICULATE MATTER CONSTITUENTS

Species, Cell Type, etc. [a]	Particle or Constituent [b]	Cell Count	Concentration	Particle Size	Exposure Duration	Effect of Particles	Reference
Human BEAS-2B	ROFA (Florida)		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)
Human BEAS-2B	ROFA (Florida) 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)
Human lung mucoepidermoid carcinoma cell line, NCI-H292	ROFA (Florida)	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 was 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%).	Longphere et al. (2000)
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)

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TABLE 7-11 (cont'd). IN VITRO EFFECTS OF ROFA AND OTHER COMBUSTION-SOURCE 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	ROFA (Boston), 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)
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)
Human lung epithelial (A549) cells	TiO_2, Fe_2O_3, CAP (Boston), and the fibrogenic particle α-quartz	3×10^5 cells/mL	TiO_3: 40 µg/mL; Fe_2O_3: 100 µg/mL, α-quartz: 200 µg/mL; 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)
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 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/mL 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)

[a] Cell types; RTE = Rat tracheal epithelial cells; GPTE = Guinea pig tracheal epithelial cells; NHBE = Normal human bronchial epithelial cells; A549 = Human lung epithelial cell line; BEAS-2B = human airway epithelial cell line; AM = Alveolar macrophage.

[b]
DEF = Deferoxamine
CAP = Concentrated air particles
UAP = Urban ambient PM
TSP = Total suspended particles
ROFA = Residual oil fly ash
DOFA = Domestic oil fly ash
CFA = Coal fly ash
PFA = Pulverized fuel ash
DPM = Diesel particulate matter
VO = Vanadate oxide
TiO_2 = Titanium oxide
DPM = Diesel particulate matter

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very high on a cellular basis, thus requiring much caution in attempting to extrapolate in vitro findings to in vivo exposure conditions. It would be useful if in vitro studies included, in addition to the high doses, lower doses more 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 a useful approach by which to explore potential cellular and molecular mechanisms by which PM mediates health effects, allowing mechanisms identified in vitro to be evaluated later in vivo or possibly helping to confirm mechanisms suggested by the results of in vivo studies.

The following subsection discusses the more recently published studies that provide an in vitro approach toward identifying potential mechanisms by which PM mediates cardiovascular and respiratory health effects. Based on these available data the ensuing subsection then discusses the potential mechanisms in relation to PM size or chemical characteristics.

7.4.2 Ambient Particles Effects

Newly available in vitro studies since the 1996 PM AQCD include many that 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 respiratory and/or cardiovascular pathophysiological responses seen in vivo. The ambient PM evaluated includes 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).

Because soluble metals from ROFA-like particles have been shown to be associated with pathophysiologic/toxic effects, several new studies have investigated whether the soluble components of ambient PM may exert similar effects. For example, 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 only ~0.1% of the mass, are responsible for the biological activity of the extracted PM components. These North Provo 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 human airway epithelial (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. Also, 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 such Provo-PM extracts (Ghio et al., 1999a,b).

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) with effects seen at all doses. 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).

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 at the lowest concentration of 62.5 μg/mL 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.

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 a PM concentration of 500 µg/mL. 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 at doses of 50, 100, or 200 µg/mL 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 that was present at all doses. 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 at concentrations ≥ 50 µg/mL 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 TNF-α) 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.

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 at concentrations of 0.03 µg/mL 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).

Becker and Soukup (1998) found interesting differences between biological activity of PM materials 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} at a concentration of 10 $\mu g/cm^2$ 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 $PM_{10\text{-}2.5}$ from the ambient air of Chapel Hill, NC, and compared the activity of these two particle size fractions. Both water soluble and insoluble components at concentrations of 100 μg/mL were assessed for cytokine production, inhibition of phagocytosis, and induction of apoptosis. The insoluble $PM_{10\text{-}2.5}$ 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.

Diociaiuti et al. (2001) compared the in vitro toxicity of fine ($PM_{2.5}$) and thoracic coarse ($PM_{10-2.5}$) fraction particulate materials extracted from sampling filters collected in an urban area of Rome (average 24-h concentrations of 31 and 19 $\mu g/m^3$, respectively). Cell cultures were exposed to the extracted PM materials suspended in saline at doses ranging from 0 to 80 µg/mL. The in vitro toxicity assays included evaluation of 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 either fine or coarse particles, but the hemolytic potential was greater for the fine than for the coarse particles in equal mass concentration, the fine particles being linearly related from 0 to 80 µg/mL, but the coarse ones not showing any effect below 50µg/mL. However, when data were expressed in terms of PM surface area per volume of suspension, the hemolytic activity of the fine fraction was not markedly different from that of the coarse fraction, thus suggesting that oxidative stress induced by PM on the blood 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 either 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 differences in the physicochemical nature of the particles, their noting that the possible causative agent in the fine fraction could be carbon-rich particles with an associated S acidic component.

7.4.3 Comparison of Ambient and Combustion Source-Related Particles

In vitro toxicology studies using 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 ambient air particles are much more potent for inducing cellular responses than individual combustion particles such as diesel or ROFA. Metals, on the other hand, do not seem

to affect cytokine production, consistent with studies showing that ROFA does not induce macrophage cytokine production. However, in some studies (Long et al., 2001), cytokine responses in alveolar macrophages appeared to be correlated with LPS content of the PM samples tested. These results may be important because LPS is an important component associated with both ambient coarse and fine particles (Menetrez et al., 2001).

Van Eeden et al. (2001) compared effects of EHC-93 atmospheric PM sample materials from Ottawa, ROFA, and different size latex particles on cytokine induction by human alveolar macrophages. The EHC-93 particles produced greater than 8-fold induction of various cytokines (including IL-1, TNF, GMCSF) at concentrations as low as 0.01 mg/mL. The other particles at concentrations of 0.1 mg/mL only induced these cytokines by ~2-fold. Using the same EHC-93 particles, Mukae et al. (2000, 2001) found that inhalation exposure of 0.1 mg/mL also stimulated bone marrow band cell-granulocyte precursor production and 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 collected from an oil-burning power plant in Florida, 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 20 $\mu g/cm^2$ ROFA for 24 h secreted substantially increased amounts of prostaglandins E2 and F2α. 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. Concentrations used were 5 to 200 µg/mL, with effects seen at $\geq$ 50 mg/mL ROFA. 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 Florida 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, these studies indicate that 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 certain particulate materials. Compared to normal cells, exposure of tumor necrosis factor (TNF)-α-primed A549 cells to ROFA (Boston area) 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 about 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.

Imrich et al. (2000) found that, when mouse alveolar macrophages were stimulated with CAPs ($PM_{2.5}$) at a concentration of 100 µg/mL, 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).

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 endotoxin-independent, transition metal-dependent mechanism for fine PM contributed to cytotoxicity effects, whereas an endotoxin-dependent mechanism was responsible for the proinflammatory response to 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 in some ambient PM_{10} samples during warm months may affect some types of macrophage activity.

In central Taiwan, Huang et al. (2002) collected $PM_{2.5}$ and PM_{10} samples, to which RAW 264.7 cells, a mouse monocyte-macrophage cell line, were then exposed at 40 µg/mL. After 6 h exposure, either with or without polymyxin B, TNF-α levels were assayed. Higher TNF-α secretion was stimulated by PM_{10}-exposed cells; and polymyxin B inhibited TNF-α by 42% and 32% in PM_{10} and $PM_{2.5}$ exposures, respectively, suggesting that endotoxin may contribute more to TNF-α stimulation by coarse than fine fraction 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-positive bacteria were required to elicit the same level of cytokine induction as gram-negative 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 likely bacterial components as a factor contributing to PM-induced AM inflammatory responses.

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 as contributing to ambient PM (especially coarse thoracic $PM_{10-2.5}$) effects on some types of AM functions.

7.4.4 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 likely underlying PM effects on 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.

7.4.4.1 Reactive Oxygen Species

These metals are capable of catalyzing the production of reactive free radicals such as the hydroxyl (·OH) radical through the following reaction:

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{3+} \cdot OH + HO^- \quad (1)$$

It should be noted that the actual reaction is more complex than given above and is commonly referred to as the iron-catalyzed Haber-Weiss Reaction or Fenton's Reaction. Fe^{3+} produced in the above reaction can be reduced to Fe^{2+} by reactions such as:

$$Fe^{3+} + O_2^{\cdot -} \rightarrow Fe^{2+} + O_2 \quad (2)$$

where $O_2^{\cdot -}$ is the superoxide radical. Hydrogen peroxide is formed by

$$2\ O_2^{\cdot -} + 2\ H^+ \rightarrow H_2O_2 + O_2 \quad (3)$$

This reaction is catalyzed by superoxide dismutase (SOD). SODs are present as Cu-Zn SOD in cytoplasm, Mn-SOD in mitochondria and extra-cellularly as Cu SOD. Iron will participate in the above reactions and hydroxyl radicals will continue to be generated so long as there are sufficient reductants and H_2O_2 present. In addition to hydroxyl radicals, electronically excited O_2 produced in the reactions given above may also be involved in promoting cellular damage.

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; but, 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 Edinborough, 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 airborne 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 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 X-ray fluorescence (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; 2000b) 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 to 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 intensely than the less soluble form, V_2O_5, in rats. Neutrophil influx was greatest following exposure to $VOSO_4$ and lowest after 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). As(III), Ni(II), and Ce(III), major components of fly ash, did inhibit the secretion of superoxide anions (O_2^-) and hydrogen peroxide, but 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, showing that the oxidation state of metal may influence its oxidant generating properties. Other fly ash components, e.g., 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 ambient PM is responsible for PM-induced cellular responses. 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. Phagocytic cells were obtained from the segmental bronchi on both sides via a second bronchoscopy at 24 hs post-instillation. Alveolar macrophage from subjects instilled with UVD, obtained by BAL 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 was 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 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.

7.4.4.2 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 in vitro studies (Quay et al., 1998; Kennedy et al., 1998) 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). 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 contribute to the initiation phase of the inflammatory cascade following some PM exposures.

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 showed 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 urban PM or ROFA (25 µg/mL) was observed by Holian et al. (1998). In addition, both urban PM and ROFA upregulated the expression of the $RFD1^+$ (immune stimulating macrophages) AM phenotype; whereas only ROFA decreased the $RFD1^+7^+$ (suppressor macrophages) 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 urban PM and ROFA 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 the 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 7-6). 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, 1000 μg/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, mitotic fraction and apoptosis, release of interleukin-8 (IL-8) measured by enzyme-linked immunosorbent assay, and release of alkaline phosphatase measured by enzymatic activity using paranitrophenol phosphate. Results indicated that (a) SiO_2 and Ni_3S_2 caused dose dependent acute toxicity and apototic changes; (b) ROFA and ND were acutely toxic only at the highest concentrations; (c) SiO_2 (30, 100, 300 μg/cm^2) and Ni_3S_2 (10, 30, 100, 300 μg/cm^2) increased IL-8 (three and eight times over the control, respectively) but suppressed IL-8 release at the highest concentration; (d) OAA and ROFA also induced IL-8 but to a lesser degree; and (e) 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 was OAA > LD = ND > ROFA >> SiO_2 = Ni_3S_2. These results indicate 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 μg/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 2- to 3-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-A 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.

7.4.4.3 Particle Charge and Stimulation of Sensory Nerve Receptors

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). Several early studies in the 1980s demonstrated the influence of surface charge on toxicity of particulates. Work by Heck and Costa (1983), for example, found crystalline NiS, Ni_3S_2, and NiO, all particulates with strong zeta potentials (i.e., electronegativity) to be more rapidly phagocytosed than amorphous positively-charged NiS. Additionally, they found that freshly suspended amorphous NiS were phagocytosed to a greater degree than particles aged for 1 to 7 days in water or culture medium. These data suggested to the authors that a loss of negative charge during aging is responsible for decreased phagocytosis, and correspondingly, decreased carcinogenicity. The significance of particulate aging was also shown by Vallyathan et al. (1988), who compared the effects of freshly-ground and aged silica in isolated rat AM. Freshly ground silica produced greater respiratory burst, hydrogen peroxide release, superoxide anion secretion, and cytotoxicity in AM than the aged dust. The authors suggested that this freshly fractured silica dust is responsible for the pathogenesis of acute silicosis. A similar relationship between fresh and aged particles was also observed with coal dust (Dalal et al., 1989). Both bituminous (72% carbon) and anthracite (95% carbon) coal were ground and assayed for free radical concentration by electron spin resonance spectroscopy. The freshly-ground anthracite coal had both greater concentrations of free radicals and greater free radical activity than the bituminous coal. This free radical activity correlated with toxicity of the coal dust. After several hours of being ground, the coal dust lost significant free radical activity. Further, Dalal et al. examined lung tissue from coal miners at autopsy and found free radicals, which they suggested may be available for biological effects years after deposition.

New insights with regard to the potential importance of particle charge have begun to emerge in connection with the delineation of the important role of sensory nerve receptors in the initiation of PM inflammation, as demonstrated in a series of newly available 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 from Birmingham, AL. The BEAS-2B cells responded with an immediate increase in $[Ca^{+2}]_i$ at 100 µg/mL ROFA, followed by a concentration-dependent release of inflammatory cytokines (i.e., IL-6, IL-8, TNF-α) and their transcripts at doses of 12-200 µg/mL (significance levels not given; Veronesi et al., 1999a). 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, TNF-α) 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.

Since VR1 irritant receptors were found to respond to acidity (i.e., protonic charge), experiments have been conducted to see if the surface charge carried by ROFA or other PM particles could biologically activate cells and stimulate inflammatory cytokine release. Oortgiesen et al. (2000) measured the mobility of ROFA particles in an electrically charged field (i.e., micro-electrophoresis) microscopically and calculated their zeta potential. 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 negative zeta potentials ($-29 + 0.9$ mV) similar to ROFA particles. These SPM acted as ROFA surrogates with respect to their size and surface charge, but lacked all contaminants thought to be responsible for its toxicity (e.g., transition metals, sulfates, volatile organics and biologicals). Concentrations of SPM at 18.8 µg/mL and ROFA particles from Birmingham, AL at 50 µg/mL 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 findings, a larger set of PM was obtained from urban (St. Louis, Ottawa), residential (wood stove), volcanic (Mt. St. Helens), 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 at a concentration of 10 µg/mL. 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 ($r^2 > 0.97$; Veronesi et al., 2002b).

A recent study (Agopyan et al., 2003) has evaluated the hypothesis that the mechanism by which negatively-charged PM acts on bronchial epithelial cells and sensory neurons is by activation of capsaicin-sensitive vanilloid or acid-sensing receptors. They used BEAS-2B cells, HEK293 cells expressing vanilloid receptors, and rat trigeminal ganglion neurons to which they exposed negatively charged 2 and 10 µm polystyrene carboxylate-modified particles. They found that the negatively-charged particles can activate capsazepine- and amiloride-sensitive proton-gated receptors. This activation leads to increases in intracellular Ca^{2+}, cyclic AMP, and IL-6 release. Corroborating this study, Veronesi et al. (2003) conducted similar experiments using positively- or negatively-charged synthetic polystyrene micells exposures to BEAS-2B cells. They reported increases in intracellular Ca^{2+} and IL-6, which they attributed to the negative charge on the particles. This negative charge, they hypothesize, acquires a cloud of protons which then activates the proton-sensitive vanilloid and acid-sensitive receptors.

Thus, both older and newer studies have provided evidence that both the charge and the age of the PM are important factors in its toxicity. Also, several newer studies demonstrate a plausible neurogenic basis for PM inflammation by which the positively-charged 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 is thought to result in an immediate influx of calcium and release of inflammatory cytokines and

neuropeptides, which then initiate and sustain inflammatory events in the airways via neurogenic inflammation (Veronesi and Oortgiesen, 2001).

7.4.4.4 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.

7.4.5 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 7-12).

Many studies assessed in 1996 PM AQCD, 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

TABLE 7-12. 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).

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 and found the ultra fine particles to be more effective in producing lung inflammatory responses as indexed by several markers.

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 interstitialization 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 $\mu g/m^3$), they found a 9-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.

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 4-fold 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 not solid in form. Droplets (e.g., sulfuric acid droplets) and organic-based

ultrafine particles exist in the ambient environment; and 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 shown 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; Muggenburg et al., 2000b).

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 4- to 5-fold increase in BAL PMNs. There was also a ROFA dose dependent increased mortality at 96 h postexposure.

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 wks 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 wks (Kodavanti et al., 1998b). Bacterial infection models mimic the chronic bacterial infections experienced by humans with other underlying

disease conditions. The animal models develop signs similar to those in humans but to a milder degree. To mimic the 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-wk to 22-mo old Fischer 344 rats and 14- to 17-mo old T_{sk} mice to 100 μg/m^3 of ultrafine carbon (UF) and/or 1.0 ppm O_3 for 6 hs after a 12 min 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 wks. 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 wks with air or 170 ppm SO_2 for 5 h/day and 5 days/wk (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 3-fold 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 CAPs 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 $\mu g/m^3$ 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 (~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 $\mu g/m^3$ for a 6-h exposure or 192 $\mu g/m^3$ 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 MCT-treated rats exposed for 6 h to 400 $\mu g/m^3$ New York City CAPs.

Kodavanti and colleagues (1998b) also have examined the effect of CAPs 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 $\mu g/m^3$ 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 CAPs. 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.

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 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), protein levels in BAL of both WKY and SH increased significantly, but the increase in SH rats was greater than that of the WKY rats (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) for 2 h to clean air or ROFA (< 2.5 μm) at 19-25 mg/m^3. 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 wks. 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 wks 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 µg/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 μg/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 2-fold), 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 1000 μg/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 particles (PM) can alter the immune response to challenge with specific antigens and suggest that PM may act as an adjuvant. For example, studies have shown that treatment with diesel particulate matter (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 wks 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).

Steerenberg et al. (2003) expanded on these findings using a range of PM collected for the EU study, "Respiratory Allergy and Inflammation Due to Ambient Particles." The Brown Norway rat (BN) was utilized as a pollen allergy model and the BALB/c mouse was used as an OVA allergy model. PM included were two DEP samples (DEP_I from Lovelace Respiratory Research Institute, DEP_{II} from NIST, SRM 2975), ROFA (collected in Niagra, NY), Ottawa dust (EHC-93), and road tunnel dust (RTD, collected in Noord tunnel near Hendrik-Ido Ambacht, NL). Endotoxins in the PM were below detection levels except for EHC-93 (50 ng endotoxin/mL) and RTD (1 ng endotoxin/mL). The animals were exposed to either just allergen or allergen and PM combined during the sensitization and/or challenge phases. In the BN pollen model only DEP_1 stimulated IgE and IgG response to pollen allergens. The pollen + PM rats had

fewer eosinophilic granulocytes than rats exposed to pollen alone. In the BALB/c OVA model all of the PM samples when coexposed with OVA during the sensitization phase (but not the challenge phase), created increases in IgE serum responses. Both histopathological examination of the lung and BAL analysis showed inflammatory response in the lung, predominantly due to an influx of eosinophilic granulocytes. Increases were also seen in BAL levels of IL-4. The authors ranked the adjuvant capacity of the particles tested based on the OVA model results as: RTD > ROFA > EHC-93 > DEP_1 > DEP_{II}.

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 any ambient PM 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 μg/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 μg/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 1000 µg ROFA 3 days prior to sensitization with house dust mite (HDM) antigen. HDM sensitization after 1000 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 (Fe, V, or Ni) prior to sensitization with instilled house dust mite. The three individual metals augmented different aspects of the immune response to house dust mite: Ni and V 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, Ni and V also produced an increase in the lymphocyte proliferative response to antigen in vitro; and 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 may be responsible for 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) compared the effects of inhalation of CAPs 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 at a concentration of 50 ng/mL significantly enhanced the airway hyperresponsiveness in OVA-sensitized mice; whereas exposure to CAPs (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 concentrations of ambient PM. Although airway responsiveness was examined at only one postexposure 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 ambient PM.

7.5.4 Resistance to Infectious Disease

Development of an infectious disease requires both the presence of an appropriate pathogen and 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 either to clear or to kill microbes. Rodent infectivity models have frequently been used to examine effects 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. Numerous studies that used the infectivity model to assess inhaled PM effects were assessed 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

to 750 μg/m^3 for 2 h/day for 4 days (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 μg/m^3) was found to have no effect on susceptibility to bacterial infection (Jakab, 1993); whereas TiO_2 (20,000 μg/m^3) decreased the clearance of microbes and the bacterial response of lymphocytes isolated from mediastinal lymph nodes (Gilmour et al., 1989a,b). Also, 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). Pritchard et al. (1996) observed in CD-1 mice exposed by instillation to particles (0.05 mL of a 1.0 mg/mL suspension) 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. Thus, the pulmonary defense responses to microbial agents has been altered at relatively high particle concentrations in animal models, with observed effects being highly dependent on the microbial challenge and the test animal studied.

There are a few more 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 first 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. Increases in lavage fluid protein and neutrophils were 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 also depressed more 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 ambient exposures 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 comprised of mixtures of particles of varying size and composition which co-exist in outdoor and indoor air with a number of ubiquitous 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 constituents with gaseous pollutants, as evaluated by studies summarized in Table 7-13. Toxicological interactions between PM and gaseous co-pollutants may be antagonistic, additive, or synergistic (Mauderly, 1993). The presence and nature of any interaction likely depends on chemical composition, size, concentration and ratios of pollutants in the mixture, exposure duration, and the endpoint being examined. It is difficult to predict *a priori* from the presence of certain pollutants whether any interaction will occur and, if so, 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 7-13. RESPIRATORY AND CARDIOVASCULAR EFFECTS OF PM AND GASEOUS POLLUTANT MIXTURES

Species, Gender, Strain Age, or Body Weight	Gases and PM	Exposure Technique	Exposure Concentration	Particle Size	Exposure Duration	Cardiopulmonary Effects of Inhaled PM and Gases	Reference
Humans; healthy 15 M, 10 F, 34.9±10 ys of age	CAPs + O_3 Filtered air (control)	Inhalation	150 µg/m^3 CAPs 120 ppm O_3 1.6 µg/m^3 $PM_{2.5}$ 8.5 ppb O_3	$PM_{2.5}$ $PM_{2.5}$	2 h 2h	$PM_{2.5}$ CAPs + 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. Lack of comparison between exposure to PM or O_3 alone precludes attribution of observed effects to PM or O_3 alone or to joint effect.	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 Ottawa 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 PM 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)
Rats, F344: male and female 9 wks old	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 wks	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)

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TABLE 7-13 (cont'd). RESPIRATORY AND CARDIOVASCULAR EFFECTS OF PM AND GASEOUS POLLUTANT MIXTURES

Species, Gender, Strain Age, or Body Weight	Gases and PM	Exposure Technique	Exposure Concentration	Particle Size	Exposure Duration	Cardiopulmonary Effects of Inhaled PM and Gases	Reference
Humans, children: healthy (N = 15) asthma (N = 26); Age 9-12 y.	H_2SO_4, SO_2, and O_3	Inhalation (chamber)	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	Positive association seen between acid dose and respiratory symptoms, but not spirometry, in asthmatic children. No significant changes in healthy children.	Linn et al. (1997)
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)
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, σ_g = 1.7) and ultrafine (0.06 µm, σ_g = 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 σ_g = 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)
Mice, Swiss: female, age 5 wks	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 σ_g = 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. (2000c)
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 σ_g = 2.0	4 h/day, 3 days/wk for 4 wks	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)

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TABLE 7-13 (cont'd). RESPIRATORY AND CARDIOVASCULAR EFFECTS OF PM AND GASEOUS POLLUTANT MIXTURES

Species, Gender, Strain Age, or Body Weight	Gases and PM	Exposure Technique	Exposure Concentration	Particle Size	Exposure Duration	Cardiopulmonary Effects of Inhaled PM and Gases	Reference
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)
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 µg/m^3 0.152 ± 0.003 ppm + 49.9 ±7.0 µg/m^3		4 h/d 3 d/wk 40 wk	Increased lung putrescine content in all exposed rats. Synergistic effect.	Sindhu et al. (1998)

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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.

One newly available controlled exposure study evaluated the effects of a combined inhalation exposure to $PM_{2.5}$ 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) ys of age, to filtered ambient air containing 1.6 μg/m^3 $PM_{2.5}$ and 8.5 ppb O_3 (control) or to unfiltered air containing 150 μg/m^3 $PM_{2.5}$ CAPs and 120 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). This finding suggests that combined exposure to a mixture of $PM_{2.5}$ CAPs plus O_3 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. The likelihood that analogous vasoactive responses could be found at ambient $PM_{2.5}$ and O_3 levels typically found in some U.S. urban locations is enhanced by the fact that such responses would likely have been seen at distinctly lower exposure levels had the PM and O_3 exposures occurred during light, moderate, or 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 1569 μg/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. No significant increases in airway inflammation were seen 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 μg/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 Ottawa urban ambient 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 ambient PM, although at high concentrations, are consistent with studies showing interactions 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), in extending their previous work, 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 in senescent rats. Exposures were nose-only, 4 h/day, 3 consecutive days per week for a total of 4 wks. Decreases in lung collagen, and increases in macrophage respiratory burst and phagocytosis were observed. They found small changes in macrophage function and in injury to cells of the lung parenchyma, with exposures to just carbon and ammonium bisulfate. With the addition of O_3, changes in those biological responses became significant. These results suggest that (a) ozone may enhance the toxicity of inhaled particles in terms of the above types of pathophysiologic responses and/or (b) conversely, $PM_{2.5}$ exposure may enhance O_3-induced toxicity in aged rats. 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. Bronchioalveolar epithelial permeability and cell proliferation were increased. Altered breathing patterns were also seen, but with some adaptation evident over the course of repeated O_3 exposure.

Other studies have also 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 µg/m^3 carbon particles in combination with ammonium bisulfate and O_3 for 4 h/day, 3 days/wk for 4 wks. Despite 4 wks 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 h 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 contributed to 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 $\mu g/m^3$) 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.

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.

In summary, the newly available combined (PM and gaseous co-pollutant) studies provide only relatively limited evidence for additive or interactive joint PM/gaseous pollutant effects on one or the other few health endpoints evaluated. For example, recent studies have demonstrated that co-exposures of CAPs and O_3 cause potentiation of proliferative changes in the epithelium of terminal bronchioles (Vincent et al., 1997) and enhanced septal cellularity (Bouthillier et al., 1998) seen with O_3 exposure alone. Both combined CAPs/O_3 and O_3-alone exposure in a mouse asthma model (Kobzik et al., 2001) showed increases in airway responsiveness and pulmonary resistance, thus indicating a lack of synergism with the combined exposure. Mixtures of elemental carbon particles, O_3, and ammonium bisulfate showed changes in lung collagen, AM respiratory burst, and phagocytosis (Kleinman et al, 2000), the results are ambiguous as to whether PM was enhancing the effects of O_3 or the converse. A short exposure of combined

carbon particle/ SO_2 caused depressed AM phagocytosis and suppressed intrapulmonary bactericidal activity which lasted for a week (Jakab et al., 1996; Clarke et al., 2000c). On the other hand, other studies using co-exposures of PM and gases have demonstrated no changes in histopathological (Moss et al., 2001) or biochemical and morphometric endpoints (Last and Pinkerton, 1997).

The mechanisms by which interactions between PM and gases occur is thought to be by: (1) formation of secondary products by chemical interactions between the gas and the particle, (2) adherence of material to the particle and subsequent transport to sensitive sites, and/or (3) pollutant-induced change in the local microenvironment of the lung (e.g., by decreasing the pH). All of these interactions have the potential to create antagonistic, additive, or synergistic interactions between PM and gases, which can greatly modify their individual effects.

7.7 QUANTITATIVE COMPARISONS OF EXPERIMENTAL PM EFFECTS ON CARDIOVASCULAR/RESPIRATORY ENDPOINTS IN HUMANS AND LABORATORY ANIMALS

7.7.1 Introduction

The extensive literature assessed in the foregoing sections provides considerable new information on experimentally-induced effects of various types of PM on cardiovascular and respiratory endpoints. The ensuing subsections attempt to characterize salient exposure/dose-effect relationships; including comparisons between lowest observed effect levels (LOELs) reported thus far for normal and compromised subjects. In the sections that follow, for both the cardiovascular/systemic and the respiratory effects, the LOELs derived from inhalation studies are first discussed and then those from instillation studies, followed by discussion of in vitro observed effect levels. In addition, efforts are made to delineate key factors important in attempting to extrapolate observed effects across species (rat to human) and/or to human ambient exposure conditions and to provide illustrative examples of some extrapolation modeling outcomes.

7.7.1.1 Cardiovascular and Systemic Effects of Inhaled Particulate Matter

Newly available studies examining the cardiovascular and systemic effects of inhaled PM have for the most part not carried out dose-response evaluations using multiple exposure levels in the same study. However, various types of effects reported across a wide range of concentrations for various types of PM tested at single exposure levels do allow one to gain some impressions about possible lowest-observed-effect-levels (LOELs).

Concentrated ambient particles were used in a number of studies examining cardiovascular and systemic endpoints, but none were done in a manner so as to allow clear delineation of dose-response relationships for the endpoints evaluated. Probably the lowest concentrations of U.S. ambient air observed to experimentally induce any cardiovascular effect in humans were those in the study by Ghio et al. (2000a). Increases in plasma fibrinogen were observed in healthy adult subjects exposed to Chapel Hill CAPS at concentrations in the range of 23.1 to 331.1 $\mu g/m^3$. Another very small human exposure study was also reported by Petrovic et al. (2000) to show a trend toward increased blood fibrinogen in two of four human adults exposed for 2 h to Toronto CAPs ranging up to ~125 $\mu g/m^3$. Also, in laboratory animal studies, Godleski et al. (2000) used

CAPs from the Boston area and found effects on heart rate and ECG at a CAPs dose of ~100 to 1000 μg/m^3 in some dogs. Both normal dogs and dogs compromised by coronary occlusion were reported to be affected. However, a study exposing both F344 rats and hamsters to CAPs collected in Manhattan at concentrations ranging from 132 to 919 μg/m^3 had contrasting findings (Gordon et al., 2000). That is, in hamsters and in rats, both normal and MCT-treated, there was an increase in HR and peripheral blood cell differential counts, but no other cardiovascular effects were observed. In contrast to these studies, a number of other studies have examined a wide range of cardiovascular endpoints and found no changes in cardiovascular parameters, e.g., following inhalation exposures of dogs to Boston CAPS at 3 to 360 μg/m^3 (Clarke et al., 2000a), of rats to NYC CAPs at 95 to 341 μg/m^3 (Nadziejko et al., 2002), and of humans to ultrafine carbon particles at 10 μg/m^3 (Frampton, 2001).

A number of other studies have examined cardiovascular and systemic endpoints at much higher concentrations, using UAP and ROFA. For example, two other new studies showed effects of Ottawa UAP on levels of endothelin. Bouthellier et al. (1998) found that 40 mg/m^3 Ottawa UAP in Fischer 344 rats caused an increase in plasma endothelin-1 levels without causing acute lung injury. Also, Vincent et al. (2001) found that 48 mg/m^3 Ottawa UAP caused increases in both endothelin-1 and endothelin-3 in Wistar rats, the endothelins being likely powerful cardiotoxic agents. However, the relevance of these effects of exposures to such extremely high ambient PM concentrations to evaluation of current ambient PM exposures in the U.S. is questionable.

Two studies reported arrhythmias in response to ROFA exposure. Wellenius et al. (2002) exposed healthy SD rats and rats with a model of myocardial infarction to 3 mg/m^3 Boston ROFA and found arrhythmias, ECG abnormalities, and decreases in heart rate variability in the compromised animals. Watkinson et al. (2000b) exposed healthy SD rats and rats with cold stress, O_3 pre-exposure, or MCT to 15 mg/m^3 ROFA (source not reported). They observed increased arrhythmias, decreased heart rates, and hypothermia in the compromised animals. The same concentration in SH rats caused cardiomyopathy, monocytic cell infiltration, and increased expression of cardiac cytokines IL-6 and TGF-β. The ROFA-exposed SH rats also showed increased ECG abnormalities compared to air-exposed SH rats. In another study, Muggenburg et al. (2000a) exposed beagles to 3 mg/m^3 Boston ROFA and found no effects on ECG and a

trend toward decreased heart rate, these overall results not being consistent with the Godleski et al. (2000) findings noted above.

Comparisons were made also between normotensive WKY rats and SH rats following an exposure to 15 mg/m^3 Boston ROFA (Kodavanti et al., 2002). The SH animals demonstrated increased plasma fibrinogen and small but consistent decreased total white blood cell numbers, the decrease being due mostly to decreased numbers of lymphocytes. Boston ROFA was used in another study by Kodavanti et al. (2003) at a concentration of 2, 5, or 10 mg/m^3 (for 6 h/day for 4 consecutive days) to compare cardiovascular endpoints in normal SD, WKY, and SH rats. In this exposure paradigm, no significant effects were seen for any of the rat strains. However, with a second exposure paradigm (10 mg/m^3 for 6 h/day for 16 wks) WKY rats showed cardiac lesions in the form of randomly distributed foci of fibrosis and inflammation in the ventricles and the interventricular septum. Also, normal SD and MCT-treated rats exposed to 0.58 mg/m^3 Boston ROFA showed increased expression of MIP-2, predominantly in heart macrophages, in the MCT-treated animals.

In general, in the studies noted above, lower inhalation doses of CAPs than ROFA have been found to elicit at least some cardiovascular effects. Some $PM_{2.5}$ studies have demonstrated effects (increased blood fibrinogen) at concentrations as low as ~25 to 330 µg/m^3, whereas other studies of CAPs at similar or higher concentrations did not show effects at such levels. Studies of UAP or ROFA at much higher concentrations have also reported effects, but the relevance for evaluation of health effects associated with current ambient PM levels in the United States is unclear. The lack of more data from studies completing dose-response evaluations highlights a need for more rigorous future evaluation of dose-effect relationships.

7.7.1.2 Cardiovascular and Systemic Effects of Instilled Particulate Matter

Recent studies characterizing the cardiovascular and systemic effects of instilled PM show that most effects have been seen in a concentration range of 0.7 to 9 mg/kg body weight. To better compare studies here, all reported instillation study doses were converted to mg/kg body weight.

Urban air particles (UAPs) were used in several studies that evaluated changes in heart rate, temperature, and blood parameters. Ottawa UAP at a concentration of 7 mg/kg was instilled in aged (15 mo old) SH rats (Watkinson et al., 2000a, 2000b). Effects seen at this dose

were hypothermia and bradycardia. Mukae et al. (2001) exposed female New Zealand White rabbits to 2 mg/kg Ottawa UAP and found a number of altered cardiovascular endpoints; e.g., increases in circulating PMN band cell numbers and in the size of the bone marrow mitotic pool of PMNs, as well as a shortened transit time of PMN through the postmitotic pool in marrow. Suwa et al. (2002) evaluated similar endpoints with the same UAP, using 1.6 mg/kg in female Watanable heritable hyperlipidemic rabbits. The same increases in PMN parameters were observed in this study, along with progression of atherosclerotic lesions, increases in plaque cell turnover, extracellular lipid pools, and total lipids in aortic lesions.

Dose-response evaluations were carried out in several ROFA studies. Boston or Florida was the origin of ROFA used in many of the IT studies that investigated cardiovascular and systemic effects, (though several research groups neglected to report the source of the ROFA). Arrhythmias were observed with both normal and MCT-treated rats at a concentration of 3 mg/kg of Florida ROFA (Watkinson et al., 1998). In another study by the same investigators, a concentration of 0.7 mg/kg of ROFA (origin not reported) in SH rats was shown to create significant arrhythmias, whereas 7 mg/kg induced arrhythmias in normal rats (Watkinson et al., 2000a, 2000b). One study demonstrated ECG abnormalities in SD rats compromised by either MCT-pretreatment or cold stress (10°C) at a concentration of 3 mg/kg ROFA (origin not reported). Bradycardia was seen in a number of studies at concentrations of 0.7 to 7 mg/kg ROFA. Campen et al. (2000) observed a decreased heart rate at 3 mg/kg ROFA (origin not reported) in normal SD rats and at 0.7 mg/kg in rats compromised by cold stress, O_3-pre-exposure, or MCT. Florida ROFA, tested at 7 mg/kg only, caused bradycardia in both normal SD and MCT-treated rats (Costa and Dreher, 1997). Aged SH rats displayed decreased heart rates when exposed to 1.4 mg/kg ROFA (origin not reported; Watkinson et al., 2000a).

The systemic response of hypothermia has been observed in normal SD rats and SD rats compromised by cold stress, O_3-pre-exposure, or MCT when exposed to 0.7 mg/kg ROFA (origin not reported; Campen et al., 2000). Watkinson et al. (2000b) observed the hypothermic response in compromised rats at 1.4 mg/kg and in normal SD rats at 7 mg/kg. Increases in plasma fibrinogen have been observed in normal SD rats following exposure to 8.3 mg/kg Florida ROFA (Gardner et al., 2000). However, other hemostatic parameters and cardiovascular risk factors, such as activated partial thromboplastin time, prothrombin time, plasma viscosity, and complete blood count, were unaltered by the exposure. Kodavanti et al. (2002) compared

the response to Boston ROFA at concentrations of 1 and 5 mg/kg in normal WKY and spontaneously hypertensive (SH) rats. Both strains demonstrated increased plasma fibrinogen at the 5 mg/kg concentration, while only the WKY rats showed increased hematocrit at that dose. The SH rats demonstrated decreases in blood lymphocytes and increases in blood neutrophils at 5 mg/kg. Lethality was observed in MCT-treated SD rats exposed to 3 or 7 mg/kg Florida ROFA (Costa and Dreher, 1997).

Overall, then, some cardiovascular and systemic effects of instilled ambient PM were observed at instilled doses of ~1.5 to 7 mg/kg body weight. Some effects were also apparent with exposures to ROFA in a concentration range of 0.7 to 9 mg/kg body weight. In many cases the compromised animals in these studies showed effects at lower doses than their normal counterparts.

7.7.1.3 Respiratory Effects of Inhaled Particulate Matter

The few available inhalation studies of ambient PM respiratory effects in humans have yielded consistent results in finding little or no indications of pulmonary function decrements or increased respiratory symptoms among healthy adults exposed for 2 h to CAPs from several locations (Toronto, Los Angeles, Chapel Hill, NC) at concentrations across a range of ~25 up to ~300 $\mu g/m^3$ (Ghio et al., 2000a; Petrovic et al., 2000; Gong et al., 2000; Gong et al., 2003). On the other hand, some of these studies did find indications of mild lung inflammatory responses, although some were of unclear health significance.

Relatively few laboratory animal studies have been done to examine the respiratory effects of inhaled PM, versus instillation studies. Mongrel dogs were exposed to Boston CAPs for 6 h/day for 3 days at concentrations varying from ~100 to 1000 $\mu g/m^3$ (Godleski et al., 2000). The only small effects seen were decreased respiratory rate over time and some increases in BAL neutrophils. Also, Clarke et al. (1999) exposed SD rats, both normal and SO_2-pretreated bronchitic rats to Boston CAPs for 5 h/day for 3 days at concentrations of 200, 600, and 730 $\mu g/m^3$ (mean CAPs level for each day). With 3 day exposures to those CAPs concentrations, PEF, and TV were increased in the bronchitic rats; and increased levels of BAL protein and percent neutrophils were seen in both normal and bronchitic rats. Comparing this same bronchitic model to normal SD rats, Kodavanti et al. (2000b) observed similar responses to CAPs collected in Research Triangle Park, NC. That is, at a concentration of 650 $\mu g/m^3$,

bronchitic rats had increased levels of BAL protein and neutrophils compared to CAPs-exposed normal SD rats. To test the effects on the respiratory system of combined bacterial infections and CAPs exposures, F-344 rats were exposed for 3 h to NYC CAPs at a mean concentration of 225 $\mu g/m^3$ (Zelikoff et al., 2003). The CAPs exposures had little effect on respiratory parameters when they preceded lung infection with IT-administered *Streptococcus pneumoniae*; but CAPs exposure of previously-infected rats caused reductions in basal superoxide, decreased percentages of neutrophils, and increased bacterial burdens. These CAPs studies most clearly provide indications that exposure to ambient PM from several locations for 1 to 6 h/day for 1 to 3 days at concentrations across a range of ~200 to 700 $\mu g/m^3$ can cause (a) some lung inflammation in normal and compromised animals and (b) exacerbation of preexisting respiratory infection.

In a study of combustion emission source materials, Killingsworth et al. (1997) exposed by inhalation both normal SD and MCT-treated SD rats to Boston ROFA at a concentration of 580 $\mu g/m^3$. Consequent respiratory effects included increases in neurotrophils in MCT-treated rats and increases in MIP-2 mRNA in normal SD rats. Kodavanti's group (1999, 2000b, 2002) also completed a number of studies that examined a range of endpoints using a concentration of 15 mg/m^3 of Boston or Florida ROFA. One inhalation study (Kodavanti et al., 2002) used Boston ROFA inhalation at a concentration of 15 mg/m^3 in both WKY and SH rats to compare normal and cardiovascular compromised animals. Effects seen at this concentration were increases in PMN, AM, BAL protein, LDH, and lung lesions in both rat strains. Only the WKY rats showed increased glutathione in this study. This group also completed two inhalation studies utilizing Florida ROFA, also at a concentration of 15 mg/m^3. Comparisons made of normal SD and MCT-treated rats (Kodavanti et al., 1999) showed that both the normal and MCT-treated rats display lung lesions at this dose; and both groups displayed increases in BAL protein, LDH levels, and IL-6 levels. The healthy SD rats also showed increased MIP-2 expression. A subsequent comparison of WKY and SH rats (Kodavanti et al., 2000a) showed that Florida ROFA had very similar effects on most respiratory parameters in both strains. Airway hyper-reactivity, lung lesions, AM counts, RBCs in BAL, BAL protein, BAL AM, BAL oxidants, and IL-6 all increased with ROFA exposure. As in the previous study, only the normal animals exhibited increased MIP-2 expression. Only one study of combustion source materials (Dormans et al., 1999) reported completing a dose-response evaluation. This laboratory used

doses of 0, 10, 30, and 100 mg/m^3 CFA and only observed a fibrotic reaction at 100 mg/m^3, thus confirming the relatively inert nature of CFA in comparison to ROFA.

Thus, from among the growing number of animal studies in the literature describing the respiratory effects of inhaled PM, dose-response characterizations were generally not reported. So possibilities for reliably estimating LOELs from these data or for attempting extrapolations to human exposures are limited. Probably of most pertinence, for present purposes, are (a) indications from several studies that inhalation exposures to CAPs of several species (rats, hamsters, dogs) for 1 to 6 h/day for 1 to 3 days had little or not effect on pulmonary function, but induced some signs of lung inflammation in healthy animals and enhanced inflammatory responses in chronic bronchitic rats at CAPs concentrations varying across a range of ~100 to 1000 μg/m^3 (with the inflammatory responses being most clearly shown in a range of ~200 to 700 μg/m^3); and (b) some exacerbation of respiratory infection following acute (3 h) exposure to New York City CAPs at ~225 μg/m^3. On the other hand, analogous but more intensive inflammatory responses were reported for ROFA responses at a concentration of 15 mg/m^3, and coal fly ash was not found to produce any effects until indications of fibrotic changes were seen at 100 mg/m^3.

7.2.1.4 Respiratory Effects of Instilled Particulate Matter

Recent studies characterizing respiratory effects of instilled PM indicate that most effects are observed for endpoints such as PMN, AM, protein, and LDH accumulation in BAL at a concentration range of 0.7 to 10 mg/kg body weight in rats, mice and hamsters. Changes in cytokines and oxidant formation have been seen in a similar concentration range in rats. Dose-response evaluations were carried out in about one third of these studies. To better compare studies, all concentrations from the instillation studies were converted to mg/kg body weight in the instances where researchers reported a dose per animal and an average weight for the animals.

Three studies examined the respiratory effects of instilled ambient PM collected in the Utah Valley near a steel mill that was closed during 1987. Filters were collected before, during, and after the closing and the PM was water-extracted for use in the studies. Ghio and Devlin (2001) intrabronchially instilled the Utah ambient PM in healthy humans at a concentration of 0.007 mg/kg and found increases in the cytokines IL-8, TNF, and IL-lβ following exposure to

the extracted PM collected while the plant was open. Other parameters increased by this PM exposure were fibrinogen, fibronectin, PMN, and BAL protein, and tissue factor. Dye et al. (2001) found analogous increases with this Utah ambient PM sample in male SD rats at much higher exposure levels. Exposure concentrations of 3 mg/kg increased BAL LDH, PMN, and total cells counts, whereas concentrations of 8 mg/kg increased lung lesions and airway reactivity. Sprague-Dawley rats exposed to pre- and post-closure Utah PM demonstrated increased BAL PMN and protein at a concentration of 1.8 mg/kg and increased oxidant formation at a dose of 3.6 mg/kg (Ghio et al., 1999a).

The respiratory effects of UAPs were compared to the effects of ambient PM collected from the Kuwaiti oil fires of 1991 in male Syrian golden hamsters (Brain et al., 1998). Doses of 1.5, 7.5, and 37.5 mg/kg were used; and at the lowest concentrations, increased in PMNs were observed. At a dose of 7.5 mg/kg, effects seen were increases in BAL AM, protein and LDH.

Instillation of ROFA has been used in many of the newer studies examining the respiratory effects of PM. Residual oil fly ash collected at a temperature of 250-300°C, downstream from the cyclone of a power plant in Florida burning low-sulfur residual oil was the ROFA most commonly used in the following studies. Kodavanti et al. (1997a) found increases in lung lesions, PMN, AM, MIP-2, and IL-6 following instillations of 8.3 mg/kg ROFA in male SD rats. Evaluating possible strain differences, Kodavanti et al. (1997a) found similar inflammatory cell infiltration and alveolar, airway, and interstitial thickening in SD, Wistar, and F-344 rats at the same 8.3 mg/kg ROFA concentration. Another comparison of SD and F-344 rats showed increases in neutrophils in both strains at 8.3 mg/kg (Kodavanti et al., 1996). Florida ROFA at a slightly higher concentration, 9.4 mg/kg, caused increases in airway hyper-reactivity, AM, PMN, LDH, and protein in male SD rats (Gavett et al. (1997). The same laboratory (Gavett et al., 1999) found similar effects in BALB/cJ mice at a concentration of 3 mg/kg. Kadiiska et al. (1997) exposed male SD rats to 3.3 mg/kg Florida ROFA and found increases in both PNM and protein. A dose-response study was completed by Kovavanti et al. (2001) that showed that much lower exposures to Florida ROFA could elicit the same effects. At 0.83 mg/kg there were increases in BAL protein, LDH, and PMN in both WKY and SH rats. In the same study, increases in AM were seen at 0.83 mg/kg in SH rats and at 3.3 mg/kg in WKY rats. Another rat strain, Brown Norway, was shown to have increased production of LDH at 5.8 mg/kg and increased BAL protein at 1.1 mg/kg Florida ROFA, (Lambert et al., 1999), thus demonstrating

some similarity of observed effects across rat strains. In line with these studies, Madden et al. (1999) found increased production of acetaldehyde at a concentration of 3.6 mg/kg Florida ROFA. In another study (Costa and Dreher, 1997), comparing Florida ROFA, domestic oil fly ash (DOFA), coal fly ash (CFA), and four UAPs (St. Louis, Washington DC, Dusseldorf, and Ottawa), instillations of 7 mg/kg caused increases in PMNs albumin and LDH.

One study reported on respiratory effects of diesel particles (Ghio et al., 2000c) instilled into SD rats at a concentration of 1.8 mg/kg. Effects seen included increases in BAL protein, LDH, PMN, MIP-2, TNF, and total cells. A decrease in glutathione was also observed.

Overall, the studies of respiratory effects of instilled PM materials have provided limited but interesting information. Perhaps of most importance are the observations of increased levels of inflammatory indicators in BAL samples from adult humans exposed via instillation to as little as 0.007 mg/kg (i.e., 7 μg/kg) of Utah Valley ambient PM_{10} extract obtained during the time the nearby steel mill was operating. Analogously, increased oxidant formation and BAL LDH, PNM and total cell counts were seen in rats instilled with 1.8 or 3.6 mg/kg of the pre-closure Utah ambient PM. As for other laboratory animal studies, effects in animals were observed for the commonly assayed respiratory endpoints, in a PM concentration range of 0.7 to 10 mg/kg body weight. ROFA was the most commonly used PM for instillation studies, reflecting a significant data gap for other types of PM and leaving open the question of how relevant many of the results might be for assessing ambient air PM effects at concentrations pertinent to current U.S. conditions.

7.7.1.5 In Vitro Effects of Particulate Matter on ng/Cell Dose Basis

A number of the in vitro studies described previously have reported cell numbers used in the exposures. Based on this information, it is possible to determine the actual dose applied on a per cell basis. This information is important if any comparisons are to be made across studies and, further, so that extrapolations between in vitro studies and in vivo studies may be attempted. In most of these experiments, cells are plated at 1×10^4 to 5×10^6 cells per mL of media, with an average of about a half million cells per experiment. Studies where cell counts were reported, but wherein cells were given additional time to proliferate or possibly grow until confluent, were not used to make PM/cell determinations. Researchers in most cases have carried out dose-response evaluations, so that LOELs can be determined for the endpoints studied. Overall, there

is some consistency among studies as to concentrations of PM required to elicit effects, most of which fall within a range across an order of magnitude difference between 0.02 and 0.2 ng/cell.

Urban air particles, ROFA , and CAPS have been most commonly used in these studies. In studies with exposures of both rat and human AM to UAP from St. Louis, Ottawa, Dusseldorf, and Florida, effects on cytokine production were consistently seen at doses as low as 0.02 ng/cell (Becker et al., 1996; Van Eden et all, 2001; Mukae et al., 2000). Washington DC and Boston UAP were both shown to increase TNF-α production in rat AM at doses of 0.1 ng/cell (Imrich et al., 2000). Interestingly, it appears that cytokine production is induced at slightly lower concentrations in human AM than in rat AM (Becker et al., 1996). Oxidant formation is also induced by UAP exposure in vitro. St. Louis, Ottawa, and Dusseldorf UAP all were shown to induce reactive oxygen species at doses of 0.05 to 0.5 ng/cell in human AM and blood monocytes (Becker et al., 1996; Becker and Soukup, 1998). Exposure of human AM and blood monocytes to St. Louis and Ottawa UAP inhibited phagocytosis as doses of 0.5 and 0.02 ng/cell, respectively (Becker and Soukup, 1998; Van Eeden et al., 2001). Washington DC UAP has been shown to decrease viability in rat AM at doses of 0.05 ng/cell (Nadeau et al., 1996) and 0.1 ng/cell (Imrich et al., 2000). Washington, DC UAP has also been shown to increase levels of apoptosis at 0.2 ng/cell in human AM (Holian et al., 1998) and to deplete ATP at 0.5 ng/cell in rat AM (Nadeau et al., 1996).

In vitro effects on a wide range of endpoints have been observed with ROFA from Florida and Boston. Tumor necrosis factor-α has been induced with Florida ROFA in human AM at 0.02 ng/cell (Van Eeden et al., 2001) and with Boston ROFA in mouse RAW 264.7 cells at 0.2 ng/cell. Alabama ROFA has been shown to induce IL-6 production in BEAS-2B cells at 0.08 ng/cell (Oortgeisen et al., 2000). Oxidant formation has been induced by Florida ROFA in human AM and human blood monocytes at 0.05 ng/cell (Becker et al., 1996; Becker and Soukup, 1998). A slightly higher concentration of Florida ROFA (0.15 ng/cell)was shown to induce oxidant formation in rat AM (Ghio et al., 1997a; Becker et al., 1996). Boston ROFA was found to induce both oxidant formation and inhibition of phagocytosis at 0.1 ng/cell in hamster AM (Goldsmith et at., 1997; Goldsmith et al., 1998). Florida ROFA in human lung mucoepidermoid carcinoma cells induced mucin secretion at 0.01 ng/cell and lysozyme at 0.03 ng/cell (Longphere et al., 2000). Other observed effects of Florida ROFA include increased apoptosis in human AM at 0.025 ng/cell (Holian et al., 1998), increased acetaldehyde production

in BEAS-2B cells at 0.04 ng/cell (Madden et al., 1999), and increased calcium release in BEAS-2B cells at 0.08 ng/cell (Oortgiesen et al., 2000).

Fewer studies have used in vitro exposures to CAPs. Goldsmith et al. (1998) induced oxidant formation in hamster AM with Boston CAPs at 0.08 ng/cell. This group (Goldsmith et al., 1997) also demonstrated oxidant formation and inhibition of phagocytosis in hamster AM with Boston CAPS at doses of 0.01 ng/cell. One PM_{10} study was found that reported cell numbers (Soukup and Becker, 2001). In that study, Chapel Hill PM_{10} (both the soluble and insoluble fractions) caused increased production of IL-6, TNF-α, and MCP-1 at 0.01 ng PM_{10}/cell and inhibition of phagocytosis at 0.05 ng/cell in human AMs. Long et al. (2001) reported that Boston area $PM_{2.5}$ caused increase production of TNF-α at 0.2 ng/cell. Kennedy et al. (1998) exposed BEAS-2B cells to Provo total suspended particulate (TSP) and found increased IL-6 release at 2.5 ng/cell and increased IL-8 release at 1.0 ng/cell. They also exposed human primary tracheal epithelial cells to TSP and saw effects at 0.06 ng/cell, but did not report dose-response information for these effects.

Thus, it appears that the most commonly studied in vitro endpoints have very similar LOELs across many types of PM evaluated, which range from about 0.02 to 0.2 ng/cell. As more in vitro studies are completed with information regarding specific PM exposure parameters and cell numbers used, clearer patterns should begin to emerge with regard to relative toxicities by PM class, cell type, and endpoints affected.

7.7.2 Interspecies Comparisons of Experimental Results

7.7.2.1 Introduction

Much of the new toxicologic data assessed in this chapter has been derived from either (a) in vivo exposures of human subjects or laboratory animals via inhalation exposures or instillation of PM materials or (b) in vitro exposures of various (mostly respiratory tract) cells or tissues to diverse types of PM. The experimental exposure conditions used in these studies are typically different from those experienced through inhalation of airborne PM by human populations in ambient environments. Most notably, the exposure concentrations used in many of the experimental studies are well above ambient PM levels. Therefore, consideration of the relevance of effects demonstrated under experimental conditions compared to the effects observed in humans following ambient PM exposures is useful, especially to the extent that

quantitative extrapolation of experimental results across species or to ambient conditions may be feasible based on currently available data.

Appendix 7A provides an analysis of the relationship between rat and human lung doses predicted for various exposure scenarios ranging from ambient PM exposures to PM instillations into the lung. In many studies, both toxicologic and epidemiologic, health endpoints are presented and analyzed as a function of exposure concentration. However, it is generally accepted that the dose to target cells or tissues, rather than exposure concentration per se, is responsible for adverse responses. As discussed in Appendix 7A, establishing a firm linkage between exposure and dose requires that consideration be given to particle characteristics and biological normalizing factors. Optimally, the dose metrics and normalizing factors should be based on the biological mechanisms mediating an effect. For some effects, the mass of soluble PM depositing in a region of the lung may be an appropriate dose metric. For example, an appropriate normalizing factor for soluble PM could be the surface area of the airways for irritants, whereas body mass might be more suitable when considering systemic effects.

There are two principle applications for the dosimetric assessments presented in Appendix 7A. First, experimental exposure concentrations can be estimated that should result in the same tissue dose in a rat as received by a human exposed to various levels of ambient PM as a function of dose metric, normalizing factor, and level of human exertion. As no single dose metric nor normalizing factor appears to be appropriate for all situations, numerous scenarios were considered in Appendix 7A. The parameters chosen can dramatically affect the rat exposure concentration estimated to be required to provide a normalized dose equivalent to that occurring in a human, as illustrated in Tables 7A-7a through 7A-9b in Appendix 7A. Second, the dose to the lung can be estimated for both animal and human inhalation studies. These analyses make it possible to compare biological responses as a function of dose rather than just exposure. Equal lung doses should not be assumed in comparing studies, even if PM mass concentrations, animal species, and exposure times are identical. Differences in the aerosol size distributions to which animals are exposed also affect dose delivered or retained. For example, in a comparison of several CAPs studies, one study was estimated to have 1.7 times the alveolar dose of another study despite a 10% lower exposure concentration in the first study. Thus, to make accurate estimates of dose, it is essential to have accurate and complete information regarding exposure conditions, i.e., not only concentration and duration of exposure, but also the

aerosol size distribution and the level of exertion (and hence breathing rates) for exposed subjects.

It is obviously not feasible, given the complexity involved, to attempt extrapolation modeling for more than a few illustrative health endpoints that were evaluated in the multitude of studies assessed in this Chapter. Nor would such an effort necessarily be particularly useful for present purposes. However, providing some modeling results that estimate comparative exposure concentrations/doses demonstrated experimentally in animal or human studies to be effective in producing a few important types of health endpoints should be of value in helping to provide a context by which to gauge the potential relevance of experimental results for ambient human exposure conditions.

7.7.2.2 Dosimetric Intercomparison for PMN Influx as a Marker for Lung Inflammation

Various types of particulate materials (both ambient PM and combustion source particles) have been shown to cause inflammation of the lung by migration of PMNs (predominantly neutrophils) into the airways. These cells are initially produced by bone marrow and, along with AM, constitute an important defense mechanism triggered by invasion of PM, bacteria, or some other foreign matter. The PMNs, once in the lung, ingest PM and then degranulate, forming hydrogen peroxide and superoxide anions. Excessive quantities of PM in the lung can cause the lysosomal enzymes in PMNs to enter the extracellular fluid, creating further inflammatory responses. Additionally, PMN produce thromboxanes, prostaglandins, and leukotrienes.

Three recent studies provide data on PMN increases following CAPs exposure that allow comparison of rat to human responses. Kodavanti et al. (2000b) exposed both healthy SD rats and rats with SO_2-induced bronchitis to CAPs collected in Research Triangle Park, NC. The particle size distribution in this study averaged 0.98 μm MMAD (σ_g = 1.41), the average concentration was 740 μg/m^3, and exposures consisted of whole-body inhalation of 6 h/day for 2 or 3 days. Inflammation was assessed immediately after exposure or 18 h later. Increases in BAL PMNs were seen only in the CAPs-exposed bronchitic rats compared to healthy CAPs-exposed rats in 2 of 4 separate experiments when rats were lavaged immediately postexposure. The healthy CAPs-exposed rats had no significant differences in PMN counts compared to healthy air-exposed rats. In a similar study, Clarke et al. (1999) exposed healthy and SO_2-induced bronchitic rats to Boston CAPs at an average concentration of 515 μg/m^3.

Particle size averaged 0.18 µm MMAD (σ_g=2.9) and exposures consisted of whole-body inhalation for 5 h/day for 3 consecutive days. PMN levels were assayed 24 h after exposure. Increases in PMNs (both in terms of total PMN counts and as PMN as percent of total cell count) in both the normal and bronchitic rats were seen with CAPs exposure. It is possible to compare these two rat studies to a human study, wherein Ghio et al. (2000a) exposed human subjects to Chapel Hill CAPs. In that study, healthy human volunteers were exposed to ~120 µg/m^3 for 2 h, with 15 minute periods of exercise alternating with 15 minutes of rest. Particle size was 0.65 µm MMAD (σ_g=2.35) and BAL analysis was at 18 h PE. Consistent with data from the rat studies, the total numbers of PMNs increased with the human CAPs exposure.

Appendix Section 7A.7.2 compares tissue doses predicted to occur in human and rat CAPs exposures using the Ghio et al. (2000a), Kodavanti et al. (2000b), and Clarke et al. (1999) studies. The Kodavanti et al. (2000a) study consisted of five separate CAPS experiments and the retained CAPs determinations were made from the experiment that utilized the 18 h PE time point. Comparisons of these rat and human studies indicate that in order to obtain the noted similarities in PMN responses observed, rats actually received a far greater alveolar tissue dose than humans. That is, 60 to 500% increases in PMN numbers were observed in the rat studies with estimated retained alveolar tissue doses of 28 to 47 µg/m^2 of CAPs PM; whereas a 267% increase in PMNs was seen in humans with estimated doses of only 0.7 µg/m^2 of alveolar tissue. This suggests that even healthy humans are notably more susceptible to the inflammatory effects of CAPs than are rats. Table 7-14, allows more specific comparisons.

TABLE 7-14. CAPS: RAT AND HUMAN INHALATION STUDY COMPARISONS

Study	Species	Particle	Exposure Conc. (µg/m^3)	MADD (σ_g)	Exposure duration	Analysis PE	Change in PMN	Estimated alveolar dose per surface area
Kodavanti et al. (2000a)	SD rat SO_2-SD	RTP CAPs	740	0.98 (1.41)	6 h/day for 2-3 days	< 3 h 18 h	255% ↑PMN in 2 of 4 exp (bronchitic rats only) no change in PMN	ND 28 µg/m^2 retained
Clarke et al. (1999)	SD rat SO_2-SD	Boston CAPs	515	0.18 (2.9)	5 h/day for 3 days	24 h	500% ↑PMN 367% ↑PMN	47 µg/m^2 retained
Ghio et al. (2000a)	humans	Chapel Hill CAPs	120	0.65 (2.35)	2 h	18 h	267% ↑PMN	0.7 µg/m^2 retained

7.7.2.3 Inhibition of Phagocytosis by PM Exposure

Phagocytosis is a form of endocytosis wherein bacteria, dead tissue, or other foreign material (e.g., inhaled ambient particles) are engulfed by cells such as AM, MO, or PMN as part of normal lung defense mechanisms. Hence, increases in numbers of AM, MO, or PMN cells in lung tissue represent one indicator of mobilization of lung defenses in response to infection or deposition of inhaled particles. Once ingested by AM, lysosomes act to digest engulfed materials. Inhibition of the phagocytosis by AM would signal interference with lung defense mechanisms by which inhaled bacteria and viruses are killed or other foreign particles are detoxified and/or cleared from the lung. Also, if an AM is overwhelmed by the amount or toxicity of ingested material, that material may be released along with the AM's digestive enzymes onto the alveolar surface and numbers of AM or their phagocytic activities may decrease.

A number of experimental (especially in vitro) studies have demonstrated, that in some instances, one or another type of PM has caused an inhibition of phagocytosis. As with other endpoints affected by PM, this inhibitory effect is determined by the size and composition of the specific particulate materials tested.

For example, Becker and Soukup (1998) exposed human AM to UAP from St. Louis (0.2 to 0.7 µm MMAD) and ROFA from Florida (0.5 µm MMAD). Exposures periods were 18 to 20 hs at 100 µg/mL per 2×10^5 cells/mL for a dose per cell of 0.5 ng/cell. AM had a 50% decrease in phagocytosis of *Saccharomyces cerevisiae* with St. Louis UAP and a 30% decrease with ROFA, which the authors attributed to the toxicity of ROFA. The authors noted decreased phagocytosis in cells with both high and low particle burden, and further, that inhibited phagocytosis was more pronounced in the cells with a low burden. They attributed this effect to soluble fine constituents of the UAP more so than to particle-bound insoluble constituents. These researchers (Soukup and Becker, 2001) extended these findings with human AM exposures to Chapel Hill CAPs. They separated the CAPs into $PM_{2.5}$ and PM_{10}, (soluble and insoluble components) and exposed 2×10^5 cells/mL to 12.5, 25 or 100 µg/mL for 18 h. Phagocytosis was then assayed with fluorescein-tagged, zymosan particles. They observed a dose-dependent decrease in uptake with the insoluble PM_{10} (12% at 12.5 µg/mL, 30% at 25 µg/mL, and 50% at 100 µg/mL). This correlates with doses per cell of 0.06, 0.12, and 0.5 ng/cell, respectively. There was a similar percentage of AM that appeared to be undergoing

apoptosis. They postulated that the decrease in phagocytosis is due to the cells undergoing programmed cell death. In another study by this group, Soukup et al. (2000) found decreased phagocytosis of yeast particles in human AM exposed to Provo PM_{10} (Utah Valley Dust) collected before a steel plant closed. Exposures of 100 µg/mL to 2×10^5 cells/mL (for a dose of 0.5 ng/cell) caused a 30% decrease in phagocytosis. Particles collected during and after the steel mill closure did not cause a similar change in phagocytosis, even though the amount of particles engulfed was the same for all samples of the dust. They suggested that the metal content may not be predictive of decreases in AM phagocytic responses. In another study, Van Eeden et al. (2001) studied human AM exposed to UAP (Ottawa) and ROFA (Florida), both reported to be < 10 µm in diameter, and found an inhibition of phagocytosis at 100 ug/mL, which they attribute to toxicity to the cells. At 24 h PE, phagocytosis was determined by visual inspection of the cells. Cells were plated at a concentration of 0.5×10^6 and phagocytosis was decreased at a dose of 0.2 ng/cell.

These in vitro studies of human AM may be compared to three available studies that investigated animal AM responses to vitro PM exposures. In one, Renwick et al. (2001) used a mouse macrophage cell line (J774.2 MΦ) to evaluate inhibition of phagocytosis by both fine and ultrafine particles. They used fine carbon black CB (260.2 nm diameter), ultrafine CB (UCB, 14.3 nm), fine TiO_2 (250.0 nm) and ultrafine TiO_2 ($UTiO_2$, 29.0 nm). The cultured cells at a concentration of 5×10^6 cells/mL were exposed to particles at concentrations of 15.6, 31, 63, or 125 µg/mL for 8h, after which phagocytosis was assessed using 2 µm fluorescent latex beads. Phagocytosis was inhibited by UCB at 63 µg/mL and by all the particles at 125 µg/mL, which corresponds to doses of 0.013 and 0.025 ng/cell, respectively.

More recently, Renwick et al. (2004) sought to further characterize the effect of particle size on inhibition of phagocytosis in vitro. Using the same particles just described, rats were instilled with 125 or 500 ug of each of the particle types. AM were removed from BAL and cultured at concentrations of 5×10^5 cells/mL. Phagocytic activity was assayed in vitro with 2 µm fluorescent latex beads. All of the particles significantly inhibited phagocytosis at the higher dose. No effect of particle size was seen in this study. It is important to stress that this study differs from others being discussing in that the PM exposure took place in vivo, whereas the other studies utilized an in vitro exposure.

Goldsmith et al. (1997) exposed hamster AM to Boston CAPs (1 μm) or Boston ROFA (0.1 to 2.5 μm) for 30 minutes. They measured right angle light scatter (RAS) to determine cell granularity, as an indicator of phagocytosis. At concentrations of up to 20 ug/mL of CAPs and 200 ug/mL of ROFA, they observed no inhibition of phagocytosis. They listed two limitations of this type of assay to quantify phagocytosis; (1) it provides only a relative measure, not absolute numbers or mass of particles and, (2) it requires cells to be in suspension; whereas in the previously mentioned studies, the AM are adherent and thus capable of functioning in a more realistic manner. Further, as the exposure was only 30 minutes, it is difficult to compare their results to those of studies using exposures over 18 h.

To make comparisons between rodent and human studies investigating the inhibition of AM phagocytosis by PM, an understanding of the species-specific differences in AM should be noted. Appendix 7A Section 7.3 discusses the function of rat AM function versus volumetric loading. The volume, and presumably the capacity, of AM in rodents are smaller than for human AM. A human AM has an internal volume (not including the cell nucleus) of ~1350 μm^3, compared to the SD rat (1010 μm^3), F344 rat (760 μm^3), hamster (420 μm^3), or mouse (370 μm^3; Miller, 2000). The phagocytic activity of an AM is thought to slowly decrease above a particle loading of ~6% of its interior volume. At about 60% loading, alveolar macrophages become immobile. Considering the phagocytosis of a single particle, a human AM would likely become immobilized following the ingestion of a 11.6 μm diameter particle (0.816 ng assuming unit density), whereas a mouse AM could only ingest a 7.5 μm diameter particle (0.221 ng, unit density). Typically, considerably smaller particles than these (7.5 to 11.6 μm particles) deposit in the alveolar region of the lung and become phagocytosed by AM. In addition to the volume occupied by engulfed particles, another 30% or more of an AM capacity is lost to void spaces between particles packed within an AM . Table 7-15 compares several in vitro studies of human and rodent AM function and makes estimations of the AM loads based on reported PM characteristics.

Only one study was found that used both rat and human AM to compare the effects of PM on AM phagocytosis. Seemayer et al. (1990) exposed AM isolated from BAL to UAP from Duisburg (F.R.G). Both species demonstrated a reduction in phagocytic activity (% cells with > 2 particles) and phagocytic capacity (particles per cell), with little effect on cell viability. Of note, this study indicated a greater inhibition of phagocytosis in human AM compared to rat AM

TABLE 7-15. INTERSPECIES COMPARISONS OF NON-EFFECTIVE AND EFFECTIVE DOSES (estimated as ng/cell) PRODUCING NO CHANGE OR DECREASED PHAGOCYTOSIS BY HUMAN OR RODENT ALVEOLAR MACROPHAGE CELLS

Study	Species	PM	Conc	Exposure duration	Particle size	Change in phagocytosis	Estimate dose/cell	Estimated % of cell filled
Becker and Soukup (1998)	human	UAP (St. Louis) ROFA (Florida)	100 µg per 2 ×10^5 cells	18-20 h	0.2-0.7 µm 0.5	↓50% ↓30%	0.5 ng	53
Soukup et al. (2000)	human	CAPs (Chapel Hill - separated into soluble and insoluble components	12.5 25 100 µg per 2 ×10^5 cells	18 h	2.5 or 10 µm	↓12% ↓30% ↓50% with insoluble PM_{10} only	0.06 ng 0.12 0.5	6.3 13 53
Soukup and Becker (2001)	human	PM_{10} (Utah Valley)	100 µg per 2 × 10^6 cells	overnight	10 µm	↓30%	0.05 ng	5.3
Goldsmith et al. (1997)	hamster	CAPs (Boston) ROFA (Boston)	4 µg 10 20 25 50 100 200 per 0.5×10^6 cells	30 min	1 µm 0.1-2.5	no change	0.008 ng 0.02 0.04 0.05 0.1 0.2 0.4	2.7 6.9 14 17 34 69 140
VanEeden et al. (2001)	human	UAP (Ottawa) ROFA (Florida)	10 µg 100 per 0.5×10^6 cells	2,4,8,12,24 h (only 24 h data reported)	< 10 µm	no change ↓	0.02 ng 0.2	2.121
Renwick et al. (2001)	mouse macrophage cell line J774.2 MΦ	CB UCB TiO2, $UTiO_2$	15, 31,63, or 125 µg per 5×10^6 cells	8h	0.260 µm 0.014 0.250 0.029	↓@125 µg ↓@63 ↓@125 ↓@ 125	0.025 ng 0.013 0.025 0.025	9.7 5.0 9.7 9.7

CB = carbon black
UCB = ultrafine carbon black
TiO_2 = titanium dioxide
$UTiO_2$ = ultrafine titanium dioxide

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by both criteria, suggesting that human AM are more sensitive to the effects of PM than rat. Unfortunately, given that the study only reported the volume of air from which particles were collected and not particle mass, size, or composition, it is not possible to compare the data with more recent studies. In summary, the above comparisons provide interesting results suggesting that human AM are at least as sensitive to ambient PM as to ROFA.

Whereas two studies reported no change in AM phagocytosis with exposures of human cells to doses of Ottawa ambient PM or ROFA (Florida) ranging up to 0.4 mg/cell, other studies showed decreased phagocytosis in human AM's exposed to 0.05 to 0.5 mg/cell of Chapel Hill CAPs, Utah Valley PM_{10} extract, St. Louis ambient PM, or Florida ROFA. However, additional more systematic work is necessary to fully characterize the phagocytic dose-response to various species of PM. The efficient removal of inhaled PM by viable, functioning AM cells is a critical respiratory defense mechanism, one thought likely to be impaired by at least some types of ambient PM constituents.

7.8 MUTAGENICITY/GENOTOXICITY EFFECTS

The majority of newly-published PM research since the 1996 PM AQCD have focused on acute cardiovascular or respiratory 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. In view of these new ambient PM-carcinogenicity findings (and others from earlier epidemiologic studies), salient results both from some older studies (pre-1996 PM AQCD) and newly available ones are discussed below with regard to evaluations of mutagenic or other genotoxic effects of ambient PM, its constituents, and/or combustion emission source particles thought to be useful as indices of likely carcinogenic potential of such materials. The pertinent studies discussed below are summarized in Tables 7-16, 7-17, and 7-18.

7.8.1 Ambient Particulate Matter Effects

A limited number of new in vitro studies have examined the mutagenic and/or other genotoxic potential of ambient PM from various geographic locations in the U.S. or elsewhere; and, in general, they show some 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.

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, ambient PM constituents and/or PM emission source constituents. These studies, listed in Table 7-18, have focused mainly on the ability of the organic fraction of ambient PM to induce mutagenic effects in mammalian cell lines and bacteria.

Probably of most direct relevance and usefulness for assessing U.S. ambient air carcinogenic potential, Hannigan et al. (1997) examined the mutagenicity of PM from five monitoring sites in southern California. San Nicholas Island in upwind Los Angeles was considered to be a background site with low levels of PM. Central Los Angeles was

TABLE 7-16. MUTAGENIC/CARCINOGENIC EFFECTS OF AMBIENT PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle or Constituent	Exposure Technique	Concentration Dose (µg/mL)	Particle Characteristics Size (µm); μ_g	Exposure Duration	Effects of Particles on Mammalian Cells or Bacteria	Reference
Human h1A1v2	Ambient PM from Los Angeles, San Nicolas Island, Long Beach, Azusa, and Rubidoux, CA	in vitro	120 µg EOC per 12 mL assay	< 2µm	72h	No seasonal variation in mutagenic potency, leading authors to suggest that mutagenicity is due to ubiquitous emission sources like vehicle traffic or stationary combustion rather than isolated point sources. LA air had 10 times more mutagenicity than background levels (San Nicolas)	Hannigan, et al. (1997)
Human h1A1v2	Composite of ambient PM from Los Angeles, San Nicolas Island, Long Beach, Azusa, and Rubidoux, CA. Fractionated into nonpolar, polar and semipolar components	in vitro	300 - 1200 µg EOC per 12 mL assay	< 2µm	72 h	Most of the mutatenic potency was in the unsubstituted PAC fraction. 2-nitrofluoranthene and 6H-benzo [cd] pyrene were semipolar mutagens.	Hannigan et al. (1998)
Cultured tracheal epithelial cells from Hamster (Syrian golden, young) or rat	Ambient PM: industrial or high traffic areas (Germany)	in vitro	Not given	Dichloromethane extraction of high volume samples.	Dilutions of extracted organic phase of particles incubated with cells for 48 hs.	Dose-related increases in sister chromatid exchanges seen in both species. PM from industrial sample had LOEL of 0.11 m^3 air/mL medium. PM from high traffic area had LOEL of 0.16 m^3 air/mL medium.	Hornberg et al. (1996)
Human bronchioepithelial cell line (BEAS-2B)	Urban $PM_{2.5}$ Urban PM_{10} Industrial $PM_{2.5}$ Industrial PM_{10} Rural $PM_{2.5}$ Rural PM_{10} (Germany)	in vitro	6.6 to 26.5 µg/mL 1.7 to 6.9 µg/mL 10.8 to 43.2 µg/mL 5.8 to 23.1 µg/mL 7.8 to 34.1 µg/mL 3.7 to 14.4 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 hs.	Significant increases in sister chromatid exchanges were greater from all sampling sites at all doses of PM_{10} and $PM_{2.5}$ from urban and industrial regions. Extraction phase of coarse particles produced fewer sister chromatid exchanges than did the fine particles.	Hornberg et al. (1998)

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TABLE 7-16 (cont'd). MUTAGENIC/CARCINOGENIC EFFECTS OF AMBIENT PARTICULATE MATTER

Species, Gender, Strain Age, or Body Weight	Particle or Constituent	Exposure Technique	Concentration Dose (µg/mL)	Particle Characteristics Size (µm); μ_g	Exposure Duration	Effects of Particles on Mammalian Cells or Bacteria	Reference
Kidney cells from hamster (Syrian golden, 8-10 wks old)	Ambient PM from urban and industrial areas (Germany)	in vitro	Not given	Dichloromethane (DCM) extraction of high volume samples.	Dilutions of extracted organic phase of particles incubated with cells for 18 hs 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/extracted from 4 m^3 air.	Seemayer and Hornberg (1998)
Cultured hepatoma cells	Ambient PM (Netherlands)	in vitro	Not given	Acetone/DCM extraction of high volume samples.	Dilutions of extracted organic phase of particles incubated with cells for 6 or 48 hs.	Extracts of ambient PM both upwind and downwind of highway had genotoxic effects, although PAH content was greater in the downwind samples.	Hamers (2000)
Liver tumor cell line (HEPA1c1c7)	Urban air PM	in vitro	6 - 12 µg	Aqueous and organic extraction from filters of particles collected with high volume samplers.	4 hs	Inhibition of gap-junctional intercellular communication (GJIC) only significant in cells treated with aqueous extract of diesel, compost, or rubber particles.	Alink (1998)
	DEP		17 - 37 µg				
	Rubber industries PM		36 - 47 µg				
	Metal industries PM		32 - 175 µg				
	Poultry/swine farm		81 - 137 µg				
	Compost		42 µg				

PAC = polyaromatic compounds.

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TABLE 7-17. MUTAGENIC/CARCINOGENIC EFFECTS OF WOOD AND COAL COMBUSTION-SOURCE PM

Species, Gender, Strain Age, or body weight	Particle or constituent	Exposure Technique	Mass Conc µg/mL or µg/m3	Particle Characteristics Size (µm)	Exposure Duration	Effects of particles on mammalian cells or bacteria	Reference
Salmonella	Emissions from wood (birch, pine, and spruce) combustion	in vitro Ames assay	32 to 100 µg/m^3 of PM and 2.6 to 200 µg organics	PM and organic fractions from wood stoves combustion	48 hs	Organic fraction: mutagenic potency of 0.5-21 revertants/µg. The PM fraction demonstrated only very low mutagenicity	Löfroth et al. (1986)
Salmonella: TA98 TA100	Wood, diesel, and coal emissions	in vitro Ames assay (comparing standard plate and spiral assays)	200 µg/plate woodsmoke, 500 µg/plate DE and 200 µg/plate coal		72 h	DE had greatest mutagenicity under all conditions, creating both frameshift and base-pair substitution mutations. Coal just slightly less mutagenic than diesel creating indirect-acting frameshift mutations. Woodsmoke only weakly mutagenic.	Houk et al. (1991)
Salmonella: TA98 Human WBC	Emission from open fireplaces	in vitro Ames assay ^{32}P-postlabelling analysis of DNA adducts		PM extracted with methanol	1 wk	Control: 28 revertants/30 m^3 (-S9) and 69 (+S9). Combustion: 153 (-S9) and 369 (+S9). No change in DNA adducts.	Heussen et al. (1994)
Salmonella: TA98 TA100	Wood smoke condensate (Sigma)	in vitro Ames assay	0, 125, 250, 500, 750 and 100 µg/plate		48 hs	Not mutagenic at all doses	Putnam et al. (1999)
Salmonella: TA100	Emission PM - collected throughout year from burning fields	in vitro Ames assay	130 units µg/m^3 (winter) to 15 (summer), 170 (winter), 37 (summer)	$PM_{2.5}$ and PM_{10}	48 hs	$PM_{2.5}$: 30.6 revertants/m^3 air volume (winter) to 0.1 (summer) with increased mutagenicity with S9 activation. PM_{10}: 28.1 (winter) to 0.7 (summer), revertants/m^3 air volume, S9 activation increasing the mutagenicity.	Vinitketkumnuen et al. (2002)
Salmonella: TA98 TA100	Wood burning emission PM and gas phase	in vitro Ames assay		Two smoke samples collected, PM and gas phase	48 hs	12×10^6 revertants/kg using TA100-S9 and 3.5×10^6 revertants/kg using TA98-S9. Emissions can cause both frameshift and base pair substitution mutations. The gas phase of the wood smoke emission contributed to more than 60% of the direct-acting mutagenicity	Kim Oanh et al. (2002)

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TABLE 7-17 (cont'd). MUTAGENIC/CARCINOGENIC EFFECTS OF WOOD AND COAL COMBUSTION-SOURCE PM

Species, Gender, Strain Age, or body weight	Particle or constituent	Exposure Technique	Mass Conc µg/mL or µg/m3	Particle Characteristics Size (µm)	Exposure Duration	Effects of particles on mammalian cells or bacteria	Reference
Salmonella: TA98 TA100 TA1535 TA1537 TA1538	Coal fly-ash from fluidized-bed (FBC) and conventional combustion (CC) plants	in vitro Ames assay		< 3µm FBC mean diameter 0.54 µm CC mean diameter 1.05 µm	72 hs	FBC mutagenic in TA98 (3.32 revertants/mg) and TA 1538 (3.31), both without activation. S9 decreased the mutagenicity of FBC in TA98 and TA 1538. FBC had no mutagenic response in TA1537 and TA1535.	Munford and Lewtas (1982)
Salmonella: TA98 TA100 TA104	Extracts from smoky coal, China	in vitro Ames assay PCR and DNA sequencing				TA98 + S9 mutagenic at ≥ 10 ug/plate; TA98-S9 not mutagenic. Mutation spectrum hotspot. TA100 + S9 mutagenic at ≥ 10 ug/plate; TA100-S9 at ≥ 50 ug/plate. Mutation spectrum: GC → TA or GC → AT transversions. TA104: no mutagenicity	Granville et al. (2003)

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TABLE 7-18. MUTAGENIC/CARCINOGENIC EFFECTS OF MOBILE COMBUSTION-SOURCE PM

Species, Gender, Strain Age, or body weight	Particle or constituent	Exposure Technique	Mass Conc µg/mL or µg/m3	Particle Characteristics Size (µm)	Exposure Duration	Effects of particles on mammalian cells or bacteria	Reference
Salmonella	PM diesel gasoline	in vitro Ames assay			48 hs	DE mutagenic response was 800 (PM fraction) revertants/g fuel used and 210 (condensate fraction). Gasoline 24 (PM fraction) and 39 (condensate).	Löfroth (1981)
Salmonella: TA98 TA100	PM from Diesel, Gasoline, Gasoline + alcohol, liquified petroleum	in vitro Ames assay			48 hs	LP cars: 10 rev/L exhaust. Gasoline and gasoline + alcohol: 10-50 rev/L exhaust). Light-duty diesel:50-250 rev/L7	Rannug (1983)
Salmonella: TA98 TA98NR	Fractionate exhaust of gasoline and diesel engines	in vitro Ames assay	Particle emission values for the vehicles were 0.021 g/km and 0.23 g/km		48 hs	Most polar subfraction was most mutagenic TA98-S9: 7.8 rev/m^3 (g); 6.1 (d). TA98+S9: 3.3 (g);1.5 (d). TA98NR-S9: 3.7 (g); 4.1 (d) TA98NR response generally lower than TA98 response. Both had similar TA98NR-S9 response, but differed significantly in TA98-S9 response.	Strandell et al. (1994)
Mutagenicity: Salmonella T. Microsome assay Cytotoxicity: Mouse fibroblast cell line < 1.292	Diesel exhaust particles (DEP): petroleum DEP vs. rapeseed oil methyl ester (RME) DEP	in vitro Ames assay	Not given	Dichloromethane extraction of particles collected from diesel engine run with diesel fuels with low or high sulfur and plant oil fuel.	48 hs incubation with TA98 and TA100 strains.	Revertants were 2- to 10-fold higher with high sulfur diesel fuel particles. Cytotoxicity in fibroblast cells higher for RME.	Bunger (2000)

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TABLE 7-18 (cont'd). MUTAGENIC/CARCINOGENIC EFFECTS OF MOBILE COMBUSTION-SOURCE PM

Species, Gender, Strain Age, or body weight	Particle or constituent	Exposure Technique	Mass Conc µg/mL or µg/m3	Particle Characteristics Size (µm)	Exposure Duration	Effects of particles on mammalian cells or bacteria	Reference
Salmonella: TA98 TA100	PM and SVOC of exhaust from diesel and gasoline engines	in vitro Ames assay	25-500 µg/plate			Mutagenicity rankings: TA98: current diesel at 30 °F > high emitter diesel > gas emitting white smoke > normal gasoline 72 °F > normal diesel 72 °F > gas emitting black smoke. TA100:current diesel at 30 °F > gas emitting white smoke > high emitter diesel > normal diesel 72 °F > gas emitting black smoke > normal gasoline 30 °F > normal gasoline 72 °F	Seagrave et al. (2002)
Salmonella: TA98 Calf thymus DNA	PM and SVOC of exhaust from diesel and gasoline engines	in vitro Ames assay Adduct formation	Ames assay:30-500 µg/PM (-S9) 10-1000 µg/PM (+S9) Adduct:150 µg/PM gasoline extracts. high doses (42-150 µg/PM) and low doses (7.5-18.5 µg/PM) of gasoline and diesel extracts.			Gas SVOC fraction less mutagenic than PM fraction, but formed more DNA adducts. Diesel PM and gasoline extracts formed more S9-mediated adducts with increasing doses, but no dose response. Diesel extracts formed higher levels of adducts than gasoline extracts, especially in the presence of XOResults suggest high nitro-PAH levels in diesel extract.	Pohjola et al. (2003)

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characterized as a region of high PM resulting from heavy vehicle traffic. Long Beach was another high PM site studied with PM originating from power plants and oil refineries. The two other high PM sites chosen were Azusa and Rubidoux, which were considered receptor sites located downwind from high density primary emission sources. Mutagenic activity of air samples collected in 1993 were assayed using a cultured human cell assay in addition to the standard Ames bacterial mutation assay, which has limitations in terms of relevancy to human mutagenicity. The human cell assay utilizes h1A1v2 cells, which test mutagenic activity at the thymidine kinase locus. The cells contain a plasmid pHSRAA with two copies of human CYP1A1 cDNA, which confers resistance to 1-histidinol. CYP1A1 is a cytochrome P450 capable of activation of PAH. Air samples were collected throughout the year at all sites using a dichotomous sampler. Both seasonal and spatial differences in component elemental and organic carbon were observed. However, both the human cell mutagenicity assay and a Salmonella TM677 forward mutation assay showed no systematic seasonal pattern of changes in mutagenicity. These results suggested to the authors that the proportion of mutagenic compounds in the fine organic aerosol mass does not change throughout the year and that perhaps the emission sources that show seasonal variation do not contribute in a major way to the mutagenicity of the PM. They thusly concluded that, in the Los Angeles area, primary particulate emissions from sources that operate on a year-round basis are the important human cell mutagens. Further, since they found very similar mutagenic potencies at all four widely-separated high PM sites, they suggested that the mutagenicity is due most likely to ubiquitous emissions sources rather than to isolated point sources.

To ascertain which components of the Los Angeles area PM were responsible for the observed mutagenicity, Hannigan et al. (1998) extended these findings by combining the four high PM samples and a background site sample described above into a composite sample that was then separated by liquid chromatography into fractions of organic chemicals of similar polarity and functionality. A primary fractionation separated the composite sample into four fractions, designated nonpolar 1, nonpolar 2, semipolar, and polar. To further isolate the mutagens, additional fractionation steps were done by HPLC. The mutagenic potency of the unfractionated sample was 150 induced mutant fraction (IMF) per mass of fine particulate organic carbon or IMF ($\times 10^6$)/mg of EOC. They found that six unsubstituted polyaromatic compounds (PACs) were responsible for much of the mutagenicity. These included benzo [k]

fluoranthene, indeno [1, 2, 3-cd] pyrene, benzo [b] fluoranthene, benzo [g, h, i] perylene, benzo [z] pyrene, and cyclopenta [cd] pyrene. Benzo [k] fluoranthene and benzo [b] fluoranthene sources include vehicle exhaust and natural gas combustion. The source of the other four PACs is mainly noncatalyst-equipped gasoline vehicles. Additionally, two semipolar mutagens were identified: 2-nitrofluoranthene and 6H-benzo[cd]pyren-6-one. In the Los Angeles area, the first compound is a product of atmospheric chemical reactions and the other is emitted by noncatalyst gasoline-powered vehicles. The authors estimated that greater than half of the mutagenicity may be attributed to the semipolar and polar fractions of the sample.

Some additional evidence for mutagenetic properties of ambient PM derive from several European studies. 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) or in another area (Düsseldorf) of Germany dominated by high density vehicular traffic. Exposure to ambient PM extracted (by dichloromethane or DCM) from filters from both types of locations induced highly significant dose-dependent increases in SCE 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 just 0.11 to 3.5 m^3 air for the samples from the heavy industry area and from 0.16 to 10.2 m^3 for the heavy traffic area induced significant genotoxic effects (i.e., ~2-fold increases in SCE).

Hornberg et al. (1998) also evaluated 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 Germany. Both ambient PM_{10} and especially $PM_{2.5}$ extracted (by DCM) from filters for all three areas significantly increased SCE in the human bronchioepithelial cell line (BEAS-2B) cultured in vitro for a 72 h exposure. The authors noted that the fine fraction ($PM_{2.5}$) exerted stronger genotoxic activity than the PM_{10} 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 $PM_{2.5}$ (equivalent to airborne PM substances from < 0.5 m^3 of air) exerted strong genotoxicity. The $PM_{2.5}$ and PM_{10} extracted PM from the filters were reported to have been drawn from ambient air having

concentrations of: 18.4 and 4.8 μg/m^3 for Düsseldorf; 45 and 24.1 μg/m^3 for Duisburg, and 21.8 and 10 μg/m^3 for Borken, respectively (all of which were in 1 mL of medium for exposures).

Based on the above results, Hornberg et al. (1996, 1998) concluded that the increases observed in SCE of tracheal 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. However, insufficient information was provided by which to estimate the actual exposure doses to the cell cultures in the Hornberg studies. Nevertheless, their results still appear to provide qualitative evidence for mutagenic effects of ambient PM (especially the fine fraction drawn from heavily industrialized or trafficked areas). The authors also noted that the tracheobronchial epithelium is the site of one 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 findings, is derived from a study by Seemayer and Hornberg (1998), which employed a bioassay for enhancement of malignment cell transformation in vitro. Exponentially growing cell cultures from the Syrian golden hamster were exposed for 18 h to varying concentrations of PM materials extracted (by DCM) from hi-vol sampler filters that collected ambient PM from Düsseldorf or Duisburg, Germany in winter, 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. Positive control cultures pretreated with benzo-a-pyrene (BaP) showed similar dose-dependent enhancement of malignant cell transformations. The authors 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 that interactions of environmental carcinogens and viruses should be considered in human carcinogenesis.

Using a different type of bioassay from Hornberg and colleagues, Hamers et al. (2000) evaluated the genotoxicity of ambient PM collected by hi-vol sampler at several sites in The Netherlands: (1) one site next to a highway traffic point (density of vehicle passages/day = 63×10^3); (2) another next to a higher density (93×10^3 vehicle passages/day) traffic point; and (3) a third in a natural conservation area (with extensive non-manured grasslands and cattle

grazing) 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 *S. typhimurium*). Arylhydrocarbon-receptor activation was also assessed by DR-CALUX-assay, using a stably 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 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 showed that the presence of pollutants with genotoxic or PAH-like characteristics pose an undesirable mutagenic risk.

In another study using a less conventional endpoint, Alink, et al. (1998) compared effects on gap-junctional intercellular communications (GJIC) in liver tumor (HEPA1c1c7) cells of in vitro exposures to PM from urban air (geographic area not stated), rubber and metal industries, diesel exhaust, and biological sources (i.e., poultry/pig farming, compost industry). Only diesel and rubber sample filter extract suspensions significantly inhibited GJIC, with up to 83% of the inhibition attributed 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 indexed by GJIC inhibition) may vary widely depending on particle source and type, possibly due to the particles per se or to surface-bound bio-active material.

Taken together, the results of the above studies provide strong new evidence indicative of ambient PM (especially the fine fraction) having mutagenic properties, thus supporting the plausibility of epidemiologic evidence linking ambient PM (especially fine PM) to lung cancer. The results further suggest likely contributions to the observed mutagenicity of ambient PM of industrial or motor vehicle combustion sources (which are important emission sources for fine PM). The ensuing subsections discuss studies that evaluated the mutagenic/genotoxic potential of several types of major combustion sources known to contribute to ambient PM (especially fine PM) in many U.S. regions.

7.8.2 Wood and Coal Combustion-Source Effects

Emissions from the combustion of wood and coal, as well as combustion of oil fuels (diesel and gasoline) by mobile source vehicles, all contribute to ambient PM. A number of studies have been done to evaluate the mutagenicity and genotoxicity of these combustion emissions and to compare their relative mutagenic/genotoxic potentials. Table 7-17 summarizes wood/coal combustion studies, discussed first below. These include some earlier studies, conducted prior to the 1996 PM AQCD, given the only very limited more recent evaluation of wood/coal combustion mutagenic/genotoxic effects.

7.8.2.1 Biomass/Wood Burning

Early studies by Löfroth et al. (1986) used the Ames Salmonella assay to determine the mutagenicity of emissions from wood (birch, pine, and spruce) burned in conventional wood stoves. Both the PM fraction and the condensable organic fraction were applied in doses of 0.6 to 4.3 liter flue gas per plate (for a range of 32 to 100 mg/m^3 of PM and 2.6 to 200 mg organics). The wide range of doses was due to use of both updraft and downdraft stoves, the latter generating far less combustion emissions. The organic fraction had a mutagenic potency of 0.5 to 21 revertants/ug (rev/ug). The PM fraction demonstrated only very low mutagenicity. The authors compared these results to earlier studies by Löfroth (1981) and Rannug (1983) of gasoline and diesel cars. On a revertant per hour basis, wood stoves produced 6×10^6, gasoline cars 0.5 to 3×10^6, and diesel cars 3 to 20×10^6 rev/µg.

In testing the spiral Salmonella assay (Houk et al., 1991) compared the mutagenicity of wood smoke, automotive diesel exhaust and coal combustion emission. This automated bacterial mutagenicity assay dispenses Salmonella, the test agent, and the S9 mix in a spiral pattern on an agar plate, creating a uniform density of bacteria and a gradient of test agent. Doses of 200 µg/plate woodsmoke, 500 µg/plate DE and 200 µg/plate coal emission were used on standard Ames assay plates to compare the two assays. Exposures to strains TA98 and TA100, both with and without metabolic activation by S9, showed that DE had the highest mutagenicity. Results from the TA98 and TA100 experiments suggested to the authors that the mutagenic activity is due to both nitrated polynuclear aromatics creating frameshift mutations and nonpolar compounds creating base-pair substitution mutations. Coal was just slightly less mutagenic than diesel; and the data suggested that indirect-acting frameshift mutations were

occurring, which is in agreement with Mumford et al. (1987). Woodsmoke was found to be only weakly mutagenic in both strains.

Heussen et al. (1994) collected respirable PM from homes in Wageningen, NL, a region with no significant industrial pollution. For a 1-mo control period, the fireplaces were not used in five homes. Wood was then burned in open fireplaces for 4h/day during the evening for 1 wk. PM was collected during these same periods in the homes and, also, from some outdoor sampling sites to correct for possible infiltration of ambient mutagens into the homes. Non-smoking subjects from these homes gave blood samples during the control period, at the beginning of the combustion period, at the end of the combustion period, and 1 wk later. The blood was assayed using ^{32}P-postlabeling analysis of DNA adducts from white blood cells. PM samples were assayed by the Ames test (using strain TA98), both with and without S9 activation. The mutagenicity of samples from all 5 homes was increased after the week of fireplace usage. Control values averaged 28 revertants/30 m^3 (-S9) and 69 (+S9), whereas combustion samples averaged 153 (-S9) and 369 (+S9), indicating stronger indirect mutagenicity. However, there was no correlative combustion-related increase in formation of DNA adducts in white blood cells. The authors suggest several reasons for a lack of correlation between the two endpoints: (1) the exposure was too short and/or the dose was too low; (2) white blood cells may not be a suitable cell type for detection of adducts with this type of exposure; and (3) the actual genotoxic damage that occurred my not be detectable by this method of ^{32}P-postlabeling analysis.

The mutagenicity and toxicity of wood smoke condensate was assayed by Putnam et al,, (1999) using the Ames assay and neutral red uptake, respectively. The wood smoke condensate (Sigma #W2000) was prepared from hardwoods by distillation and filtration, removing 'insoluble tars' and low boiling point substances. The wood smoke condensate was tested for cytoxicity at concentrations of 0, 10, 25, 50, 75, 100 and 150 µg/mL and found to be toxic beginning at 25 µg/mL. Mutagenicity was tested using *S. typhimurium* strains TA98 and TA100, both with and without S9 activation. Concentrations of 0, 125, 250, 500, 750 and 100 µg/plate were used and the wood smoke condensate was found to be non-mutagenic at all the doses.

More recent studies have examined the mutagenicity of biomass combustion in Chaing Mai, Thailand (Vinitketkumnuen et al., 2002). Large open fires created by farmers burning fields and grass in the winter months correlate with increased in PM at that time of year. $PM_{2.5}$ and PM_{10} were collected on Teflon filters at four outdoor sampling sites. Twenty-four hour filter

samples were collected over a period of 1 mo and then pooled, dissolved in 1 mL DMSO and used for the Ames assay (TS100 strain/0.05 mL sample per assay). Monthly averages of $PM_{2.5}$ ranged from ~130 μg/m_3 (winter) to 15 μg/m_3 (summer) and for PM_{10}, 170 μg/m_3 to 37 μg/m_3, respectively. Mutagencity, expressed as number of revertants/m^3 air volume, for $PM_{2.5}$ ranged from 30.6 (winter) to 0.1 (summer), with the mutagenicity being increased with S9 activation. For PM_{10} the mutagenicity ranged from 28.1 (winter) to 0.7 (summer) revertants/m^3 air volume, again with S9 activation increasing the mutagenicity. One of the collection sites showed a much higher mutagenicity level than the other three, which might be explained by a local source of diesel exhaust. Mobile source emissions contribute to ambient PM levels in Chaing Mai, but as they remain constant throughout the year, the authors suggest that the increased mutagenicity observed in the winter months is due to biomass combustion.

Kim Oanh et al. (2002) examined the mutagenicity and toxicity of emissions from various cooking sources, including wood and kerosene. The wood (Pterocarpus indicus) was burned in a single-stage ceramic cookstove and two samples were collected, a PM phase consisting of the PM collected on the filter and the PM rinsate and a gas phase consisting of XAD-2, the condensate knockout and the rinsate. Toxicity was assayed using a Microtox bioassay and mutagenicity was assayed using the Ames test with TA98 and TA100 strains, both with and without metabolic activation by S9. The highest mutagenicity factor was observed from wood fuel which produced 12x 10^6 revertants/kg using TA100-S9 and 3.5 x 10^6 revertants/kg using TA98-S9. These results indicate that the wood smoke emissions can cause both frameshift and base pair substitution mutations. The gas phase of the wood smoke emission contributed more than 60% of the direct-acting mutagenicity.

7.8.2.2 Coal Combustion

Coal fly-ash samples (< 3 μm) from a southern U.S. conventional combustion (CC) plant and from a Linden, NJ fluidized-bed combustion (FBC) plant (both burning Pennsylvania eastern bituminous coal) were collected by Munford and Lewtas (1982). They compared the mutagenicity of PM from the two sources using the Ames assay with test trains TA98, TA100, TA1535, TA1537, and TA1538. FBC was mutagenic in TA98 (3.32 revertants/mg) and TA 1538 (3.31), both without S9 metabolic activation. S9 decreased the mutagenicity of FBC in TA98 and TA 1538. FBC had no mutagenic response in TA1537 and TA1535, with or without

S9. Thus, FBC fly ash appeared to create direct-acting frameshift mutations. In all of the five strains utilized, the CC fly-ash demonstrated no mutagenicity.

More recent studies characterizing the health effects of coal emissions have focused mainly on the mutagenicity and carcinogenicity of coal smoke exposure in regions of China with a predominance of indoor burning of "smoky" coal (Mumford et al., 1987; Chapman et al., 1988; Mumford et al., 1999). These regions have very high rates of lung cancer mortality that have been linked to exposure to unvented coal smoke.

Mumford et al., (1987) collected indoor air samples from homes burning smoky coal and from homes burning wood and smokeless coal in open hearths in kitchens. The PM levels inside the homes burning smoky coal averaged 23 mg/m^3, compared to 1.8 mg/m^3 for homes burning smokeless coal. The distribution of PM size in the homes burning smoky coal was bimodal, with half the particles < 1 μm and half 1 μm to 10 μm, whereas particle size in wood-burning homes ranged from 1 to 30 μm. Fractionation of the filter extracts created aliphatic, aromatic, moderately polar, and polar components. High concentrations of organic matter were present in the smoky coal (72 to 82%) compared to 27% for the smokeless coal and 55% for the wood sample. Further, the highest PAH levels were found in smoky coal samples. Both neat samples and fractions were tested for mutagenicity by the Ames assay using strain T98, with and without metabolic activation by S9. Most of the samples required S9 activation for mutagenicity, suggesting to the authors the presence of PAH. Smoky coal samples had the highest mutagenicity (60-17 revertants/m^3 air) compared to wood (11) and smokeless coal (1.3). The fractions that displayed the most mutagenicity were the polar and aromatic, which were shown by GC/MS to consist primarily of nitrogen- and oxygen-containing compounds (polar fraction) and PAH, methylated PAH, and nitrogen heterocyclic compounds (aromatic fraction).

A retrospective epidemiologic study (Lan et al., 2002) evaluated the incidence of lung cancer in a cohort of farmers born in Yunnan Province. The farmers were raised in homes burning smoky coal in unvented firepits and 81% changed to homes utilizing stoves with chimneys that reduced indoor levels of PM_{10} (2.08 mg/m^3 to 0.71 mg/m^3, respectively). After stove improvement, Lan et al. observed a long-term reduction in lung cancer incidence, calculating risk ratios of 0.59 in men and 0.54 in women.

Very recent work (Granville et al., 2003) has focused on the mutation spectra of coal smoke emissions from the Yunnan Province. Smoky coal extracts from the same source as

above (Mumford et al., 1987) at doses of 0, 10, 25, 50, and 100 ug/plate were used in the Ames assay with strains TA98, TA100, and TA104, both with and without S9 activation. Molecular analysis of the revertants was then done to identify the mutations. Coal smoke extract was mutagenic in TA98 in the presence of S9 at doses ≥ 10 ug/plate and not mutagenic without S9 activation. The extract was mutagenic in TA100 with S9 at doses ≥ 10 ug/plate and without S9 at doses ≥ 50 ug/plate, but it was not mutagenic in the TA104 strain. The authors interpreted these results to suggest that the coal extract induced mutations primarily at GC sites and that PAHs were probably involved in the mutations because of the greater mutagenicity in TA100+S9 compared to TA98+S9. The mutation spectrum in TA98 showed that the extract induced only the hotspot mutation, which is a 2-base deletion in an 8-base GC repeat. This suggested to the authors that about 70% of the mutations in TA98 were due to standard PAH compounds in the coal smoke. The mutation spectrum in TA100 showed that most of the mutations were GC → TA or GC→AT transversions. The authors then compared these mutation spectra with KRAS and TP53 mutation spectra observed in lung tumors from nonsmoking women exposed to coal smoke emissions. They found similarities in the GC → TA transversions in TA100 with human mutations due to PAH exposures, thus linking smoky coal exposures to human cancer.

7.8.3 Mobile Combustion-Source Effects

Numerous studies have linked mutagenic/carcinogenic effects to diesel and gasoline exhaust and/or to particles contained therein, as summarized in Table 7-18 and discussed below.

7.8.3.1 Diesel

Results such as those noted in Table 7-18 add further to an extensive database on diesel-related mutagenicity that was thoroughly reviewed in the 2002 U.S. EPA Diesel 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, use of mutagenicity data as an 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 test agent concentrations orders of magnitude greater than encountered in environmental situations. With diesel emissions or other mixtures, other complications also arise due to the complexity of the materials tested.

Since 1978, more than 100 publications have been reported for genotoxicity assays used with whole diesel emissions (DE), the volatile and particulate (DPM) fractions (including extracts), or individual chemicals found in diesel emissions. Interest in the contribution of mutagens to carcinogenicity was high in the early 1980s and the lack of long- term rodent carcinogenicity data for DE led to use of semiquantitative mutagenicity (and in vitro cell transformation) data for DE to augment epidemiology studies of diesel-related carcinogenic effects.

The number of chemicals in diesel emissions is very large; and many have been shown 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 of diesel exhaust are ethylene, benzene, 1,3-butadiene, acrolein and several PAHs, all of which are also present in comparable or greater amounts in gasoline exhaust. 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.

Numerous studies have evaluated mutagenic effects of DE and/or DPM. In one early study, Huisingh et al. (1978) showed 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 were reported by several other using the salmonella frameshift-sensitive strains TA1537, TA1538, and TA98 (Siak et al., 1981; Claxton, 1981; Dukovich et al., 1981; Brooks et al., 1984). Mutagenic activity

was also 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 difficult 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 content, certain PAHs (1-nitropyrene) and naphthenes.

Matsushita et al. (1986) tested particle-free DE gas and 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 most 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 increased significantly with S9 present. These latter results suggest 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 were confirmed in a numerous 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.

Belisario 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 mutagenic increases were seen 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 wks (Pereira et al., 1981a) or Fischer 344 rats exposed to DPM at a concentration of 1.9 mg/m^3 for 3 mos to 2 ys (Ong et al., 1985) was negative in Salmonella strains.

Specific-locus mutations were not induced in (C3H × 101)F1 male mice exposed to DE 8 h/day, 7 days/wk for either 5 or 10 wks (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.

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 combustion of conventional petroleum diesel fuel or diesel fuel containing rapeseed oil methyl ester (RME), were extracted (by dichloromethane) and their cytotoxicity was then evaluated by the neutral red assay and their mutagenicity by the S. typhinuiam assay. The diesel petroleum 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 petroleum 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 sulfur and PAH content in the RME emissions.

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; 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, respectively. Levels of DNA adducts determined by ^{32}P-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 wks (6 hr/day, 5 days/wk) and measured *hprt* mutations in alveolar

type II cells in animals immediately after exposure and at 12 and 32 wks after the end of exposure. The two higher exposures caused significant increases in mutant frequency. Whereas the mutant frequency from the 7.1 mg/m^3 group returned to control levels by 12 wks, that of the high-exposure group was still higher than controls even after 32 wks. 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 wks. 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 ^{32}P-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.

Reed et al. (2004) exposed both male and female A/J mice to DE generated from a 2000 model diesel engine. Whole body exposures included dilutions at concentrations of 30, 100, 300, and 1000 µg/m^3 for exposure periods of 6 h/day, 7 days/wk, for 6 mos. The mice were then held for 6 mos without DE exposure to allow time for tumors to form; but no significant increase was found for the incidence or multiplicity of tumors. The authors conceded that the model used for assessing carcinogenesis is somewhat uncertain in this study. Another index of carcinogenic potential, micronucleus formation, was also assessed, but demonstrated no significant DE exposure effects on micronucleated reticulocyte counts.

Other diesel studies have evaluated chromosome effects. Mitchell et al. (1981) and Brooks et al. (1984), for example, 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.

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 mos of inhalation exposure to DE particles at 6 mg/m^3. Also, Pereira et al. (1981a) exposed female Swiss mice to by inhalation DE 8 h/day, 5 days/wk for 1, 3, and 7 wks. 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 mos of exposure to DE (Pereira et al., 1981b).

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 2-fold greater than the DPM extract.

In a study using 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 DE. In the dominant lethal test, T-stock males were exposed for 7.5 wks and immediately mated to females of different genetic backgrounds. There were no differences from controls in any of the parameters measured. For heritable translocation analysis, T-stock males were exposed for 4.5 wks 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 $\leq$ 1/1,000.

A number of studies have measured other types of genotoxic effects (e.g., increased DNA adducts) in animals exposed to DPM, carbon black or other particles, 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 ^{32}P 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 compared to nonexposed controls (Nielsen and Autrup, 1994). Furthermore, the findings were concordant with 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 versus 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).

7.8.3.2 Gasoline

In addition to the above studies of DE and DPM effects, other studies have also evaluated mutagenic/genotoxic effects of gasoline combustion emissions and/or compared the potentcies of such emissions to DE or DPM potencies.

In an early study, Löfroth (1981) compared the mutagenic activity of PM from diesel and gasoline engine exhaust and found both to be mutagenic in the Ames assay, in the absence of mammalian metabolic activation. Both particulate and condensate fractions were tested. Expressed in units of revertants/g fuel used, diesel exhaust mutagenic response was 800 (PM fraction) and 210 (condensate fraction), which was far greater than the mutagenic response of gasoline (24 and 39, respectively). In another older study, Rannug (1983) collected both particulate and gas phase components from motor vehicle exhaust from medium- and heavy-duty diesel vehicles and light-duty cars burning gasoline and other fuels. The Ames assay was used with strains TA98 and TA100, both with and without S9 activation. The particulate phase of the

exhaust created < 20,000 revertants/km (corresponding to 10 rev/L exhaust) in cars burning liquified petroleum and cars with catalysts, classified by the authors as the low mutagenicity group. Light-duty diesels produced > 100,000 rev/km with the highest effect of up to 700,000 rev/km seen with TA100-S9 (corresponding to 50-250 revertants/L exhaust) and were classified by the authors as the high mutagenicity group. Engines burning gasoline or gasoline-alcohol fuels created exhaust from which the particulate phase gave 10,000 to 20,000 rev/km (or 10-50 rev/L exhaust). In general, the newer vehicles tested produced exhaust with slightly less mutagencity than older models. Also, more mutagenesis was seen in exhaust from cold starts (0 °C) than in starts at 23 °C.

Strandell et al. (1994) fractionated the extracts of gasoline and diesel exhaust from Volvos to find the most potent mutagens among the subfractions. Particle emission values for the vehicles were 0.021 g/km and 0.23 g/km, respectively. Mutagenicity testing was done with the Ames assay with strain TA98, both with and without S9 metabolic activation, and with strain TA98NR, the nitro reductase-deficient strain used to determine the presence of nitro aromatic mutagens. The subfraction that was most polar also demonstrated the most mutagenicity (51% of the total mutagenicity for gasoline and 39% of the total for diesel). This fraction contained low-boiling point components and some phenol derivatives. Quantitatively, this subfraction's mutagenicity using TA98-S9, TA98+S9 and TA98NR-S9 was 7.8, 3.3 and 3.7 rev/m^3, respectively, for gasoline and 6.1, 1.5, and 4.1 for diesel. The TA98NR response was generally lower than the TA98 response, which the authors suggested was due to the presence of nitro-PAH in the fractions. Both fuels had a similar TA98NR-S9 response, but differed significantly in their TA98-S9 response, which suggests difference in some nitro-reductase-dependent mutagens. A reduction in mutagenicity was observed with the addition of S9 activation, which the authors attribute to enzymatic deactivation of direct-acting mutagens or possible activation or deactivation of unknown compounds.

A more recent study (Seagrave, et al., 2002) using vehicles including automobiles, SUVs and pickup trucks from 1976 to 2000 evaluated the genotoxicity of gasoline and diesel emissions from normal vehicles, high emitters, and gasoline vehicles emitting smoke. Both PM and semivolatile organic compound (SVOC) fractions were collected, both at room temperature and in a cold environment. The PM and SVOC fractions were recombined and tested for mutagencity using the Ames assay with strains TA98 and TA100, both with and without S9

activation. All of the samples caused mutations in both strains with doses from 25 to 5000 µg/plate (LOEL not given). With most samples evidence pointed to a direct-acting mutagenesis effect due to the results of TA98 both with and without S9 activation. The assay using TA100 showed greater mutagenicity in the exhausts from the high emitter diesel, the white smoker gasoline, and the black smoker gasoline. As seen in the Rannug (1983) study, emissions samples collected from cold engines were more mutagenic than those collected at room temperature. The authors ranked the mutagenic potency based on the TA98 results as: current diesel at 30 °F > high emitter diesel > gasoline engine emitting white smoke > normal gasoline > normal diesel 72 °F > gasoline engine emitting black smoke. The mutagenic potency based on the TA100 results were: current diesel at 30 °F > gasoline engine emitting white smoke > high emitter diesel > normal diesel 72 °F > gasoline engine emitting black smoke > normal gasoline 30 °F > normal gasoline 72 °F. The authors' goal with this work was to examine the various bioassays available to ascertain which are most useful in determining differences in mutagenicity, toxicity, and inflammation. Significant findings indicate that both diesel and gasoline exhaust emissions are mutagenic, with diesel being more mutagenic in general. The increase in mutagenicity of gasoline samples with S9 activation indicates the role of PAH in this effect. Decreased mutagenicity in the diesel sample collected at 30 °F suggested to the authors that the mutagenicity of the exhaust may be due to nitroarenes.

Pohjola et al., (2003) used the extractable organic material from the PM and SVOC gasoline and diesel exhaust fractions to examine their ability to induce mutations in Salmonella strain TA98 and to form adducts in calf thymus DNA. Doses used in the Ames assay were 30 to 500 µg/PM for the -S9 experiments and 10 to 1000 µg/PM for the +S9 experiments. Doses used for oxidative and reductive activation of PAHs were 18-300 µg PM for gasoline and 75-1,500 µg PM for diesel. Using the ^{32}P-postlabeling method, 4 µg of DNA was analyzed for bulky aromatic DNA adducts. Only the gasoline was tested in the Ames assay. The PM fraction had higher mutagenicity, which averaged 431 rev/mg PM (-S9) and 487 rev/mg PM (+S9). The SVOC fraction had only 106 rev/mg PM (-S9) and 98 rev/mg PM (+S9). However, the SVOC fraction formed more DNA adducts. PAH-DNA adduct formation with 150 µg PM gasoline extracts in calf thymus DNA ranged from 3.7 to 8.3 (-S9), 7.7 to 56 (+S9), 5.2 to 18 (-XO), and 19-60 (+XO) adducts/10^8 nucleotides/mg PM. Comparisons were made of PAH-DNA adduct levels using high doses (42 to 150 µg PM) and low doses (7.5 to 18.5 µg PM) of gasoline and

diesel extracts. Diesel PM and gasoline extracts formed more S9-mediated adducts with increasing doses, but gasoline did not have a linear dose-response. The authors suggested that complex interactions and/or inhibition of S9 caused lower concentrations of both gasoline and diesel extracts to bind DNA with greater efficiency than 8-fold higher doses. Diesel extracts formed higher levels of adducts than gasoline extracts, especially in the presence of xanthine oxidase (reductive activation), indicating possible high levels of nitro PAHs. Diesel extracts were also more mutagenic than gasoline extracts in the -S9 (direct-acting) assays, which further suggests higher concentrations of nitro-PAHs in diesel exhaust. This study corroborates earlier research demonstrating that diesel exhausts extracts are more mutagenic than gasoline extracts, and that diesel's mutagencicity can be attributed, in part, to DNA adduct formation.

7.8.4 Summary of Mutagenic/Genotoxic Effects

A number of recent in vivo and in vitro studies have suggested that ambient urban PM is mutagenic. Research evaluating the mutagenicity of ambient PM from the Los Angeles area has pointed to ubiquitous emission sources as being responsible for mutagenic activity observed in vitro (Hannigan et al., 1997, 1998). Fractionation of those ambient samples and subsequent mutagenicity assessments have indicated that six unsubstituted polyaromatic compounds and two semi-polar compounds are the likely mutagens. Mutagenicity of urban air from Germany has also been demonstrated (Hornberg et al., 1996, 1998; Seemayer and Hornberg, 1998), with evidence showing that the fine fraction of PM exerted greater toxicity. Additionally, ambient PM from high traffic areas in The Netherlands also induced genotoxic activity.

Emissions from wood/biomass burning have been shown to be mutagenic. Studies of human exposures in China (Vinitketkumnuen et al., 2002) and The Netherlands (Heussen et al., 1994), examining both chronic seasonal and acute exposures, have demonstrated increased mutagenicity with environmental exposures. Characterization of wood smoke fractions to assign mutagenicity have shown that the organic fraction is more mutagenic than the PM component and that the condensate is not mutagenic (Putnam et al., 1999). Wood smoke emissions can cause both frameshift and base pair mutations but have not yet demonstrated the production of DNA adducts.

Emissions from coal combustion have been shown to be mutagenic, especially the polar and aromatic fractions. Research in China examining populations with high lung cancer rates

have shown that emission samples from homes burning smoky coal are mutagenic in the Ames assay, and implicate PAHs as contributors to the mutagenicity (Mumford et al., 1987, 1999; Lan et al, 2002). Recent work (Granville et al., 2003) characterizing the mechanism of genotoxicity has examined the mutation spectra of coal smoke emissions from these Chinese homes. Sequencing the revertants has demonstrated that the mutations in Salmonella exposed to coal smoke extract are similar to mutations seen in lung tumors of women exposed environmentally to the coal smoke.

Extensive studies have demonstrated mutagenic activity in both particulate and gaseous fractions of DE. By sequential fractionation of DE, apportionment of the mutagenicity is possible, which has implicated nitrated polynuclear aromatic compounds as being responsible for a substantial portion of the mutagenicity. Other mutagenically active compounds include enthylene, benzene, 1,3-butadiene, acrolein, and several PAHs in the gas phase. In addition to Ames assay studies, the induction of gene mutations has been reported in several in vitro mammalian cell lines after exposure to extracts of DPM. Structural chromosome aberrations and SCE in mammalian cells have been induced by DE particles and extracts.

Early studies comparing the mutagenicity of gasoline and diesel exhaust showed that the PM component of the exhaust is more mutagenic than the condensate fraction, and that overall, diesel exhaust is more mutagenic than gasoline exhaust. More mutagenicity is also observed in exhaust from cold starts than from exhausts at room temperature. Examining the fractional mutagenicity of gasoline and diesel exhausts, it was shown that, as with coal smoke, the polar component has the most mutagenicity, and further, that nitro-PAH is present in the fraction. A comprehensive study comparing gasoline and diesel exhaust genotoxicity, using both the PM and SVOC fractions, demonstrated that both exhausts are mutagenic, but, in general, diesel exhaust is more mutagenic. Further, the study implicates PAH and nitroarenes in the genotoxicity. Another current study corroborates these finding, and includes data suggesting that DNA adduct formation is a component of the mutagenicity.

Exact comparisons of the mutagenicity of combustion emissions of these fuels are not possible because data provided in the studies vary so greatly in units in which mutagenicity is expressed. However, a very general ranking of these mutagenicity of these emissions may be: diesel > coal >> wood > gasoline. Thus, there is qualitative evidence for the mutagenic/ genotoxic potential of both ambient PM and some fuel combustion products. Many of the

published in vitro studies failed 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 was done or reported in many studies. Thus, any quantitative extrapolation of the reported findings would be quite difficult. Nevertheless, they collectively do appear to provide extensive credible evidence substantiating the biologic plausibility of, and/or elucidating potential mechanisms underlying reported epidemiologic associations between long-term human exposure to ambient PM and lung cancer.

7.9 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 types of interactive effects of particles and gaseous copollutants have been demonstrated?

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

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

One overarching issue in the interpretation of toxicology study results is the relevance of findings from experimental human or animal studies using controlled exposure/dose concentrations that are high relative to the much lower ambient pollutant exposure concentrations that apply within the context of pertinent epidemiology studies. To provide insight on this issue, EPA conducted a series of illustrative analyses using dosimetric modeling of the type discussed in Chapter 6; these analyses are described in detail in Appendix 7A. First, taking into account certain key points regarding dose metrics, one of the publically available dosimetry models (the MMPD model) discussed in Section 6.6.4 of Chapter 6 and in Appendix 7A Section 3 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-4. The second approach involved 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. Given that rats clear PM from the respiratory tract much faster than humans, MPPD modeling indicates that higher exposure concentrations in the rat may be needed, in certain cases, in order to evaluate toxicological endpoints predictive of health outcomes in humans and to investigate biological mechanisms. The higher doses needed depend on the health endpoint measured. For example, the modeling results indicate that higher PM concentration exposures in rats are needed to achieve nominally similar inflammatory responses relative to the human.

7.9.1 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.

7.9.1.1 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: (1) lung injury and inflammation; (2) increased airway reactivity and exacerbation of asthma; and (3) impaired lung defense mechanisms and increased susceptibility to respiratory infections.

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 $\mu g/m^3$). Ghio and Devlin (2001) say an active person might have an average ventilation rate of 15 l/min and thus inspire 21.6 m^3 of air per day. 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 airway 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 among some ambient PM constituents identified as 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; Veronesi 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 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., negative charges surrounded by a proton cloud).

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 both organic fraction and the carbon black fraction of DPM are 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. Thus the available evidence is inconclusive with regard to increased airway activity as a possible PM mechanism.

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. (2003) showed that brief exposures (3 to 5 h) of Fischer rats to New York City CAPs (~225 $\mu g/m^3$) 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.

7.9.1.2 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 sequelae 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 Affect 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. However, whether or not ROFA or key constituents of it causes such effects at relevant ambient or near-ambient exposure concentrations remains unclear.

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 to 18 h after surgery; and exposure to ROFA (but not to 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 high concentrations of 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 to 0.34 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., Muggenburg et al., 2000a,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, and more basic information on the effects of mild pulmonary injury on cardiovascular function is needed to more fully evaluate this hypothesis.

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 μg/m^3) in healthy nonsmokers causes increased levels of blood fibrinogen. Gardner et al. (2000) have also shown that a high dose (8300 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 to 400 μg/m^3) for 3 h. These differences in blood parameters were present at 3 h PE but not 24 h PE.

On the other hand, exposure of normal dogs to concentrated ambient PM from Boston (~100 to 1000 μg/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 μg/m^3), as reported by Nadziejko et al. (2002). Frampton (2001) also reported finding no effects on fibrinogen or clotting Factor VII in healthy, nonsmoking human adults exposed to 10 μg/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.

7.9.1.3 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,b); (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 $Ca^{+2}$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. (Muggenburg et al., 2000b; Frampton, 2001).

7.9.1.4 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.

Ambient urban PM from sources such as the Los Angeles area, Germany, and The Netherlands has been shown to possess mutagenic activity in both in vivo and in vitro assays. The mutagenicity is dependent upon the chemical composition of the PM and also the size of the particles. Both unsubstituted polyaromatic compounds and semi-polar compounds are thought to be highly mutagenic components of urban ambient PM. Additionally, the fine fraction appears to be more mutagenic than the coarse fraction.

Emissions from wood/biomass burning have been shown to be mutagenic in studies of human exposures in China and The Netherlands. The organic fraction appears to be more

mutagenic than the PM component. Mutagens from wood smoke emissions can cause both frameshift and base pair mutations but have not yet demonstrated the production DNA adducts.

A large body of work examining emissions from coal combustion in China has demonstrated the mutagenicity of both the polar and aromatic fractions. Populations with high lung cancer rates have been linked to exposures of the PAH component of coal smoke. By sequencing of mutations, a direct link has been established between the mutagenesis assay results and human lung cancer.

The U.S. EPA Diesel Document (U.S. Environmental Protection Agency, 2002) was cited earlier in this chapter as discussing a number of studies utilizing mutagenicity/genotoxicity 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 genotoxic 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 (PAHs) 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 Driscoll 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, which the authors suggest leads to increased epithelial cell proliferation and consequently, mutating. Overall, the new studies are highly indicative of mutagenic and other genotoxic effects of ambient PM and/or various specific constituents (e.g., comparisons of gasoline and diesel exhaust show that the PM component is more mutagenic than the condensate fraction in both exhausts). Additionally, the polar component has the most mutagenicity. Other components of both gasoline and diesel exhaust thought to contribute to the mutagenicity are PAHs, nitro-PAH, and nitroarenes. DNA adduct formation is one mechanism whereby these mobile combustion products are thought to induce carcinogenesis.

In summary, both ambient PM and combustion products of coal, wood, diesel, and gasoline are mutagenic/genotoxic. Exact comparisons of the mutagenicity of combustion emissions of

these fuels are not possible, but a very general ranking of these emission may be: diesel>coal>>wood>gasoline. Additionally, the data currently available allow some clues as the to potential mechanisms underlying these health effects.

7.9.2 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.

7.9.2.1 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 (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 interesting new information. Despite the difficulties associated with extrapolating from the bolus delivery used in such studies, they have provided evidence indicating 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 (shown by many studies to be relatively inert toxicologically). Similarly, dose-dependent increases in polymorphonuclear leukocytes (PMNs), other markers of lung inflammation, and decreases in alveolar macrophages (AMs) 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 have provided important exposure-response information for some PM size fractions (especially $PM_{2.5}$), they have not, to date, been very helpful in identifying specific toxic components in urban PM. Insufficient attention has been accorded to characterization of day-to-day variations in specific PM constituents in order to relate such variations to observed variable health responses to CAPs exposures. Also, 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 factor analyses on exposure/response matrices. This may also hinder principal component analysis techniques that are useful in identifying particle components responsible for health 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. CAPs studies also hold promise for helping to identify susceptibility factors in animal models, and permit examination of mechanisms related to PM toxicity.

7.9.2.2 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).

7.9.2.3 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 exposures to ambient PM extracts, ROFA, other combustion source emission materials (e.g., CFA, etc.), or specific 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, some water soluble metals (e.g., Ni, V, Zn, Fe) 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 collected from the State/Federal sampling sites yielding aerometric data used to ascribe the impact of PM on hospital admissions and population mortality rates showed remarkable qualitative coherence with toxicological and clinical endpoints (BAL fluid markers, lung dysfunction) among the human and rodent test subjects. Moreover, the data were themselves consistent with the hypothesized underlying mode of action (oxidant generation, inflammation) for metal-associated PM cardiorespiratory effects (Frampton et al., 1999; Dye et al., 2001; Ghio and Devlin, 2001; Soukup et al., 2000; Wu et al., 2001; Pagan et al., 2003). Studies comparing human (Ghio and Devlin, 2001) and rat (Dye et al., 2001) exposures to both high and low metal content PM collected near a steel plant, showed convincingly that the metal content of the PM, and not the mass, was a major determinate of the toxicity of the PM. Both species showed similar inflammatory responses to exposures from PM with high metal content (collected while the steel

mill was operating). Hence, this rich data set provides an important linkage across study disciplines 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 and appeared to be influenced by the ambient PM metal content in the respective cities. 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 exposure/dose discrepancies, not to mention species and other differences, is unclear, but the data and findings 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 (typically $\leq$ 0.5 to 1.0 $\mu g/m^3$) associated with current U.S. ambient PM mass concentrations exhibit greater toxicity than other PM components typically present in ambient air. In studies in which various ambient and emission source particulates were instilled into rats, the soluble metal content did appear to be one important determinant of lung injury (Costa and Dreher, 1997). However, one published study (Kodavanti et al., 2000b) 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.

7.9.2.4 Diesel Exhaust Particles

As described in Section 7.5.3, there is growing toxicological evidence that, analogously to several other types of PM (silica, carbon black, road dust, etc.), 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 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., PAHs), consistent with qualitative findings in several studies of increased lung cancer effects being associated with long-term, occupational exposure to diesel emissions.

7.9.2.5 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 exhaust particles from various sources to the fine PM fraction (Dreher, 2000). Strategies have been proposed for examining the health effects of this potentially important constituent (Turpin, 1999).

7.9.2.6 Ultrafine Particles

Studies of various types of ultrafine particles have demonstrated a significantly greater inflammatory response than that seen with fine particles of the same chemical composition at similar mass doses (Oberdörster 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 µg/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; Oberdörster et al., 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.

7.9.2.7 Bioaerosols

Bioaerosols, from sources such as plants, fungi, and microorganisms, range in size from 0.01 to µm to > 20 µm. They comprise a small fraction of ambient PM, but have been shown to contribute to the adverse health affects from PM exposure. Pollen from flowering plants, trees and grasses, deposits in upper airways to induce allergic rhinitis. Ruptured pollen grains can enter the deep lung, where they can exacerbate asthma. Synergistic interactions between pollen debris and other ambient PM (e.g., the polycyclic hydrocarbon component of DE) are thought to be a mechanism that may explain the increased incidence of asthma morbidity and mortality.

Human handling and burning of plant material contributes to increased bioaerosol levels, which have been shown to have adverse health effects.

Animals and insects produce bioaerosols capable of producing hypersensitivity diseases. Most notably, exposure to dust mite and cockroache material has been linked to sensitization in children. Fungal spores are the largest and most consistently present outdoor bioaerosol. They cause allergic rhinitis and asthma, which is highly dependent on seasonal variations in concentration. Exposures have been linked to asthma hospitalization and death.

Bacterial and viruses are significant bioaerosols. Much of the toxicity of bacteria is due to the endotoxins present in the outer cell membrane, which trigger production of cytokines and a cascade of inflammation. Concentrations of endotoxins are seasonal and tend to be higher in samples of coarse-mode than in fine-mode ambient PM. Another cell wall component of bacteria and fungi, (1→3)-β-D-glucan, has also been shown to cause respiratory inflammation.

7.9.3 PM Interactions with Gaseous Co-Pollutants

Particulate matter exists in an atmospheric milieu of ubiquitous co-pollutant gases, all of which have the potential for antagonistic, additive, or synergistic interactions with PM and which can modify the toxicologic health effects. The mechanisms by which interactions between PM and gases are thought likely to occur are by: (1) formation of secondary products by chemical interactions between the gas and the particle, (2) adherence of material to the particle and subsequent transport to sensitive sites, and/or 3) pollutant-induced change in the local microenviroment of the lung (e.g., by decreasing the pH). All of these interactions have the potential to create antagonistic, additive, or synergistic interactions between PM and gases, which could potentially greatly modify their individual effects.

Several new controlled human exposure toxicology studies provide interesting findings on effects of combined exposures to PM and other pollutants. One study, by Linn et al. (1997), found a positive association between acid concentration and respiratory symptoms (but not spirometry) among allergic/asthmatic children (in comparison to clean-air exposure results) following a single 4-h 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. However, the experimental design did not include comparison of effects of the overall mixture versus those of individual components (e.g., H_2SO_4 alone, O_3 alone), thus precluding discernment of possible

interactive effects. Another human exposure study by Brook et al. (2002) reported that combined exposure (for 2 h via inhalation) to $PM_{2.5}$ CAPs (150 μg/m^3) and O_3 (120 ppm) increased brachial artery vasoconstriction over filtered air control values, but it too did not compare the CAPs/O_3 effects versus CAPs or O_3 alone.

A few recent animal studies have demonstrated that co-exposures of CAPs and O_3 causes potentiation of proliferative changes in the epithelium of terminal bronchioles and enhanced septal cellularity seen with O_3 exposure alone. Two studies showed that co-exposures at much higher concentrations of CAPs (5, 40, 50 mg/m^3) and O_3 (0.8 ppm) cause potentiation of proliferative changes in the epithelium of terminal bronchioles (Vincent et al., 1997) and enhanced septal cellularity (Bouthillier et al., 1998) over that seen with the O_3 exposure alone. However, the reported effects at such extremely high CAPs and O_3 exposures are not likely of much relevance to the understanding of possible interactive effects of ambient PM or O_3 present at much lower levels in contemporary U.S. urban airsheds. In another study, both combined CAPs/O_3 and O_3-alone exposure in a mouse asthma model (Kobzik et al., 2001) showed similar increases in airway responsiveness and pulmonary resistance, thus indicating a lack of synergism with the combined exposure. Mixtures of elemental carbon particles, O_3, and ammonium bisulfate showed changes lung collagen, AM respiratory burst, and phagocytosis (Kleinman et al, 2000), although the results were ambiguous as to whether PM was enhancing the effects of O_3 or the converse. A short exposure of combined carbon particle/SO_2 caused depressed AM phagocytosis and suppressed intrapulmonary bactericidal activity which lasted for a week (Jakab et al., 1996; Clarke et al., 2000c).

Other studies using co-exposures of PM and gases have demonstrated no changes in histopathological (Moss et al., 2001) or biochemical and morphometric endpoints (Last and Pinkerton, 1997). Additionally, 4-wk exposures of rats to a mixture of carbon particles, ammonium bisulfate, and O_3 caused no changes in BALF parameters and only a small decrease in plasma fibronectin compared to O_3 alone (Bolarin et al., 1997).

7.9.4 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, 2000a,b; Kodavanti et al, 1998, 2000a, 2001; Gordon et al., 2000; Ohtsuka et al., 2000b; 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 is difficult to know what disease models to use in evaluating the biological plausibility of adverse health effects of PM.

Compromised hosts are a significant susceptible population which includes individuals with asthma, individuals with pneumonia or other lung infections, and the elderly with chronic cardiopulmonary disease. To better understand the effects of PM in this population, researchers are increasingly using compromised host models such as a rat model of cardiopulmonary disease that utilize MCT-treatment to induce pulmonary vasculitis/hypertension. This model has demonstrated ROFA-induced increased neutrophilic inflammation, exacerbated lung lesions, increased lung edema, alveolar thickening, and decreased phagocytosis of particles in some studies (Costa and Dreher, 1997; Kodavanti et al., 1999; Madl et al., 1998). Another report using MCT-treated rats did not find similar inflammatory responses or changes in pulmonary function following CAPs exposures (Gordon et al., 2000),

Animals infected with bacteria or viruses are used to model humans with respiratory infections; and they have been shown to have increased inflammatory response with PM exposure (Kodavanti et al., 1998b; Elder et al., 2000a,b). Rats pre-treated with high SO_2 exposures have been used to model chronic bronchitis and have shown interaction of CAPs with preexisting lung injury which produce changes in cellular and biochemical markers in lavage fluid and increases in tidal volume (Clarke et al., 1999; Saldiva et al., 2002; Kodavanti et al., 1998b).

Genetic susceptibility to the effects of PM are becoming increasingly apparent as various strains of rodents are characterized for strain-specific responses. Rat strains such as SD, Fischer-344, and Wistar demonstrate unique inflammatory and histological responses to ROFA (Kodavanti et al., 1996, 1997b). Also, genetically predisposed spontaneously hypertensive (SH) rats have been used to model cardiovascular disease to evaluate potential increased susceptibility to PM-associated effects. SH rats demonstrate greater oxidative stress and cardiovascular responses than their normal counterparts in response to ROFA exposure. Inter-strain differences

in airway hyperresponsiveness, inflammation, Fc-receptor-mediated AM phagocytosis, and mortality, too, have been demonstrated in mouse strains such as BALB/c, C57BL/6, C3H/HeJ, and others (Veronesi et al., 2000; Ohtsuka et al., 2000a,b; Prows et al., 1997; Leikauf et al., 2000, Wesselkamper, et al., 2000) in response to PM exposure. The extent to which genetic susceptibility plays a role in humans remains to be determined. Use of genomics, proteomics, and bioinformatics technologies will allow further characterization of the differences in susceptibility to PM.

Newly available studies also suggest that individuals with allergic disorders are likely more susceptible to PM effects than are non-allergic persons. Relatively little is known about the effects of inhaled PM on humoral (antibody) or cell-mediated immunity. However, both in vivo and in vitro studies have shown that various types of PM can alter immune responses to challenge to antigens and may act as an adjuvant (Van Zijverden et al., 2000; Van Zijverdan and Granum, 2000). Steerenberg et al. (2003) found adjuvant activity to be associated with several types of PM tested (e.g., road tunnel dust, ROFA, DPM).

ROFA has been shown to enhance allergic sensitization in a number of studies (e.g., Hamada et al., 1999; Lambert., 1999; Gilmour et al., 2001), but the applicability of these findings to ambient PM is not certain. A study has shown that a single exposure to ROFA elicits a greater effect on airway hyperresponsiveness than a 3 day exposure to CAPs (Goldsmith et al., 1999)

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 Zijerden 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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