8 EFFECTS ON VEGETATION

The research community acknowledges the effect of suspended particles on vegetation in the research community, however, researchers have not focussed the same level of effort as has been devoted to the study of the effects of phytotoxic pollutants (NO_2, SO_2 and O_3) (Farmer, 1993). The high deposition of inert particles from the use of fertilizers and lime, and by the emissions of cement dust from cement works in the early 1900s prompted the first research on the effects of dust on vegetation (Pierce, 1909; Duggar and Cooley, 1914).

Stomata are the primary entry point of atmospheric pollutants into vegetation. Typical stomatal openings are 10-12 µm long by 2-8 µm wide (Smith, 1990; Farmer, 1993). Some corn and cereal stomata are up to 20-32 µm long and 8-12 µm wide (Krajickova and Mejstrik, 1984). The primary mechanisms by which particles affect vegetation (Pyatt and Haywood, 1989; McCune, 1991; Farmer, 1993) are by:

- physical smothering of the leaf surface,
- physical blocking of stomata through particle lodging or penetration of stomatal apertures,
- inducing a chemical effect due to particle composition, and
- indirect effects on soil pH and ionic composition.

There are three ways particles can deposit upon leaf surfaces: by sedimentation under the influence of gravity, by impaction under the influence of eddy currents and by deposition under the influence of precipitation (Bache, 1981; Smith, 1990). For particles of about 10 µm, impaction is efficient, and interception by fine hairs on vegetation is likely an effective retentive mechanism. The magnitude of the effect of the particles will be determined by the deposition rate under conditions of variable microclimates.

The physical smothering of the leaf surface causes reduced light transmission, affecting photosynthetic processes (Thompson et al., 1984; Pyatt and Haywood, 1989; Farmer, 1993). It is estimated that typical roadway depositions are <2 g dust/m^2, and the resultant effect of PM_{10} from automobile exhaust on the photosynthesis of trees is likely to be small (Thompson et al., 1984). Increases in the temperature of particle-covered leaves result in a positive impact on respiration and a negative impact on photosynthesis and productivity (Eller, 1977).

Chemical effects and metal accumulation have been observed from particle deposition. Sphagnum accumulated Al, Cr, Fe, Ni, Sc, Sm and V from a nearby gravel road with dust particles of 3-100 µm diameters (Santelmann and Gorham, 1988). Saline particles on vegetation caused accumulation of Cl, production of foliar lesions, and changes in levels of plant mineral nutrients and metabolites, physiological processes and growth and reproduction (McCune, 1991).

The physical obstruction of the stomata has been observed to reduce stomatal resistance, resulting in the potential for higher uptake of pollutant gases, and it may also affect the exchange of water vapour. There are several examples of this. In a deciduous forest, *Quercus petraea* (Mattuschka) Leibl (oak) leaves were studied for stomatal blockages (Williams et al., 1971). At mean deposition rates of 11.5 µg/cm^2 and 70.7 µg/cm^2, approximately 8% and 40%, respectively, of stomata were blocked open with particulate debris from a smokeless fuel plant. That same study also observed that accumulation of particles at a rate of 200–600 mg/m^2 interfered with normal stomatal behaviour by lowering stomatal resistance at night, and higher levels of total leaf sulphur were observed (Ricks and Williams, 1974). A study of laurel leaves near a steel works operation found that average daily smoke concentrations of 17–30 µg/m^3 caused approximately 50% blockage of stomata (Pyatt, 1973). In one instance, exposure to fly ash particles (83% were <8 µm) did not obstruct stomata, however, the particles did accumulate on the surface of guard cells, stimulating the mechanism regulating the opening and closing of the stomata and blocking the closing of the stomata (Krajickova and Mejstrik, 1984).

Particle accumulation on leaf surfaces may cause plants to become more susceptible to other stresses. Sassafras and grape leaves were observed to be more susceptible to leaf spot diseases caused by fungi than were leaves of comparable plants with no visible dust deposits; numbers of microflora were

higher on dusty leaves; and second-year dusty hemlock needles were frequently more chlorotic (Manning, 1971).

Much literature describing the effect of cement dust on vegetation is available; however, it often fails to show the size distribution of the particles. Cement particle diameter ranges can be typically 1-100 µm (Hinds, 1982). It is estimated that 80-90% are less than 30 µm. Relatively high deposition rates (from 1 to 7 g/m^2 for up to 130 days) of cement dust on cereals have resulted in decreased respiration, catalase activity, oil content and overall yields (Borka, 1980; Singh and Rao, 1981; Shukla et al., 1990). Estimating whether the negative effects are governed by the sheer volume of particulate loading or by the size of the particles is difficult. A review of the effects of cement dust on trees showed that the dust caused physical damage to the leaves, reduced fruit setting and generally reduced growth (Farmer, 1993).

Small particles (10 µm) are of significance to forest ecosystems owing to long-range transport, preferential contamination with toxic metals and efficiency of vegetative capture via impaction (Smith, 1990).

Accumulation of metals and stomatal interference have been observed for particles in the 10 µm size range for a variety of vegetation types and particle sources. Insufficient information is available to estimate a relationship between ambient concentrations of particles less than 10 µm in diameter and vegetation effects. This relationship will be extremely complex, given the interactions between particle size, particle composition and vegetation species variability.

Conclusions

This review has focussed on the impact of inert particles and as such, describes the limited information available. Indirect and possibly significant effects on vegetation from: reduced light intensity (through particle loading of the atmosphere), secondary aerosols and nutrient imbalances through particle deposition to soils are not addressed. Neither are the potentially significant impacts of particles that are of similar or smaller size than the stomatal openings.

Conclusive information about the effects of PM on vegetation is quite limited and robust quantitative dose-response information is lacking.

9 EFFECTS ON MATERIALS

The deposition of particulate matter on materials can reduce their aesthetic appeal, increase their rate of physical and chemical degradation. Particles can act as catalysts for the conversion of SO_2 and NO_x to sulphuric acid and nitric acid (Santachiara et al., 1989). These acidified particles can accelerate the chemical degradation of the susceptible material surface on which they are deposited.

Most available information in the literature is on the effects of particle exposure in combination with SO_2. The following discussion will, however, focuses on the effects associated only with particulate matter exposure. Two categories of effects are discussed: 1) corrosion and erosion, and 2) soiling and discolouration.

9.1 CORROSION AND EROSION

9.1.1 Metals

Most studies (e.g., Sanyal and Singhania, 1956; Yocom and Grappone, 1976) have linked the presence of particulate matter in the air to the enhanced speed of corrosion on metal surfaces. Goodwin et al., (1969) found that damage to steel, protected with a nylon screen and exposed to quartz particles, became substantial when the steel was exposed to particles of diameter 5 µm or larger. The corrosion of iron in an atmosphere of 0.15% by volume SO_2 and at varying relative humidity values between 55% and 95% was found to increase in samples with carbonaceous particulate matter (Skerry et al., 1988a, 1988b; Askey et al., 1993).

There are two different views on how particulate matter, together with SO_x, plays a role in the corrosion of metals. The first states that the catalytic species in particulate matter (e.g., ash) accelerate the oxidation of SO_x. Therefore, SO_x alone will not lead to an acceleration in corrosion (Walton et al., 1982). The second view states that PM increases the conductivity of the metal surface moisture layer after the dissolution of soluble anions contained within the particle. The net effect is to enhance corrosion. The increase in atmospheric corrosion rates in this case is predominantly due to the quantity of species that can be leached from the particulate. If the leachate is of higher pH or contains significant quantities of inhibitive ions (e.g., Ca^{2+}), then the corrosion rate may not increase significantly (Askey et al., 1993).

9.1.2 Paint

Existing evidence shows that paint durability is altered by the presence of particulate matter (National Research Council, 1979). Some studies also suggest that more corrosive pollutants are carried on the particulate matter, allowing them to reach the underlying surfaces or to serve as concentration sites for other pollutants on painted surfaces (Cowling and Roberts, 1954). General Motors conducted field tests to study the effect of particulate matter on finishes of automobiles. The tests concluded that damage on the paint surface was mostly due to the reaction of calcium from dust with sulphuric acid in rain or dew, forming calcium sulphate deposits on the surface of the paint. Damage increased with increased exposure time; however, washing the panels within 24 hours of a wetting event significantly reduced the damage (Wolff et al., 1990).

9.1.3 Stone

Heavy metals contained in dust convert SO_2 to sulphuric acid and could thus accelerate stone corrosion (Luckat, 1972). Slow erosion of stone surfaces, similar to sandblasting, is also possible under strong wind conditions (Yocom and Upham, 1977).

The surfaces of buildings contain zones protected from rainfall and surface runs. With time, a hard, irregular, black crust usually covers these zones. This crust was composed of aerosols and particulate matter of atmospheric origin and is thought to play a role in the deterioration of the stone surface. Del Monte et al., (1981) plotted the particulate size distribution of a thin section of crust from northern Italy; they found the median number diameter to be very close to 10 µm. Following a series of analyses, the authors concluded that oilfired boilers played a substantial role in the formation of this crust. Generally, the crust composition will vary from site to site depending on the composition of the particulate airborne pollutants

in the area (Del Monte et al., 1981; Sabbioni and Zappia, 1992).

9.1.4 Electronics

Particulate matter of natural and anthropogenic origin, ranging in size from tens of angstroms to 1 µm, can cause corrosion and failure of electronics. Failure can be caused by several factors, including the hygroscopic property of the particles or the formation of a conductive moisture film on insulating surfaces, thus leading to electrical leakage. Sinclair (1992) demonstrated from data collected in the 1980s that the indoor concentration of anthropogenic particles and their rate of arrival at surfaces is comparable to those of many corrosive gases in urban environments.

9.2 SOILING AND DISCOLOURATION

Soiling of materials is a concern, because of its potential aesthetic impact and because it precipitates more frequent cleaning and repainting, thereby reducing its lifetime usefulness and increasing costs associated with maintenance of the material. Haynie and Lemmons (1990) describe soiling as the contrast in reflectance of particles on a substrate to the reflectance of the bare substrate. Deposition studies have shown that more than 50% of the deposited matter could be due to particulates greater than 10 µm in diameter. However, it does not follow that these particulates are the major source of soiling, as it is known that fine particulates have greater light extinction characteristics (see chapter 7) and may be bound more strongly to surfaces.

Soiling also refers to degradation that can be remedied by cleaning, washing and/or repainting. However, determining the level of particulate matter accumulation that leads to increased cleaning is difficult. In a study by Hancock et al. (1976), visually perceived dust accumulation and a telephone survey were used to draw the conclusion that a dustfall rate of less than 0.17% surface coverage (effective area coverage) would be tolerated by the general public. Hamilton and Mansfield (1993) state that cleaning and repainting are usually triggered following a 30% loss of reflectance.

9.2.1 Building Materials

Theoretical dose-response relationships have been developed with the intent to describe the soiling of surfaces by airborne particles and predict the necessary cleaning frequencies. For example, Beloin and Haynie (1975) conducted experiments in five locations in the US over a two year period. Six different building materials were tested: painted wood, concrete block, brick, limestone, asphalt shingles and window glass. TSP levels among the sites ranged from 60-250 mg/m^3. Surface reflectance was used as a measure of soiling. The authors found that, except for asphalt, soiling of all the material was directly proportional to the square root of the TSP dose, with TSP dose equal to the product of TSP level and exposure time. For asphalt, soiling was directly proportional to the TSP dose. Van Aalst (1986), in a review of the literature, stated that the major fault in models presented to date was the lack of experimental verification.

Hamilton and Mansfield (1991,1993) attempted to address this issue, focussing their work on white-painted wood. A number of equations were presented for the calculation of reflectance of a surface based on experimental data and for the calculation of soiling rate based on models. These theoretical models describe soiling as a function of either TSP (Haynie, 1986) or elemental carbon (EC) (Lanting, 1986). The conclusion drawn was that for model output and experimental results to agree, many factors must be considered in choosing the right equations to apply. Two factors appear to be especially critical:

1) The physical and chemical composition of the airborne particles, especially the proportion of EC, since their strong light absorption properties will affect the soiling rate. Recent increases in the proportion of fine particles and EC (from diesel emissions primarily) in ambient PM requires some modifications to the model equations which were derived at a time when coarse particles comprised a larger fraction of PM and when emissions were predominantly from coal combustion.

2) Whether or not the surface is sheltered from the rain, which if not protected, will promote cleaning, by washing PM (primarily coarse particles) off the surface of the material. Exposed surfaces, therefore, tend to have slower soiling rates than sheltered surfaces although the experimental data demonstrated that the effect of rainfall on soiling has some complexities to it (e.g., rainfall may lead to redistribution of particles on surfaces with a consequent increase in localized soiling).

Table 9.1 summarizes some of the experimental data presented in Hamilton and Mansfield (1993). Soiling

Table 9.1 A Summary of Soiling Rates for White-painted Wood in a Tunnel and an Ambient Environment (Hamilton and Mansfield, 1993)

Exposure time	% loss in reflectance		
	Road tunnel [C_{tsp} = 208 µg/m^3]	Ambient atmosphere [C_{tsp} = 45 µg/m^3]	
		Sheltered wood	Exposed wood
50 days	25	11	9
100 days	45	18	15

was greater in both of the sheltered environments than in the exposed situation; however, the five-fold difference in TSP levels between the two experimental sites was not matched by a proportional change in reflectance (the latter changed by a factor of two to three). The authors report that the difference in observed reflectance is consistent with soiling being proportional to the square root of TSP dose, as described above, for example, by Beloin and Haynie (1975).

Haynie and Lemmons (1990) also conducted a soiling study aimed at determining the environmental factors that play a role in the soiling of white-painted surfaces. Five conclusions were reached by these authors:

- Coarse particles (between 2.5 and 10 µm) initially contribute more to soiling of both horizontal and vertical surfaces than fine particles ($\leq$2.5 µm).
- Coarse-mode particles, however, are more easily removed by rain than are fine particles.
- For sheltered surfaces, reflectance changes are proportional to surface coverage by particles, and particle accumulation is consistent with deposition theory.
- Rain interacts with particles to contribute to soiling by dissolving particles and leaving stains.
- Very long-term remedial actions are probably taken because of the accumulation of fine rather than coarse particles.

Similar results were also reported by Creighton et al. (1990).

9.2.2 Fabrics

There is no recent information in the published literature on the effect of particulate matter on fabrics. Earlier studies show that abrasive particles are damaging to fabrics. Also, the soiling of fabrics by particulate matter increases washing frequency, thereby reducing their life usefulness.

9.2.3 Indoor Soiling of Works of Art

Potential soiling of artwork in five southern California museums was studied by Ligocki et al. (1993). Buildings with sophisticated ventilation systems and particle filters showed much lower seasonally averaged concentrations of particulate matter. For example, museums with good ventilation/filter systems typically had indoor fine particulate concentrations of less than 10 µg/m^3, whereas one museum with no control system had indoor fine particulate concentrations averaging 60 µg/m^3. The authors found fine elemental carbon and soil dust particles from the outdoor environment present indoors.

Nazaroff and Cass (1991) proposed several means by which to reduce the hazard of art soiling, including reducing the building ventilation rate, increasing the effectiveness of particle filtration systems, the use of display cases and the elimination of indoor particle sources. The authors predicted that such control measures could reduce the soiling rate by at least two orders of magnitude.

9.3 CONCLUSIONS

The limited amount of scientific literature supports observational data that particles affect materials either by soiling, eroding or corroding the surface. Specific information on the effects of chemical composition and quantitative dose-response relationships are not currently available, nor is it currently possible to quantify the particle exposure level at which materials damage and soiling are perceived. It should be noted, however, that the soiling potential of fine particles is greater than that of coarse particles, given the greater rate of light extinction.

10 TOXICOLOGY

Toxicological studies involve the controlled testing of experimental animals (or tissue samples) to known quantities of PM under laboratory conditions. Whereas the human epidemiological studies discussed in Chapter 12 reflect exposure to the full range of ambient particle mixtures, the studies with experimental animals have, for the most part, been restricted to well-defined particle species. Although not directly comparable to the complex ambient particle mix, the animal toxicology studies provide valuable information about mechanistic aspects of particle toxicity, including the fate of particles in the lower respiratory tract, and information describing the biological responses that particles can elicit in the lung. In toxicological studies, PM are administered either by inhalation, or by intratracheal instillation.

Inhalation studies themselves can be conducted as whole-body exposures or as nose/mouth-only exposures. Generally, the interpretation of whole-body exposure studies can be complicated by the systemic effects of ingestion of the test substance during preening. Nevertheless, for the most part, the effects of concern observed in the published studies are limited to the airway itself, minimizing any potential confounding effects of ingestion.

Particle size is believed to be the most important characteristic influencing deposition in the respiratory system (Lippmann, 1977; Dockery and Pope, III 1994; Anderson et al., 1990). Since fine particles ($PM_{2.5}$) readily enter the respiratory system they are considered to pose a greater health risk than coarse particles. Up to 80% of PM_{10} and 60% of the total suspended particles (TSP) are made up of $PM_{2.5}$. Therefore, in this review we have paid great attention to particle sizes used for animal toxicology studies.

It should be noted that for the purposes of this review, the animal toxicology assessment will be limited to the particle effects *per se*. Where individual chemical compounds are identified on particles, the toxicology assessment will be limited to the chemical's effect as an integral part of the particle, except where the toxicity of the identified chemical is central to the toxicity of the particle.

It should also be noted that considerable uncertainty is involved in extrapolating results from animal inhalation studies and applying these results to humans for purposes of quantitative risk assessment. These uncertainties relate to differences in the sensitivities of specific target cells, in cell populations in the individual airway generations of animal species, in metabolic activities of lung cells, in the lifespans between laboratory animals and humans, and also in the dosimetry in the respiratory tract. The issue of animal to human extrapolation is addressed in Section 10.10.

This review on animal toxicology studies is a summary based extensively upon a contract report prepared for Health Canada, entitled "Particulate Air Pollution: Animal Toxicology" (Oberdörster et al., 1994b). Recently, a large body of literature on particle toxicity studies have come to light, providing further evidence concerning the impact of particles on experimental animals. In particular, data from several animal studies have shed light on the potential underlying mechanism of particle-induced cardiovascular diseases. This may provide biological plausibility for the recent epidemiological findings of a consistent association between fine particle air pollution and increased hospital admissions due to cardiovascular diseases (Burnett, 1995; Schwartz and Morris, 1995). Thus, in this report a great effort is made to provide the latest available data, in an attempt to assess the mechanisms and concentrations of particle-induced toxicity.

The studies reviewed in this report are divided into those involving acute and short-term exposure, and those that used subchronic and chronic exposure. An acute exposure is defined as single 4 or 6 h exposure to the test chemical. A subchronic study generally involves repeated exposure for approximately one tenth of the lifetime of the species studied. Chronic studies usually mimic those encountered in the workplace with animals exposed 6 h per day, 5 days per week for their lifetime.The effects of PM on a number of different health endpoints are considered: lung function, host defences, tissue cellular and biochemical responses, mortality, cardiovascular response, effects on compromised lungs and cancer.

The effect of combined exposures of PM with other air pollutants is also considered. The chapter concludes with a discussion of the critical issue of animal to human extrapolation, and provides a discussion of the mechanistic aspects of particle-induced health effects.

10.1 VENTILATORY LUNG FUNCTION

10.1.1 Acute and Short-Term Exposure

In some widespread geographic areas of North America, silica can be found among important ambient PM constituents. Studies involving intratracheal instillation of silica (<5 µm) in rats demonstrated changes consistent with airflow obstruction, with a restrictive lung disease component, although the amounts of silica used (10-40 mg/animal) were well beyond those likely to be encountered in the environment (Gross et al., 1984; Wright et al., 1988; Churg et al., 1989). Similar levels of iron oxide (0.1 µm MMAD, 10 mg) showed no significant changes in a variety of pulmonary function parameters (Wright et al., 1988). The instillation of 100 mg latex microspheres (0.1 µm) into the lobes of sheep also failed to demonstrate any altered pulmonary function (Begin et al., 1985).

Guinea pigs exposed for 1 h to aerosols of sodium sulphite (0.36 µm MMAD, 474–972 mg/m^3) showed dose-related decreases in total lung capacity, vital capacity, functional residual capacity, residual volume and diffusion capacity (Chen et al., 1987).

The role of particle acidity in potentiating toxicity has been demonstrated in a series of studies. Inhalation of zinc oxide particles (0.05 µm, 0.87 mg/m^3) coated with sulphuric acid at a concentration of 20 µg/m^3 resulted in hypersensitivity responses of the conducting airways of guinea pigs, whereas the zinc oxide without the acid coating did not produce such effects. A 10-fold higher concentration of acid alone was required to produce similar effects (Chen et al., 1992a). A 1 h exposure to zinc oxide alone at 6 mg/m^3 also showed no effects (Chen et al., 1991a). Similar exposures to acid-coated copper oxide particles (<0.1 µm, 1-2 mg/m^3) caused a prolonged change in pulmonary mechanics (Chen et al., 1991b).

Fine H_2SO_4 aerosols have been shown to induce nonspecific airway hyper-responsiveness to stimuli, a hallmark of human asthma, in otherwise healthy animals. El-Fawal and Schlesinger (1994) exposed rabbits for three hours to submicrometre sulphuric acid aerosols (50–500 µg/m^3) and then used an *in vitro* assay to determine the airway responsiveness to acetylcholine or histamine. Hyper-responsiveness was observed following acid aerosol exposure concentrations ≥75 µg/m^3 (El-Fawal and Schlesinger, 1994). When rabbits were exposed to 250 µg/m^3 of H_2SO_4 for 1 hour per day, 5 days per week for 4 months, H_2SO_4 caused bronchial hyper responsiveness and alterations in airway diameter and secretory cell number, while pulmonary mechanics parameters did not show significant change (Gearhart and Schlesinger, 1989). The underlying mechanism for hyper-responsiveness appears to be interference with normal contractile/dilatory homeostatic processes in the airways via modulation of airway receptors involved in maintenance of airway tone. This assumption is supported by an observation of decreases in the production of pulmonary prostaglandins E_2 and $F_{2\alpha}$, and thromboxane B_2, by H_2SO_4 inhalation (250-1000 µg/m^3, 1 h/d for 5 days) in rabbits (Schlesinger et al., 1990). These eicosanoids serve as potent mediators of smooth muscle tone and inflammatory response.

When guinea pigs were exposed to fly ash (0.21 µm MMAD, ~6 mg/m^3) from low-acid Montana lignite coal or high-acid Illinois no. 6, only those exposed to the high-acid coal showed reduced pulmonary function, which persisted for up to 96 h post-exposure (Chen et al., 1990).

A possible role of endotoxin contamination of particles, which may be a component of indoor air pollution, as a cause of particle-associated changes in ventilatory function is suggested in the study by Gordon (1992). Guinea pigs exposed to machine oil aerosols (10-100 mg/m^3) contaminated with endotoxin (0.3-5.3 µg/m^3) showed significant decreased airway conductance, whereas non-contaminated oils produced no effects.

10.1.2 Subchronic and Chronic Exposure

Most subchronic and chronic exposure studies in which the ventilatory status of animals was measured involved multiple instillations of relatively high mass burdens of particles or aerosol exposures performed at mass concentrations >1 mg/m^3. For essentially all types of particles investigated to date, such exposures have been found to cause significant compromises in various lung ventilatory functions. The severity of changes observed with a given particle type generally increases with exposure duration and mass concentration.

Instillation of 100 mg of silica into the lungs of sheep every 10 days for 18 months resulted in decreased pulmonary functional status, including decreased lung compliance, vital capacity and diffusion capacity (Begin et al., 1989).

Exposure to diesel exhaust particles resulted in increased pulmonary flow resistance in guinea pigs (~6 mg/m^3, 20 h/d, 7 d/wk) for 28 days (Wiester et al., 1980). Fedan et al. (1985) showed that the contractile response to acetylcholine was increased by chronic, two-year exposure of rats (7 h/d, 5 d/wk) to diesel exhaust (2 mg/m^3), coal dust (2 mg/m^3) or a combination of the two (additive response). Mauderly et al. (1988) exposed rats for up to 30 months (7 h/d, 5 d/wk) to diesel exhaust at 0.35, 3.5 or 7.1 mg/m^3 and reported reduced lung volume and compliance at the higher doses.

Pulmonary function changes were reported in rats following two-year exposures (6 h/d, 5 d/wk) to silica (1 mg/m^3) or test toner (16 mg/m^3). However, no effects were seen after exposure to titanium dioxide (5 mg/m^3) (Heinrich et al., 1989). Guinea pigs showed a decreased tidal volume throughout a one-year chronic exposure to cotton dust (21 mg/m^3, 6 h/d, 5 d/wk) (Ellakkani et al., 1987). In rats exposed to aerosolized volcanic ash (1.6-2.5 µm MMAD, 5-50 mg/m^3) or quartz (1.7-2.5 µm MMAD, 50 mg/m^3) for up to 12 months (6 h/d, 5 d/wk), only the quartz-exposed lungs developed significantly higher lung volumes, and both quartz-exposed animals and those exposed to the highest concentration of volcanic ash studied had elevated respiratory rates (Wehner et al., 1983).

Rabbits exposed to submicrometre sulphuric acid aerosols delivered at a mass concentration of 250 µg/m^3 for 1 h/d over a period of four months showed an increase in airway sensitivity to acetylcholine (Gearhart and Schlesinger, 1986). The cumulative dose inhaled by the rabbits is similar to current peak daily doses from ambient exposure in some areas of North America.

Unfortunately, the studies done to date do not provide any insight into how different sizes of the same type of particle may affect ventilatory function, and it generally remains unknown how subchronic or chronic exposures in the range of ambient concentrations might affect lung function.

10.2 HOST DEFENCES

10.2.1 Mucociliary Clearance and Conducting Airways

Acute and Short-Term Exposure

The main lines of defence against particles that deposit in the conducting airways are a mucous lining that entraps deposited particles and a cilia-based mucociliary transport system that conveys mucus and associated constituents out of the lower respiratory tract. Acute exposures to a variety of types of particles have been shown to affect mucociliary clearance or the action of cilia that propel airway mucus. The exposure of hamsters for 2 h to nickel chloride (<1 µm) at a mass concentration of 100 µg/m^3, for example, has been shown to decrease ciliary beat frequency (Adalis et al., 1978). On the other hand, Schiff et al. (1979) found no consistent changes in ciliary beat frequency after hamsters were exposed for 3 h to carbon black (0.3 µm) at a mass concentration of 1.5 mg/m^3. A 5 d inhalation exposure to volcanic ash (0.65 µm MMAD, 9.4 mg/m^3) showed no effects in hamsters (Grose et al., 1985). Such findings suggest that changes in ciliary beat frequency following particle deposition in the airways are a function of the chemical characteristics of the particles.

Differing effects on mucociliary clearance have been observed with acid aerosols. Two studies performed with rabbits (Chen and Schlesinger, 1983; Naumann and Schlesinger, 1986) showed that 1 h exposures to submicrometre-sized sulphuric acid enhance mucociliary clearance of inert tracer particles at <1 mg/m^3 but decrease clearance at higher doses. Exposures of mice to 3.2 µm sulphuric acid droplets (15 mg/m^3) reduced mucociliary clearance, whereas exposure to 0.6 µm sulphuric acid particles (1.5 mg/m^3) had no effect (Fairchild et al., 1975). In the rat, a 4 h exposure to sulphuric acid (0.4 µm) at a mass concentration of 3.6 mg/m^3 caused no changes in short-term clearance (Phalen et al., 1980). In hamsters, however, a 2 h exposure to submicrometre-sized sulphuric acid at a mass concentration of ~860 µg/m^3 resulted in a reduction of ciliary beat frequency, which presumably would translate into a reduction in mucociliary clearance. The collective findings made in these studies suggest that lower doses, as a function of both concentration and time (C × T), increase mucociliary clearance, whereas higher doses have the opposite

effect. These studies also show that the dose-response relationship for this effect is species-dependent.

Subchronic and Chronic Exposure

Relatively few studies have been performed to examine how subchronic or chronic exposure to particles may affect the airways and mucociliary clearance. In the guinea pig, four-week exposures to sulphuric acid aerosol at a mass concentration of 1 mg/m^3 resulted in airway epithelial injury and the loss of cilia (Nagai et al., 1991). In donkeys, six-month exposures to submicrometre-sized sulphuric acid at a mass concentration of 104 µg/m^3 resulted in a slowing of bronchial clearance (Schlesinger et al., 1979). A four-month exposure of rabbit to H_2SO_4 (250 µg/m^3) also resulted in a reduction of mucociliary clearance (Gearhart and Schlesinger, 1989). Rabbits exposed to sulphuric acid aerosol at 125 µg/m^3 (0.3 µm MMAD) for up to one year showed progressive slowing in clearance times (Schlesinger et al., 1992a). An increase in the numbers of secretory cells in the small airways at 12 months was also shown.

The slowing of tracheobronchial mucociliary clearance reflects changes in mucus secretory capacity, alteration in the type of mucus produced, and disruption of normal morphology of cilia and ciliated cells, which, again, can lead to the impairment of pulmonary defense capacity (Samet and Cheng, 1994).

10.2.2 Pulmonary Macrophage Response and Function

Acute and Short-Term Exposure

The alveolar macrophage response is the primary, first-line defence of the alveolar region against inhaled particles. Particle deposition in the alveoli can cause a relatively prompt expansion in the size of the alveolar macrophage population and also an influx of polymorphonuclear leukocytes (PMN) onto the alveolar surface. In the rats that had received intratracheal instillations of polystyrene microspheres (1.9 µm), Lehnert et al. (1985) found that the free cell population size increased nearly five-fold as of one day after the instillations. Although the PMN response had subsided as of day 14 after the instillations, the alveolar macrophage population remained persistently elevated at least 30 d after particle deposition.

The importance of particle size and particle numbers in determining the magnitude of the response has been shown using intratracheal instillation studies with latex microspheres (0.1 and 1.0 µm) and carbon (0.03 µm) in mice (Adamson and Bowden, 1981) and with carbon particles and coal dust in hamsters and rats (Brain and Cockery, 1977). In all cases, the smaller particle size (or greater number) led to a greater increase in the numbers of alveolar macrophages and PMNs (with equivalent dose mass). Also, more phagocytes are recruited with increasing lung burden (Brain and Cockery, 1977). Particle size also affects phagocytosis. In mice, maximal phagocytosis of polystyrene and phenylated microspheres took place in the size range of 1.0-2.0 µm, and particles with hydrophobic surfaces were more readily phagocytosed by peritoneal macrophages (Tabata and Ikada, 1988).

Some particles, however, decrease alveolar macrophage numbers, possibly through their cytotoxic effects — e.g., silica (Civil and Heppelston, 1979), cadmium chloride (Koshi et al., 1978) and manganese dioxide (Adkins et al., 1980).

Particles can also affect the phagocytic activities of alveolar macrophages. In mice exposed for 2 h to fly ash or silica (~1.6 µm MMAD, ~22 mg/m^3) decreased alveolar macrophage activity was seen *in vitro* (Fisher and Wilson, 1980). Similar results were seen for alveolar macrophages from rats exposed to quartz (10-100 mg/m^3) for 6 h or 3 d (Warheit et al., 1991). Conversely, rats exposed to iron oxide for 2 h (1.6 µm MMAD, ~22 mg/m^3; Lehnert and Morrow, 1985) or 3 h (0.15 µm MMAD, 271 mg/m^3; Kavet et al., 1987) showed increased alveolar macrophage activity *in vitro*. Rabbits exposed to sulphuric acid aerosol (0.3 µm, 0.5 mg/m^3) showed decreased mobility of alveolar macrophages after 7 or 14 d of exposure (Schlesinger, 1987).

To determine if particle size influences toxic effects of H_2SO_4, Chen et al. (1992) examined the effects of fine (0.3 µm) and ultrafine (0.04 µm) H_2SO_4 aerosols (300 µg/m^3) on biochemical and cellular parameters of bronchoalveolar lavage fluid from exposed guinea pigs. Four days of exposure (3 h/d) to fine aerosols enhanced the phagocytic activity of recovered alveolar macrophages, while an identical exposure to ultrafine aerosols depressed phagocytic function. A reduction in intracellular pH was noted 24 h following 4 d of exposure to the ultrafine aerosols, but not the fine aerosols. Thus, acid exposure produced a change in intracellular pH and phagocytic function of the alveolar macrophages, and the effect was particle size-dependent. Since the same mass concentration of H_2SO_4 was used for aerosols of

both sizes, ultrafine H_2SO_4 aerosols as a population may have delivered a higher dose to the macrophages than fine H_2SO_4 aerosols. Enhancement of phagocytosis at a low dose and depression of phagocytosis at a higher dose have been reported in macrophages exposed to acidic media *in vitro* (Schlesinger et al., 1990). Therefore, the differences in response between fine and ultrafine aerosols may be attributable, to some extent, to the doses of acid aerosols delivered to macrophages *in vivo*.

Although the increase in lung phagocytes provides more cells to ingest particles, these same cells can also be the cause of injurious responses via the production of reactive oxygen species, hydrolytic enzymes, arachidonic acid metabolites and pro-inflammatory/pro-mitogenic cytokines.

Nadeau et al., conducted a series of *in vitro* studies (Nadeau et al., 1995; Nadeau et al., 1996) using human and rat alveolar macrophages obtained by lung lavage. Although a low dose of urban particles (up to 100 µg) induced an oxidative burst (Nadeau et al., 1996), the major effect was the suppression of phagocytosis of rat alveolar macrophages. More significantly, pre-exposure of human or rat macrophages to urban particles suppressed respiratory burst upon secondary challenge with zymosan, a stimulus (Nadeau et al., 1995). This reveals an impairment of bactericidal function.

Recently (Costa et al., 1994), the pulmonary inflammatory response and acetylcholine-induced bronchoconstriction in rats instilled with Mount St. Helen's dust, ambient particles from Düsseldorf or residual fly ash (all 2.5 mg) were suggested to be related to surface-coordinated Fe^{3+} and its role in generating oxidants in the lung. Ghio et al. (1992), using three types of silica dust with differing levels of surface-complexed Fe^{3+}, have also demonstrated that the inflammatory response in rats increased with increased Fe^{3+} levels.

Subchronic and Chronic Exposure

Subchronic and chronic exposures to many types of particles result in an accumulation of alveolar macrophages in the alveoli. Examples include diesel exhaust (six and 12 months, 250–6000 µg/m^3; Strom, 1984) to rats; carbon black (six weeks, 0.24 µm MMAD, 7 mg/m^3; Strom et al., 1989) to rats; cobaltous chloride (16 weeks, 1 µm MMAD, 2 mg/m^3; Johansson et al., 1986) to rabbits; and titanium dioxide (two years, 1.6 µm MMAD, 10-250 mg/m^3; Lee et al., 1985a) to rats. However, cytotoxic particles — e.g. nickel oxide (four weeks and four months, 0.4 µm MMAD, 15-800 µg/m^3 to rats) — can cause reductions in alveolar macrophage numbers (Spiegelberg et al., 1984).

Concurrent with the increases in alveolar macrophages observed with high lung burdens of poorly soluble particles are reductions in lung clearance rates, which indicate compromises in alveolar macrophage-mediated alveolar clearance. Rats exposed to coal fly ash (3 µm MMAD, 10.4 µg/m^3) for one month showed very little lung clearance during the 10 month post-exposure period (Matsuno et al., 1986). Similar results have been shown in 24 month studies with test toner (4 µm MMAD, 1-16 mg/m^3), titanium dioxide (1.1 µm MMAD, 5 mg/m^3) and silicon dioxide (1.4 µm MMAD, 1 mg/m^3) (Bellmann et al., 1991). This effect has become known as "particle overload." Both theoretical and experimental evidence indicate that the translocation of alveolar macrophages from the lung ceases when these phagocytes contain burdens of particles that are equivalent to ~60% of their original volume (Morrow and Mermelstein, 1988; Lehnert, 1990; Oberdörster et al., 1992b). However, such overload conditions have occurred only after chronic exposure of experimental animals to inhaled concentrations of several milligrams per cubic metre and are not expected to occur at ambient concentrations of <200 µg/m^3.

Kleinman et al. (1995) investigated exposure of rats for eight weeks to two important constituents of the fine particle fraction of PM_{10}, sulphate (0.2 µm MMAD, 70 µg/m^3) and ammonium nitrate (0.6 µm MMAD, 350 µg/m^3), and to the coarse mode of ambient aerosol (>2.5 µm MMAD) using resuspended road dust (4.0 µm MMAD, 300 and 900 µg/m^3). Decreases in alveolar macrophage function (except low-dose road dust) and increased lung permeability (only for nitrate and sulphate) were reported. Histopathological changes in the alveolar space were moderately changed in the order nitrate > sulphate > road dust. It appears that the fine fraction of PM_{10} was more toxic than the coarse fraction.

10.2.3 Antimicrobial Defence and Immunological Function

Acute and Short-Term Exposure

The acute deposition of particles in the lung can cause alterations in the lung's defence mechanisms against microbial infections; however, these effects appear to be related to the physicochemical characteristics, including acidity, of the particles deposited and not simply due to a particle effect.

The intratracheal instillation of 100 µg of urban air particles (0.4 µm) and coal fly ash particles (0.9 µm) into the lungs of mice was found to moderately enhance susceptibility to bacterial infection, whereas diesel particles (0.6 µm), silica (<5 µm) and volcanic ash (1.4 µm) caused only small increases in infectivity (Hatch et al., 1985). Even brief aerosol exposures to acid particles delivered at relatively low mass concentrations can affect mechanisms that would be expected to alter lung defence against microbes. This conclusion is illustrated in the study by Zelikoff and Schlesinger (1992) in which production of tumour necrosis factor-α (TNF-α) and superoxide anion by rabbit alveolar macrophages was decreased after the animals were exposed for 2 h to 50-125 µg/m^3 sulphuric acid (0.3 µm). On the other hand, exposure of mice for 4 h/d for four days to carbon black at a mass concentration of 10 mg/m^3 (Jakab, 1993) did not increase the animals' susceptibility to experimentally imposed infection.

Some investigations have shown that when the particle size is small enough (MMAD <0.05 µm), even the particles with little intrinsic toxicity can become very toxic. For example, Oberdörster et al. (1992) performed intratracheal instillation of fine (>0.2 µm) or ultrafine (~0.02 µm) titanium dioxide particles (both 500 µg), which are highly insoluble particles of low intrinsic toxicity. The ultrafine particles induced substantially more severe acute inflammatory reaction, than the fine particles, as determined by an increase in protein leakage and the number of inflammatory cells in lung lavage fluid. The inflammatory response due to ultrafine particles was dose-dependent in the range of 65 to 500 µg. The authors also found that inhaled ultrafine titanium dioxide translocated into pulmonary interstitium via endocytosis by pulmonary Type I and Type II cells (Ferin et al., 1992; Lehnert et al., 1993), to a greater extent than fine titanium dioxide particles (Oberdörster et al., 1992; Ferin and Oberdörster 1992), was retained in the lung for longer periods (Lehnert et al., 1993; Oberdörster et al., 1994), and induced more severe pulmonary inflammation (Oberdörster et al., 1994). Oberdörster and co-workers, in recent data, have shown that freshly generated thermodegradation products of polytetrafluoroethylene (PTFE) containing singlet ultrafine particles (MMAD 26 nm) at concentrations as low as about 9 µg/m^3 inhaled for 30 minutes caused pulmonary inflammation in rats, along with the up-regulation of several inflammatory mediators including interleukin-6 (Oberdörster, 1995). The evidence that the surface area of the retained particles in pulmonary interstitium, rather than their mass, correlates best with the inflammatory response (Oberdörster et al., 1994). This implies that the induction of mediators (e.g., chemotactic factors, growth factors, enzymes) from macrophages is a function of the particle surface area that interacts with the receptors of alveolar macrophages.

Evidence exists that ultrafine H_2SO_4 aerosols (0.04 µm) result in greater decreases in both intracellular pH and phagocytic function of alveolar macrophages than do fine H_2SO_4 aerosols (0.27 µm) in guinea pigs (Chen et al., 1992). H_2SO_4-coated ultrafine zinc oxide particles have been shown to initiate changes in production of prostaglandin $F_{2\alpha}$ and leukotriene B_4 in guinea pigs (Chen et al., 1989), which are involved in mediating the tension of bronchial smooth muscle and inflammatory responses.

Subchronic and Chronic Exposure

Subchronic or chronic exposure to high mass concentrations of even relatively "benign" dusts can result in increases in susceptibility to microbial infection. Bacterial clearance was found to be diminished in mice that were exposed to 20 mg/m^3 titanium dioxide (<1.98 µm) for 10 or 20 days. Whereas this effect was not observed in mice exposed to 2 mg/m^3 of this type of particle (Gilmour et al., 1989a, 1989b), it should be noted that species' differences evidently can play a role in the outcome of infectivity studies. Guinea pigs exposed to 23 mg/m^3 titanium dioxide (<1.98 µm) for 20 h/d for 20 days (Baskerville et al., 1988) showed no evidence of an enhanced increase in susceptibility to infection.

Kleinman et al. (1995) exposed rats to ammonium sulfate (20 or 70 µg/m^3, 0.2 µm MMAD), ammonium nitrate (90 or 350 µg/m^3, 0.6 µm MMAD), resuspended road dust (300 or 900 µg/m^3, 4 µm MMAD), or purified air, 4 h/d, 4 d/wk for 8 weeks. Exposure to sulfate and nitrate particles and road dust significantly depressed macrophage-dependent lung defence functions (antigen binding to Fc receptors

and respiratory burst activity), compared to purified air controls.

Exposures to some types of particles can also result in alterations in immunological responses in the lung and regional lymph nodes. The humoral response in rats following nickel oxide exposure (0.4 µm MMAD) was significantly weakened at 100 and 200 µg/m^3 after four weeks and at 25 and 150 µg/m^3 after four months of exposure (Spiegelberg et al., 1984). With other types of particles — e.g., diesel soot (24 months, 7 and 3.5 mg/m^3, 0.25 µm MMAD) — alterations in immunological responses were observed only after chronic exposures (Bice et al., 1985). These results indicate that particles of metals, with known cytotoxic properties, affect the immune system to a significantly greater degree. Species differences in changes in the immunological status of the lung in response to particle deposition have not been studied systematically.

10.3 TISSUE, CELLULAR AND BIOCHEMICAL RESPONSES

10.3.1 Histopathological and Cytological Changes

Acute and Short-Term Exposure

Acute exposures to particles can result in a range of histological and cellular disturbances, although most of the reported studies have involved the exposure of animals to very high mass lung burdens or mass exposure concentrations. As previously described, Wright et al. (1988) found evidence that intratracheal instillation of 10 and 30 mg quartz caused increases in intra-alveolar space sizes (i.e., emphysema) and thickening of the most peripheral airways in rats that were not detected after the instillation of 10 mg iron oxide. These histopathological lesions are considered a model of pulmonary fibrogenic, restrictive lung disease and/or chronic obstructive pulmonary disease (COPD). Callis et al. (1985) found increased levels of lung hydroxyproline 10 months after intratracheal instillation of 5 mg of quartz in mice. An equivalent lung burden of latex microspheres (0.9 µm) failed to elicit a similar response.

Particle size and surface characteristics play important roles in mediating the fibrotic response of quartz. In a study using a range of quartz particle sizes (1, 5, 7.8 and 11.2 µm) deposited at equivalent particle surface areas, larger quartz crystals were found to cause a greater inflammatory and fibrogenic response than smaller crystals (Wiessner et al., 1989), and surface charge has been shown to affect the fibrogenicity of silica particles (Wiessner et al., 1990). It must be noted, however, that the toxicological studies performed with crystalline quartz were at far greater levels than those occurring in the urban atmosphere.

In rats that were intratracheally instilled with 40 mg of three different volcanic ash samples (0.5-1.5 µm count median diameter (CMD)) obtained from three geographical locations, pure crystalline quartz (1.5 mm CMD) or soil quartz (1.6 mm CMD). The quartz caused early granuloma formation, later fibroplasia and collagen deposition, widespread lipoproteinosis and fibrosis in the regional lymph nodes (Sanders et al., 1982). The volcanic ash caused an ill-defined inflammatory reaction, with a few rats showing granuloma formation, a limited fibrosis, moderate lipoproteinosis and lymph node enlargements with evidence of collagen formation. The pulmonary response to the soil particles was least intense and qualitatively similar to the response seen with the volcanic ash. The soil particles, however, did not cause lymph node enlargements.

There are indications that surface-complexed iron on particles is involved in pulmonary injury. Guilianelli et al. (1993) examined the role of iron in PM-related lung injury using chrysotile, nemalite, and hematite particles incubated with rabbit tracheal epithelium *in vitro* (50-100 µg/cm^2). Surface available iron was correlated both with the oxidizing potency of mineral particles, and with cytotoxicity, the expression of cytokeratin-13 and the formation of cross-linked envelopes of tracheal epithelial cells (two markers of squamous metaplasia), which could be blocked by deferoxiamine, an iron chelator.

To determine potencies of specific metals, Berg et al. (1993) examined different fractions of fly ash (12.5-1000 µg/ml, MMAD <4 µm) for their ability to stimulate bovine alveolar macrophages to secrete reactive oxygen species, namely superoxide anion and hydrogen peroxide which play a key role in macrophage bactericidal function. They noted that the release of hydrogen peroxide was correlated, in descending order, with the metal contents in the fly ash particles: iron>manganese>chromium>vanadium>arsenic.

Subchronic and Chronic Exposure

Nearly all subchronic and chronic exposure studies that have been reported to date describe pathological responses to insoluble particles, regardless of the types of particle involved, the exposure mass concentrations studied, the duration of exposure or the species examined (although most studies have been conducted at particle concentrations above 1 mg/m^3). The following studies represent the types of responses across particle types and reflect the range of morphological responses reported.

Morphological changes were seen in the centriacinar regions of the lungs of cats exposed for 27 months to diesel exhaust (~6-12 mg/m^3), including the appearance of ciliated basal and Clara cells (Plopper et al., 1983). Following a six-month recovery period, peribronchiolar fibrosis in the proximal acinar region was reported in these same animals (Hyde et al., 1985). Guinea pigs exposed to diesel exhaust (~6 mg/m^3) for eight weeks showed hyperplasia of the alveolar epithelium and goblet cell hypertrophy (Wiester et al., 1980). An increase in type II cells and an accumulation of inflammatory cells in the alveoli and alveolar septal walls were seen in rats similarly exposed (White and Garg, 1981).

Kleinman et al. (1995) exposed rats to ammonium sulfate (20 or 70 µg/m^3, 0.2 µm MMAD), ammonium nitrate (90 or 350 µg/m^3, 0.6 µm MMAD), resuspended road dust (300 or 900 µg/m^3, 4 µm MMAD), or purified air, 4 h/d, 4 d/wk for 8 weeks. Lung permeability, as determined from measurements of albumin concentrations in bronchoalveolar lavage fluid, was significantly greater in rats exposed to high concentrations of road dust and nitrate, but not sulfate, when compared to air controls. Quantitative histopathologic analyses, which included measurements of alveolar nuclear density, alveolar chord length, alveolar septal thickness, and alveolar cross sectional area, showed moderate to substantial changes following exposure to particles. The severity of the responses was in the order of sulfate>nitrate>road dusts.

In a 24 month study of the exposure of rats and mice to diesel exhaust (0.35, 3.5 and 7 mg soot/m^3), a chronic inflammatory response was indicated by dose-dependent increases in inflammatory cells, cytoplasmic and lysosomal enzymes and lavageable protein at the two higher levels of exposure. Histologically, the rats developed focal areas of fibrosis associated with deposits of the soot, whereas the mice had only fine fibrillar thickening with occasional alveolar septal thickening in the high-level exposure group (Henderson et al., 1988). Similar lesions have been reported in other studies using similar exposure levels (Heinrich et al., 1986a, 1986c; Ishinishi et al., 1986; Mauderly et al., 1987; Brightwell et al., 1989; et al., 1993).

In rats exposed to aerosolized fluidized bed coal combustion fly ash (3.6 µm MMAD, 36 mg/m^3) for two or four weeks, morphological changes reported included thickening of the alveolar walls and perivascular inflammation (Shami et al., 1984). Additionally, small granulomas were observed in the alveolar region and the lung and bronchus-associated lymph nodes at 42 weeks post-exposure.

Exposure to more cytotoxic metal particles leads to more profound effects, although such effects may be related more to the inherent toxicity of the metal than to the “particle” effect. The nickel compounds provide an example of this toxic process. In rats and mice, 12 d exposures to aerosolized nickel subsulphide (~2-3 µm MMAD, 10 mg/m^3) caused a necrotizing pneumonia, occurring mainly in the centriacinar regions of the lung (Benson et al., 1987). In a subsequent study in rats and mice, histopathological changes in the lungs, including chronic active inflammation, fibrosis and alveolar macrophage hyperplasia, were associated with exposure to nickel oxide, subsulphide and sulphate hexahydrate.

The order of toxicity of the compounds studied corresponded to their water solubility (nickel sulphate > subsulphide > oxide) (Dunnick et al., 1989). Degeneration of the olfactory and respiratory epithelium, including the alveolar epithelium, was seen in rats exposed to cobalt sulphate (~1 µm MMAD, 0.3-30 mg/m^3) for 13 weeks (Bucher et al., 1990).

The effect of particle size and lung burden on the fibrotic response to silica was shown in a study in which mice were exposed to 0.74 µm particles over four weeks, resulting in a reversible inflammatory response; in contrast, 2 µm particles at the same mass burden (3.2 mg/m^3) induced no response. At a higher mass burden (10.7 mg/m^3), mice exposed to the 0.74 µm silica showed progressive inflammation with a significant perivascular influx of T-lymphocytes leading to focal fibrotic lesions (Velan et al., 1993). Exposure to titanium dioxide (0.28 µm MMAD, 3.9 mg/m^3) showed no effects.

In chronic studies with silica, Muhle et al. (1991) investigated the fibrogenicity of toner (4 µm MMAD, 1-16 mg/m^3), titanium dioxide (1.1 µm MMAD,

5 mg/m^3) and silicon dioxide (1.4 µm MMAD, 1 mg/m^3) in the lungs of rats exposed for 24 months. Using a dosimetric model, these investigators concluded that the relative fibrogenic potency of titanium dioxide, toner and silica was 1:5:418. No evidence of fibrosis was obtained with the animals exposed to the lowest toner concentration studied. A similar fibrotic response has been reported in rats exposed to aerosols of Ludox colloidal silica (2.9-3.7 µm MMAD, 10-150 mg/m^3) for four weeks (Lee and Kelly, 1992) and to cristobalite (1.4 µm MMAD, 27.5 mg/m^3) 50 weeks after an eight-day exposure (Absher et al., 1989).

Wolff et al. (1988) exposed rats to gallium oxide (Ga_2O_3) particles (0.2 µm, 23 mg/m^3) for four weeks and reported that at six months post-exposure, inhaled gallium oxide produced cytotoxic, inflammatory and fibrogenic responses similar to or greater than responses to inhaled quartz particles. The gallium oxide aerosols consisted of aggregates of ultrafine (14 nm) singlet particles.

Concerning soluble particle matter, of note is the minimal histological effects observed in rabbits exposed for one year (2 h/d, 5 d/wk) to sulphuric acid aerosols at a dose of 125 µg/m^3, (0.3 µm MMAD) (Schlesinger et al., 1992a), and 250 µg/m^3 (1 h/d, 5 d/wk)(Gearhart and Schlesinger 1989). The major responses produced by 125-250 µg/m^3 of H_2SO_4 are characterized by hyperplasia of epithelial secretory cells and hypertrophy in small airways (Gearhart and Schlesinger 1989; Schlesinger et al., 1992).

10.3.2 Biochemical Changes

Acute and Short-Term Exposure

The biochemical assays of lavage fluids of rats intratracheally instilled with silica (2.2 µm, 0.2-5 mg/100 g body weight), titanium dioxide (2.2 µm, 1 or 5 mg/100 g body weight) or aluminum oxide (5.3 mm, 1 or 5 mg/100 g body weight) indicated increased levels of lactate dehydrogenase, β-glucuronidase, N-acetylglucosaminidase and total protein, in a dose-dependent manner (Lindenschmidt et al., 1990). In an inhalation study using titanium dioxide (1.0 µm MMAD, 50 mg/m^3), no such effects were seen (Driscoll et al., 1991). The reason for the differing results is not clear. Elevated total protein is indicative of increased permeability of the alveolar-capillary barrier, whereas the elevated lactate dehydrogenase reflects cellular damage (Henderson et al., 1978). A concentration-dependent elevation in lactate dehydrogenase levels, resulting from *in vitro* treatment of alveolar macrophages with quartz (<5 µm), has also been reported, indicating that alveolar macrophage damage also contributes to increased lactate dehydrogenase levels (Chen et al., 1991). The increased lysosomal enzyme levels are believed to reflect elevated alveolar macrophage activity/numbers (Henderson et al., 1988). In most cases, these changes are seen as sensitive indicators of histological changes in the lung.

Similar increases in both total protein and enzyme levels were reported in rats after intratracheal instillation of 0.5-200 µg lanthium chloride (Suzuki et al., 1992), cobalt particles (4 µm, 0.06 mg/100 g body weight), tungsten carbide (2 µm, 1 mg/100 g body weight), tungsten carbide-cobalt powder (2 µm, 1 mg/100 g body weight) (Lasfargues et al., 1992) and a series of nickel compounds (0.01-1.0 µm Ni), including nickel subsulphide, nickel chloride, nickel sulphate and nickel oxide (Benson et al., 1986).

Other responses measured in bronchoalveolar lavage (BAL) fluid include elevated levels of phospholipid in rats following silica (6 mg) instillation, which was related to the surface-complexed iron (Fe^{3+}) (Ghio and Hatch, 1993); increased phospholipid in rats following inhalation of diesel exhaust (0.2 µm MMAD, ~6 mg/m^3) (Wright, 1986); and *in vitro* rat alveolar macrophage release of glutathione in response to quartz (Boehme et al., 1992).

Acidic aerosols, both fine (0.3 µm MMAD) and ultrafine (0.04 µm MMAD), were found to initially elevate lavageable protein, β-glucuronidase and TNF-α; however, the ultrafine aerosol was found to decrease *in vitro* alveolar macrophage phagocytosis, whereas the fine aerosol increased phagocytosis (Chen et al., 1992b).

Alveolar macrophages from rats exposed to silicon dioxide (0.9 µm MMAD, 45 mg/m^3) for eight days were found, five to seven months later, to have elevated levels of cyclo-oxygenase metabolites, including prostaglandin E_2, thromboxane B_{22} and prostaglandin D_2, "priming" the alveolar macrophages for TNF-α release (Mohr et al., 1992). Silica (1.6 µm MMAD) was also found to cause increased fibronectin and lipopolysaccharide-stimulated release of interleukin-1 (IL-1) (Driscoll and Maurer, 1991). In a summary of a number of such studies, Driscoll and Maurer (1991) suggest that IL-1, TNF-α and fibronectin correlate to exposures to toxic dust and may play a role in the pulmonary response. Increased TNF-α release has also been reported in hamsters

exposed to opsonized albumin-coated latex particles (0.1 µm) (Kobzik et al., 1993).

Subchronic and Chronic Exposure

The types of biochemical changes reported in subchronic and chronic studies are similar to those seen in the shorter-term exposures and reflect similar processes. In guinea pigs exposed to silica (6-28 mg/m^3, 75% of particles below 2 µm) or titanium dioxide (24 mg/m^3, 85% of particles below 2 µm) for three weeks, the silica-exposed animals showed elevated enzyme levels in the BAL fluid eight weeks post-exposure. The titanium dioxide induced no effects (Sjöstrand and Rylander, 1984). Elevated levels of cytoplasmic and lysosomal enzymes and total protein in the BAL fluid were similarly reported in rats up to 12 months after exposure to 0.2 µm (aggregates of 14 nm particles, as above) gallium oxide particles (23 mg/m^3) over four weeks (Wolff et al., 1988).

The fibrosis reported by Henderson et al. (1988), discussed above under histopathological effects, was accompanied by elevations in lavageable β-glucuronidase and hydroxyproline. Lavageable glutathione and glutathione reductase activities increased in both species, but the extent of these effects was greater in mice. Whole-lung glutathione was depleted in a dose-dependent manner in rats, whereas it was increased in mice. Conceivably, this depletion may have played a role in the fibrotic response seen in the rat. Lung P-450 content was not elevated in either species.

Kuhn et al. (1990) exposed rats to bituminous coal dust (4-5 µm MMAD, 80% of particles between 2 and 7 µm, 25 mg/m^3) for two weeks. Exposure to the dust resulted in increases in lavageable alveolar macrophage numbers, which, *in vitro*, showed increased production of thromboxane A_2 and leukotriene B_4 and a decrease in prostaglandin E_2. The alteration in thromboxane A_2 and leukotriene B_4 production persisted for two weeks post-exposure.

10.4 PARTICLE-ASSOCIATED INCREASES IN MORTALITY

Acute exposures to some types of particles can result in increases in mortality. A number of the heavy metal particles are lethal, by virtue of their inherent toxicity – e.g., tri- or pentavalent arsenic (LD_{50} 14.3 mg/kg), vanadium pentoxide (LD_{50} 8.1 mg/kg) and selenium and cadmium oxides (LD_{50} 0.88 mg/kg) (Rhoads and Sanders, 1985). In terms of "particle effect" toxicity, increases in mouse mortality have been reported to occur after the intratracheal instillation of only 0.4 mg of 0.4 µm diesel soot (Sagai et al., 1993), although the chemical composition of the particles played a role in mediating this response, as methanol-washed diesel particles caused no increases in mortality.

For $(NH_4)_2SO_4$ and H_2SO_4, two important components of ambient particles, fairly high concentrations are required to induce animal acute mortality. Above 4 mg/m^3 of H_2SO_4 is required for acute lethality of guinea pigs, a species believed to be the most sensitive. The animal mortality induced by H_2SO_4 is primarily due to laryngeal or bronchoconstriction (Office of Air Quality Planning and Standards et al., 1982). Pure ammonium sulphate particles are rather inert, and exposure to $(NH_4)_2SO_4$ at 1000 – 2000 mg/m^3 for 3 days did not cause death in rats (Pepelko et al., 1980).

The most lethal particles investigated to date are those ultrafine particles that are freshly generated as fumes from some polymers and perhaps metals. Oberdörster et al. (1994a) exposed rats to Teflon® (26 nm MMAD, ~60 µg/m^3) for 10 minutes at inhaled number concentrations of 5×10^5 particles/cm^3, which resulted in acute mortality from a hemorrhagic pulmonary edema. Factors that appear to impact on the toxicity of these types of particles include particle aging and aggregation (Warheit et al., 1990; Lee and Seidel, 1991). The relevance of these observations to environmentally exposed humans remains open to speculation; however, ultrafine particles are consistently present in the urban atmosphere (Brand et al., 1992). Also, humans exposed to polymer fumes and metal fumes are known to develop symptoms observed in the animals (i.e., polymer fume fever and metal fume fever) (Shusterman, 1993; Gordon and Fine1993).

A more recent study using concentrated air particles shows that ambient particles concentrated and instantaneously given to rats for inhalation may cause death (Godleski et al., 1996). In this study, normal rats, rats with monocrotyline-induced pulmonary inflammation (50 mg/kg subcutaneously), and rats with SO_2-induced chronic bronchitis (250 ppm SO_2 for 6 weeks) were exposed to concentrated air particles (~250 µg/m^3) for 3 consecutive days, 6 h/d. Death occurred during exposure and overnight. The mortality for healthy rats, rats with pulmonary inflammation, and rats with chronic bronchitis were

0, 19% and 37%, respectively. Dead animals had a significantly more prominent airway constriction than did live animals. It is speculated that reactive oxygen species accompanying fresh ambient particles in concentrated form may play an important role in the acute mortality. Thus, ambient particle inhalation can cause death in rats with disease, and airway constriction appears to be important in the response.

Few investigators have noted an increase in mortality following subchronic or chronic exposures of animals to particles. Even in instances in which such exposures have resulted in the development of pulmonary fibrosis and lung tumours, increases in mortality have not been concurrently observed as a major outcome of exposure.

10.5 PARTICLE-INDUCED CARDIOVASCULAR RESPONSES

Since epidemiological studies conducted in Canada (Burnett et al., 1995) and in the US (Schwartz and Morris 1995) have shown a consistent association between fine particulate matter air pollution and increased hospital admissions due to cardiovascular diseases, several studies using experimental animals have been carried out in an attempt to investigate the underlying mechanism of ambient particle-induced cardiovascular effects.

Animal studies demonstrate that emission residual oil fly ash causes dose-related increases in electro cardiographic (ECG) abnormalities in rats (Campen et al., 1996) and dogs (Nearing et al. 1996). In the study conducted by Campen et al. (Campen et al., 1996), healthy rats and rats with monocrotaline-induced pulmonary hypertension (60 mg/kg) were instilled with residual oil fly ash (ROFA) intratracheally at doses of 0, 0.25, 1, or 2.5 mg. Data show that body core temperature and heart rate decreased immediately post-instillation in a ROFA dose-dependent manner. Analyses of ECG's show dose-related increases in arrhythmias and skipped heart beats. Pulmonary hypertension may contribute to the adverse health effects of particles.

Nearing et al. (1996) exposed healthy adult mixed-breed dogs to a fine particle aerosol (MMAD ~ 3 µm) produced by an aerosol generator using oil-fired power plant ash, for 3 consecutive days, 3 h/d. The particle concentrations were 1.68 or 3.04 mg/m^3. Measurements of potentially toxic metals (vanadium, nickel, zinc, copper and manganese) showed no acute increases with exposure. Dogs exposed to the higher concentration of particles had significant increases in the amplitude of T-wave altemans in ECG, an index of vulnerability to ventricular fibrillation, compared to pre-exposure control measurements. All dogs exposed to particles exhibited ST-segment elevation, while controls did not show any changes.

An *in vitro* study using isolated atrium of guinea pigs has shown that diesel exhaust particles (0.01 - 0.5 mg/ml) caused a negative inotropic action followed by the cardiac arrest of the isolated atrium (Sakakibara et al., 1994).

The mechanism for particle-induced cardiovascular response is not yet clear. However, diesel exhaust particles have been reported to cause oxidative modification of low density lipoprotein *in vitro* (Ikeda et al., 1995). Oxidized low density lipoprotein is known to be very cytotoxic (Morel et al., 1983; Ocana, 1989; Ikeda et al., 1995), inducing endothelial damage, the proliferation of smooth-muscle cells, causing monocyte-endothelial interactions (Berliner et al., 1990; Cushing et al., 1990), and platelet aggregation, which all eventually may lead to adverse cardiovascular effects. Indeed, intratracheal instillation of diesel exhaust particles (0.4 mg or 0.8 mg per mouse) caused acute disruption of capillary endothelial cells and the detachment from their basement membrane (Ichinose et al., 1995). An epidemiological study also shows an increased plasma viscosity during an air pollution episode (Weinmann et al., 1995). Oxidized low density lipoprotein is also demonstrated to be a potent inhibitor of endothelial-derived relaxation of vascular smooth muscles (Ezaki et al., 1994). Thus diesel exhaust particle-induced oxidization of low density lipoprotein may play a pathological role in promotion of coronary vasospasm and hypertension through inhibition in endothelium-dependent relaxation.

10.6 EFFECTS OF PARTICLES ON COMPROMISED LUNGS

Early studies conducted using animals with compromised lungs focussed on the changes to the deposition and clearance patterns of particles, and relied on artificially induced lung lesions as models for human disease conditions.

Ferin (1971) used papain to induce emphysema in rats and found that the deposition of aerosolized titanium dioxide (0.25 µm) was approximately one-half the amount deposited in normal lungs; the papain-treated lungs cleared ~18% of the deposited

material as of 30 days after exposure, whereas normal lungs cleared ~51% of the originally deposited lung burden.

The deposition pattern of an inhaled submicrometre aerosol (^{99m}Tc-sulphur colloid, 0.46 µm MMAD) was studied in hamsters experiencing elastase-induced emphysema (Sweeney et al., 1987). Fewer particles were found deposited in the emphysematous lungs, and the pattern of deposition in the emphysematous lungs was less uniform than in control lungs. A similar effect was reported in hamsters with bleomycin-induced fibrosis, exposed to submicrometre-sized gallium oxide aerosol (Sweeny et al., 1983) or to submicrometre-sized particles of colloidal gold (Tryka et al., 1985). In the latter case, the particle clearance over five days post-exposure compared with day 0 exposure, was 45% in the fibrotic lungs, and 26% in the normal lung. The authors suggested accelerated alveolar-bronchiolar transport of particles or particle-containing macrophages may be responsible for the greater clearance in the diseased lungs.

In contrast, Greene et al. (1987) found that chronic bronchitis induced by a 60-week exposure (2 h/d, 5 d/wk) to 500 ppm of sulphur dioxide in dogs resulted in a lower deposition of inhaled ^{134}Cs-labelled aluminosilicate particles, yet their subsequent clearance from the lung was unaffected by the pre-existing disease. However, as only two sulphur dioxide-exposed and two unexposed dogs were involved in this study, the result is not very conclusive.

The influence of pre-existing emphysema on the susceptibility to the effects of chronic exposure to diesel exhaust was investigated by Mauderly et al. (1990). Rats with elastase-induced emphysema were exposed to diesel exhaust (3.5 mg soot/m^3) or air only for 24 months (7 h/d, 5 d/wk). The overall results of this detailed study did not support the hypothesis that emphysematous lungs are more susceptible than normal lungs to chronic exposure to high levels of diesel exhaust. It appeared that the decreased accumulation of particles in the emphysematous lungs may have negated any potential increase in response.

Little is known about how lung defence mechanisms against deposited particles may be altered in a background of acute lung inflammation. Slauson et al. (1989a) investigated the clearance of inhaled cobalt oxide particles (0.54-0.65 µm) using calves experiencing acute lung injury induced by parainfluenza-3 virus. Control animals had a biphasic clearance pattern, with rapid initial clearance of 50% of the initial lung burden as of seven days after exposure followed by a slower clearance component. With the virally infected calves, 90% of the initial lung burden was retained as of seven days after exposure. Virtually all of the particles lavaged from the control and experimental animals were contained in alveolar macrophages. However, histological analyses revealed that some particles were contained in macrophages in the interstitial regions of the lungs of calves with viral pneumonitis, and also increased transport to the regional lymph nodes; this outcome was not observed in the lungs of healthy calves.

In another study, Slauson et al. (1989b) studied the phagocytic activities of alveolar macrophages lavaged from the lungs of calves that were experiencing experimentally induced interstitial adjuvant pneumonitis. Compared with alveolar macrophages from healthy control animals, the alveolar macrophages from the inflamed lungs showed a higher maximum rate of particle (microbeads, 2.02 µm) phagocytosis *in vitro*, and a higher proportion of them were able to phagocytize multiple particles. These observations differ from those made by the same group in a study in which alveolar macrophages from calves experiencing virus-induced acute bronchiolitis and alveolitis were found to have depressed phagocytic activities.

Recently, Rabbe et al. (1994) exposed rats with elastase-induced emphysema to two particle atmospheres, a California-type aerosol [consisting of particles of graphitic carbon, natural clay, NH_4HSO_4, $(NH_4)_2SO_4$, NH_4NO_3, $PbSO_4$, $VOSO_4$, $MnSO_4$, and $NiSO_4$, MMAD 1.1-1.5 µm], and a London-type aerosol [consisting of NH_4HSO_4, $(NH_4)_2SO_4$, coal fly ash, and lamp black carbon, MMAD 0.8-0.9 µm]. While a 3 d exposure (23 h/d) to the London aerosol produced a significantly greater increase in pulmonary DNA and RNA contents in emphysema rats than in normal rats, there were no changes in tracheobronchial clearance or lung permeability compared to normals. A 30 d exposure to the California aerosol enhanced small airway lesions in emphysema rats, but did not alter lung hydroxyproline, tracheobronchial clearance, or small airway fibrosis.

More recently, Gilmour et al. (1997) examined whether residual oil fly ash (obtained from the stack of an oil fired power plant) could exacerbate the severity of influenza infection in rats. Female rats were given an intratracheal instillation of 1 mg residual oil fly ash along with an intranasal infection with influenza virus. Virus infection alone caused mild pulmonary inflam-

mation and increased airway reactivity 24 hours post-infection that subsided by 3 days. Residual oil fly ash instillation resulted in a stronger inflammatory response and airway reactivity that persisted at the three day time point. The combination of residual oil fly ash with virus did not affect the clearance of virus from the lung, but resulted in significantly higher numbers of inflammatory cells at day 1 than either treatment alone and a heightened airway reactivity at three days. The data indicate that the dual insult of particulate matter and virus produces more severe respiratory symptoms and lung damage than either stimulus alone. However, the effects do not appear to be more than additive.

10.7 PARTICLE-INDUCED LUNG CANCER

10.7.1 Carcinogenicity Studies

The interpretation of experimental studies relating to the carcinogenic effects of particulate matter is confounded by the fact that the studies are necessarily conducted using a single particle type. In these long-term studies, the contribution to the overall effect due to the inherent chemical toxicity of the particle itself, rather than the more general "particle effect," is more difficult to assess. For example, a number of metals that are found as components of the particle mass have been classified as carcinogenic or potentially carcinogenic to humans by the inhalation route, including some of the inorganic compounds of arsenic (Health Canada, 1989), cadmium (CEPA, 1994a), chromium VI (CEPA, 1994b) and nickel (CEPA, 1994c). This review will focus only on carcinogenicity as it has been investigated in terms of a "particle effect" of inert particles. Diesel particles have been examined, as there is some evidence of a particle-related effect.

Another confounder is the carcinogenic potential of substances adsorbed to the particle surface – e.g., PAHs on diesel particles. Again, this review is focussed on the "particle effect" alone and will deal only briefly with this issue as it relates to diesel particles.

Pott et al. (1994) instilled 30-150 mg of diesel soot, carbon black, quartz, titanium dioxide and black iron oxide (Fe_3O_4) into rats' lungs with weekly instillations and found significant increases in lung tumour incidence in all exposed animals except for the titanium dioxide-instilled animals. Carbon black and diesel soot instillations resulted in similar lung tumour incidence, indicating that the diesel exhaust-induced lung tumours seen in rat inhalation studies may be explained by a particle effect. Kawabata et al. (1986) also observed that both diesel soot and carbon black can induce lung tumours in rats after intratracheal instillation.

Heinrich et al. (1986a, 1986b) performed a two-year chronic inhalation study in female mice and rats and in both sexes of hamsters with filtered and unfiltered diesel exhaust (4.0 mg/m^3, 19 h/d, 5 d/wk). No tumours were found in the lungs of hamsters. A significantly increased incidence of malignant tumours over the control group was observed in the lungs of both diesel exhaust-exposed mice (adenocarcinoma) and rats (squamous cell tumour). In satellite groups of animals additionally treated with benzo[a]pyrene (BaP), dibenz[ah]anthracene or diphenylnitrosamine, syncarcinogenic effects of diesel exhaust were found only in the respiratory tracts of rats.

A number of investigators in Europe, the United States and Japan have confirmed that inhalation of diesel exhaust at concentrations exceeding ~2 mg/m^3 for two years can induce lung tumours in rats. The study design was essentially similar, in that freshly generated diesel engine exhaust was diluted and rats were exposed in whole-body inhalation chambers to differing concentrations ranging from 0.3 to 7 mg/m^3. In some studies, mice and hamsters were also exposed under similar conditions. Collectively, these studies showed that rats, but not mice and hamsters, had significantly increased lung tumour rates when they were exposed to the unfiltered diesel exhaust (Ishinishi et al., 1986; Heinrich et al., 1986b; Mauderly et al., 1987; Brightwell et al., 1989; Kawabata et al., 1993).

Chen and Vostal (1984) exposed rats to diesel particles (1.5 mg/m^3 and 6 mg/m^3) for 2-28 days (20 h/d, 7 d/wk) to investigate aryl hydrocarbon hydroxylase (AHH) activity in their alveolar macrophages. Additionally, AHH activity in alveolar macrophages after the intratracheal instillation of BaP (5 mg/kg body weight) or diesel exhaust extract was also investigated. After four weeks of exposure to both diesel exhaust concentrations, AHH activity in alveolar macrophages was decreased. BaP instillation resulted in a slight increase in AHH activity in alveolar macrophages, whereas the diesel extract resulted in no change in AHH activity. The results of this study suggest that the amounts of aromatic hydrocarbons associated with the diesel particles were insufficient

to induce AHH activity in the alveolar macrophages.

The question as to whether the results of the diesel exhaust studies in rats are attributable to the particle phase only or whether the organic compounds adsorbed on the particles also play a role was addressed in studies by Heinrich et al. (1992). Female rats and mice were exposed for two years (18 h/d, 5 d/wk) either to diesel soot or carbon black at concentrations ranging from 0.8 to 7.5 mg/m^3 or to ultrafine titanium dioxide particles (20 nm) at average concentrations of 12 mg/m^3. The investigators found that only rats developed both benign and malignant tumours after exposure to all three particle compounds (diesel, 22%; carbon black, 39%; titanium dioxide, 32% combined tumour incidence in high-dose groups), which indicates that the particle compounds, including the ultrafine titanium dioxide, have a carcinogenic potential in rats and that the diesel exhaust-induced lung tumours can be fully explained by the particles.

Lee et al. (1985a) exposed rats to titanium dioxide (1.5-1.7 µm MMAD at 0, 10, 50 and 250 mg/m^3) for two years (6 h/d, 5 d/wk). Bronchoalveolar adenomas and cystic keratinizing squamous cell carcinoma occurred in ~17% of the rats exposed to the 250 mg/m^3 concentration of titanium dioxide, with no increases seen at lower dose levels.

In contrast to the study by Heinrich et al. (1992) with ultrafine titanium dioxide, the titanium dioxide particles used by Lee et al. (1985a) were more than a factor of 10 larger. Lung tumours induced by high particle burdens of benign particles such as titanium dioxide can be associated with a lung particle overload effect (Morrow and Mermelstein, 1988; Morrow, 1994). However, even benign particles can become significantly more toxic if their size is in the ultrafine particle range, and, as demonstrated by the Heinrich et al. (1992) study, 20-fold lower inhaled concentrations in the rat than used in the particle overload study by Lee et al. (1985a) will induce lung tumours.

Muhle et al. (1989) exposed rats for two years (6 h/d, 5 d/wk) to crystalline silica (1 mg/m^3), test toner (0, 1, 4 and 16 mg/m^3) or titanium dioxide (5 mg/m^3). They observed that 18% of the silica-exposed rats developed benign and malignant lung tumours, significantly increased above the tumour rate of controls. No increases in tumour incidence were reported for the test toner or the titanium dioxide.

Reuzel et al. (1994) reported that chronic exposure of rats to aerosolized polymeric methylene diphenyl diisocyanate (<5 µm, 0, 0.2, 1.0 or 6.0 mg/m^3) for up to 24 months (6 h/d, 5 d/wk) caused lung tumours in the high-dose group (6 mg/m^3).

The overall evidence indicates that a number of different particle types, including titanium dioxide, carbon black, diesel soot particles and silica, when inhaled at sufficient dose levels, can induce lung cancer in rats, but not in mice or hamsters. The studies conducted with diesel particles show that the particle itself, rather than the chemical constituents or adsorbed chemicals, may be important in the carcinogenic process. Given the very high particle loads required to induce a carcinogenic response, it is unclear how these studies could be interpreted with respect to their potential extrapolation to humans.

10.7.2 Mutagenicity

Although genotoxic effects of particles have been associated with specific carcinogenic metal compounds, as discussed above, these would not be considered strictly particle effects. Potential particle-related genotoxic effects have been reported in studies examining diesel exhaust. Bond et al. (1990) examined rat alveolar type II cells from animals exposed to diesel particles or carbon black at 6.2 mg/m^3 for 12 weeks (16 h/d, 5 d/wk) and found a four-fold increase in DNA adducts for both exposures. In an earlier study, Wolff and co-workers (1989) measured DNA adducts in the lungs of rats after they were exposed for 12 weeks (4 h/d, 5 d/wk) to pure aerosols of BaP (2 mg/m^3) or BaP adsorbed on carbon black particles (97 mg/m^3). Although there was no significant difference in levels of DNA adducts, some evidence was obtained to indicate that the pattern of the DNA adducts formed in response to BaP alone and BaP adsorbed onto the carbon particles was different.

Interaction of urban particulate matter with cellular macromolecules may occur, leading to DNA damage or enzyme induction. The formation of DNA adducts has been observed following incubation of urban particles from Ottawa, Ontario, 10 µg/ml medium, with normal human bronchial epithelial cells for 72 hours (Shah et al., 1995).

Vincent et al. (1995) incubated urban particles from Ottawa, Washington, D.C., or St. Louis, or aqueous extracts of $PM_{2.5}$ (100 µg/ml medium) *in vitro* with a panel of HepG2 cell lines transfected with target gene constructs, for 18-24 hours. Urban particles caused gene expression of several enzymes includ-

ing cytochrome P4501A1, glutathione S-transferase Ya, and a xenobiotic response element, which are biologically critical in chemical biotransformation. The potency of gene induction was correlated with the content of polycyclic aromatic hydrocarbons in particles. The induction of gene expression of cytochrome P4501A1 and the xenobiotic response element was significantly potentiated by co-incubation with rat alveolar macrophages (Goegan and Vincent, 1995), indicating that alveolar macrophages may increase the bioavailability of polycyclic aromatic hydrocarbons from particles.

10.8 COMBINED EXPOSURES TO PARTICLES AND OTHER AIR POLLUTANTS

Most pulmonary toxicological studies of particles deposited as aerosols or by the intratracheal instillation route have involved exposures to single materials. However, "real-world" exposures to ambient environments involve combinations of a number of toxic and potentially toxic materials that may be inhaled together or more or less sequentially under some conditions. Possible interactions that may result from the inhalation of mixtures of toxicants conceivably can include simply additive effects, synergistic effects or even antagonistic effects. This section summarizes several studies in which combined exposures to test materials were assessed for their overall toxic effects as well as some studies in which exposure to one material was followed by exposure to another.

10.8.1 Combined Exposure with Ozone

Particulate matter and ozone are two major pollutants in ambient air. Several studies have examined how the injurious responses to sulphuric acid are altered by other pollutants. Warren et al. (1986) investigated the interaction between inhalation of ozone and an acid aerosol composed of ammonium sulphate. Rats were continuously exposed to ozone (0.2 ppm) with and without concurrent exposure to 5 mg/m^3 ammonium sulphate (0.5 µm MMAD) for two to seven days. A synergistic interaction between ozone and the acid aerosol was identified by greater than expected increases in the rate of hydroxyproline synthesis by lung tissue and lavageable protein. In another series of studies reported by Warren and Last (1987), a synergistic interaction was also observed between 0.2 ppm ozone and 100, 500 or 1000 µg/m^3 sulphuric acid aerosol after three days' exposure of rats, by the criterion of a significant increase in total lavageable lung protein. Synergistic interaction was also observed, by the criterion of increased lung protein content, in rats exposed to 0.2 ppm ozone plus 40, 100 or 500 µg/m^3 sulphuric acid aerosol for seven or nine days. Synergistic elevations in lung collagen were also observed when rats were exposed to 0.64 ppm ozone and 200, 500 or 1000 µg/m^3 sulphuric acid aerosol. The interaction between ozone and sulphuric acid evidently is one in which the damaging effects of the ozone in the centriacinar region of the lung are enhanced. In this case, the acid aerosol potentiates the effects of ozone and not vice versa.

In another study, Last et al. (1984) examined biochemical and morphometric changes in the lungs of rats exposed for three, seven or 14 days to ozone (0.64-0.96 ppm), with or without accompanying exposure to aerosolized ammonium sulphate (~1 µm MMAD, 5 mg/m^3). After three days of exposure to the mixtures, lung macrophages and monocytes increased two- to three-fold, fibroblasts increased two-fold and collagen synthesis rates increased 2.5-fold compared with values obtained from animals exposed to ozone only. Only relatively minor, if any, changes were observed with ammonium sulphate alone.

Schlesinger and co-workers (1992b) found that interactions between exposures to both sulphuric acid aerosol and ozone can be both antagonistic and synergistic. In their study, rabbits were exposed for three hours to submicrometre sulphuric acid aerosol (50-125 µg/m^3) with 0.1, 0.3 or 0.6 ppm ozone. An antagonistic relationship was demonstrated by depressed phagocytic activity of lavaged alveolar macrophages at the two highest concentrations of acid aerosol, whereas ozone alone did not affect this alveolar macrophage function. Exposure to 75 or 125 µg/m^3 sulphuric acid caused a reduction in tumor necrosis factor-α (TNF-α) production by stimulated alveolar macrophages, a response that was not observed with ozone only. All mixed exposures containing 75 µg/m^3 sulphuric acid also reduced TNF-α production by the alveolar macrophages, whereas exposures to mixtures of 125 µg/m^3 acid with 0.3 or 0.6 ppm ozone resulted in an enhanced level of production of this proinflammatory cytokine.

Chen et al. (1991a) investigated possible interactions between aerosolized sulphur oxides and ozone. In the first exposure regimen, guinea pigs were exposed for one hour to pure sulphuric acid (0.08 µm MMAD, 300 µg/m^3), to sulphuric acid layered on zinc oxide particles (0.05 µm CMD, 24 or 84 µg/m^3) or to zinc oxide particles only (6 mg/m^3); two hours later, they were exposed for one hour to air or 0.15 ppm ozone. The guinea pigs exposed to the 84 µg/m^3 concentration of acid layered on zinc oxide and subsequently exposed to 0.15 ppm ozone showed a more than additive reduction in vital capacity and diffusion capacity. In a second set of exposure regimens, guinea pigs were exposed to 24 µg/m^3 sulphuric acid layered on the zinc particles for five days (3 h/d). On days 8 and 9, the animals received two additional, three-hour daily exposures to the acid layer particles or a single one-hour exposure on day 9 to 0.15 ppm ozone. The second set of acid exposures induced greater decreases in lung volume and diffusion capacity than reported after the initial exposures. The ozone exposure induced reductions in lung volumes and diffusing capacity that were not observed in animals that received exposures to either sulphuric acid-layered particles or ozone alone. Overall, these studies indicate that single or multiple exposures to sulphuric acid-layered zinc oxide can sensitize guinea pigs to subsequent sulphuric acid or ozone exposures.

The effects of ozone exposure (0.8 ppm, 37 exposures, 6 h/d) on the fibrotic response elicited by intratracheally instilled silica (Min-U-Sil-5, ~1.5 µm, 2-50 mg) were examined in the rat by Shiotsuka et al. (1986). There was no detection of an interaction between silica and ozone in the development of pulmonary fibrosis, as determined by quantitative biochemical indices (hydroxyproline and lysyl oxidase) or by histopathological criteria.

Repeated exposure of rabbits to the mixture of H_2SO_4 (75-125 µg/m^3) and ozone (0.1-0.6 ppm), 3 hours per day for 5 days, induced synergistic/additive interactions in stimulating bronchial hyperresponsiveness and down-regulation of macrophage function (McGovern et al., 1993). In addition, repeated inhalation of mixture of ozone (0.4 ppm), H_2SO_4 (500 µg/m^3) and carbon particles (250 µg/m^3) for 5 days lead to greater lung parenchyma lesion and change in breathing pattern in rats compared with exposure to ozone alone, while there was no synergistic effect on depression of phagocytic activities of macrophages (Kleinman et al., 1993). Long-term exposure (Schlesinger et al., 1992) of rabbits to the mixture of H_2SO_4 (125 µg/m^3) and ozone (0.1 ppm) for 2 h/d, 5 d/wk for up to one year did not show any indications of interaction in terms of clearance response between acid and ozone in the group exposed to the mixture. Histological examination of intrapulmonary conducting airways showed an increase in secretory cell number after exposure to ozone alone or the mixture by 4 months, and there was evidence for synergistic interaction between ozone and acid. At subsequent times the interaction became antagonistic in nature with respect to histological changes (Schlesinger et al., 1992).

Bouthillier et al. (1996) exposed rats to a combination of ozone (0.8 ppm) and Ottawa particles (50 mg/m^3, MMAD 1.4 µm, collected from air conditioner filters) via nose-only inhalation for 1 or 3 days. Exposure to particles alone did not increase levels of protein, fibronectin and alkaline phosphatase in bronchoalveolar lavage fluid, nor did it exacerbate these effects of ozone following co-exposure. While exposure to ozone or particles alone altered some aspects of alveolar macrophage physiology, these cellular effects appeared to be attenuated by co-exposure of two pollutants. Particles potentiated the ozone-induced neutrophilic infiltration in lung lavage.

10.8.2 Combined Exposure with Other Pollutants

Castranova et al. (1985) exposed groups of rats to filtered air, coal dust (8.5 µm MMAD, 2 mg/m^3), diesel exhaust (~0.3 µm, 2 mg/m^3) and diesel exhaust (1 mg/m^3) plus coal dust (1 mg/m^3) for two years (7 h/d, 5 d/wk). Exposure to the coal dust resulted in an increase in lavaged alveolar macrophage numbers, increased the abilities of the alveolar macrophages to produce reactive oxygen and increased alveolar macrophage surface ruffling, which the investigators interpreted as indicating enhanced activity. The diesel exposure, on the other hand, decreased both the ability to generate reactive oxygen and cell surface ruffling. The combination of the coal dust with the diesel exposure resulted in a reactive oxygen generation potential that was essentially an average level between what was observed with the individual exposures.

The cytotoxic effects of carbon particles, sulphuric acid mist and the combination of the two on the tracheal epithelium were assessed by Schiff et al. (1979) using both *in vivo* and *in vitro* models. In the *in vivo* component, hamsters were exposed for three

hours to an aerosol of carbon black (99.5% pure, 0.3 µm, 1.5 ± 0.4 mg/m^3) or sulphuric acid aerosol (0.12 µm MMAD, 1.1 mg/m^3), or both pollutants. Their tracheas were removed immediately, or at 24, 48 or 72 hours after exposure; or the animals were killed less than 1 hour after exposure and their tracheal rings were studied over a 72 hour period. In the *in vitro* component, ciliary beat frequencies of tracheal rings from normal hamsters were measured in response to exposure to carbon particles and sulphuric acid solution, alone or in combination. The ciliary beat frequency of the acid-carbon group in both components of the study was significantly reduced from the control and acid and carbon alone treatments. Histological analyses of the *in vivo* and *in vitro* samples showed that epithelial damage caused by acid mist-carbon exposure, including occasional rounded, swollen epithelial cells, focal losses of ciliated cells and elevations in mucosubstances, did not differ from that produced by acid mist alone, but the damage was greater than that produced by carbon only.

Alarie et al. (1973) exposed guinea pigs (whole body) and cynomolgus monkeys (face mask) to mixtures of fly ash (5 mg/m^3) and sulphur dioxide (0.1-5 ppm) for 52 weeks (23 h/d). This same group previously found that exposures to these materials alone do not cause deleterious effects. Likewise, no evidence was found that combined exposures to sulphur dioxide and fly ash significantly affected the pulmonary function status of the animals or other endpoints, including serum biochemistry and hematology and lung histopathology.

Inhaled fine-mode volcanic ash (0.65 µm MMAD, 9.4 mg/m^3), sulphur dioxide (2.7 mg/m^3) and a combination of sulphur dioxide and ash inhalation (2 h/d for 5 days) yielded no observable effects in normal rats and rats with elastase-induced emphysema (Raub et al., 1985). In a second part of the study, coarse (12.1 µm MMAD, 50 mg and 300 µg/rat) and fine (2.2 µm MMAD, 50 mg and 300 µg/rat) volcanic ash, pure silica (<5 µm, 50 mg and 100 µg/rat) and saline were intratracheally instilled into elastase-induced emphysematous and normal rats (Raub et al., 1985). Low- and high-dose injection of ash did not produce any indication of fibrosis. However, lung wet and dry weights increased and pulmonary alveolitis was observed after the high-dose instillations. In addition, silica-instilled rats showed changes consistent with interstitial lung fibrosis.

Murthy and Holovack (1991) investigated the ultrastructural effects of cadmium oxide (0.1 mg), alone and in combination with zinc oxide (5 mg), copper oxide (0.2 mg) and nickel oxide (1 mg), one week after these compounds were intratracheally instilled into the lungs of rats. Treatments with these heavy metals caused increases in alveolar macrophages and type II cell hyperplasia, but the general design of the study precludes any clear conclusion that the metals in combination are additive, synergistic or inhibitory.

Fujimaki et al. (1992) examined how exposure to sulphuric acid aerosol in combination with nitrogen dioxide might affect the functional properties of lung mast cells. They exposed guinea pigs to 1.0 mg/m^3 sulphuric acid and 4 ppm nitrogen dioxide for two weeks. Lung mast cell suspensions were then isolated, and antigen or A23187-induced histamine release was measured. No evidence was found that the combined exposure caused an increase in histamine release by the mast cells.

Last and Warren (1987) examined interactions between respirable aerosols of sulphuric acid (0.35 µm MMAD) or sodium chloride (0.38 µm MMAD) and nitrogen dioxide. They exposed (whole body) rats for one, three or seven days (23.5 h/d) to 5 ppm nitrogen dioxide, alone or in combination with ~1 mg/m^3 sulphuric acid or sodium chloride aerosols. A synergistic interaction was found between the nitrogen dioxide and sulphuric acid or sodium chloride exposure, as indicated by lung collagen synthesis (165% increase). Neither exposure alone produced such a response. A synergistic interaction was also shown by the criterion of increased protein content in lung lavage fluids from rats exposed to the nitrogen dioxide and 1 mg/m^3 sulphuric acid after one day of exposure and between the nitrogen dioxide and sodium chloride aerosol after three days of combined exposure.

10.9 ANIMAL TO HUMAN EXTRAPOLATION

The effects reported in experimental animal studies provide a basic understanding of the potential toxicological impacts of inhaled particulate matter. However, to use this information, both to predict potential effects in humans as well as to explain the results reported in human epidemiological studies, understanding those factors that affect the extrapolation of such effects to humans is important. Examining the differences between the experimental animal species themselves is also important, as this can impact upon how the effects are extrapolated to humans.

10.9.1 Differences in Respiratory Anatomy

In considering experimental animal studies, it is important to recognize that there are significant anatomical differences between the experimental animals and humans at all levels of the airway. Three anatomical regions are important when considering the response to the inhalation of particles: the extrathoracic, nose/mouth to the entrance of trachea; the tracheobronchial, trachea to the terminal bronchioles; and the pulmonary, the parenchymal airspace of the lung, including the alveoli. The focus of the studies reported here is limited to those describing effects in the lower respiratory tract (tracheobronchial airway to parenchyma), as it is this region of the airway identified in the epidemiological studies as the target site. A detailed discussion of the inter-species differences in these regions is beyond the scope of this assessment; however, comprehensive review articles are available in the literature (Schlesinger and McFaddden, 1981; Phalen and Oldham, 1983; Tyler et al., 1985; Patra et al., 1986; Henderson et al., 1987; Plopper et al., 1989; Stone et al., 1992).

The fundamental difference in the lower respiratory tract between humans and experimental animals is that the branching of the airways in humans is primarily a regular dichotomous pattern, whereas it is monopodial in mice, hamsters, rats, dogs, horses, rabbits and monkeys. The dichotomous pattern consists of regular subdividing of the major airway into increasingly smaller airways, as opposed to the monopodial pattern, which consists of a long tapered airway with smaller side branches. These differences are important, as particles tend to accumulate at the branch points in the airway owing to impaction.

At the level of the peripheral airways, humans have several generations of well-defined bronchioles, whereas the bronchioles are ill-defined or not present at all in rats, mice and hamsters. As the bronchioles are the last generation of the conducting airway bearing cilia, this difference is manifest in differences in clearance kinetics (see below).

In addition, when assessing toxic responses to inhaled materials in rodents, it has to be kept in mind that rodents are obligatory nose breathers, whereas a significant fraction of humans are routinely mouth breathers or oro-nasal breathers (Niinimaa et al., 1981), and inspired airflow partitioning from the nasal route to the oral pathway in otherwise predominantly nose breathers occurs during many human activities (e.g., Saibene et al., 1979; Camner and Bakke, 1980; Niinimaa et al., 1981). Accordingly, results emanating from such studies may not realistically depict the responses that may occur in humans when defences offered by the upper respiratory tract are bypassed via mouth breathing during exposure to toxic gases or particles.

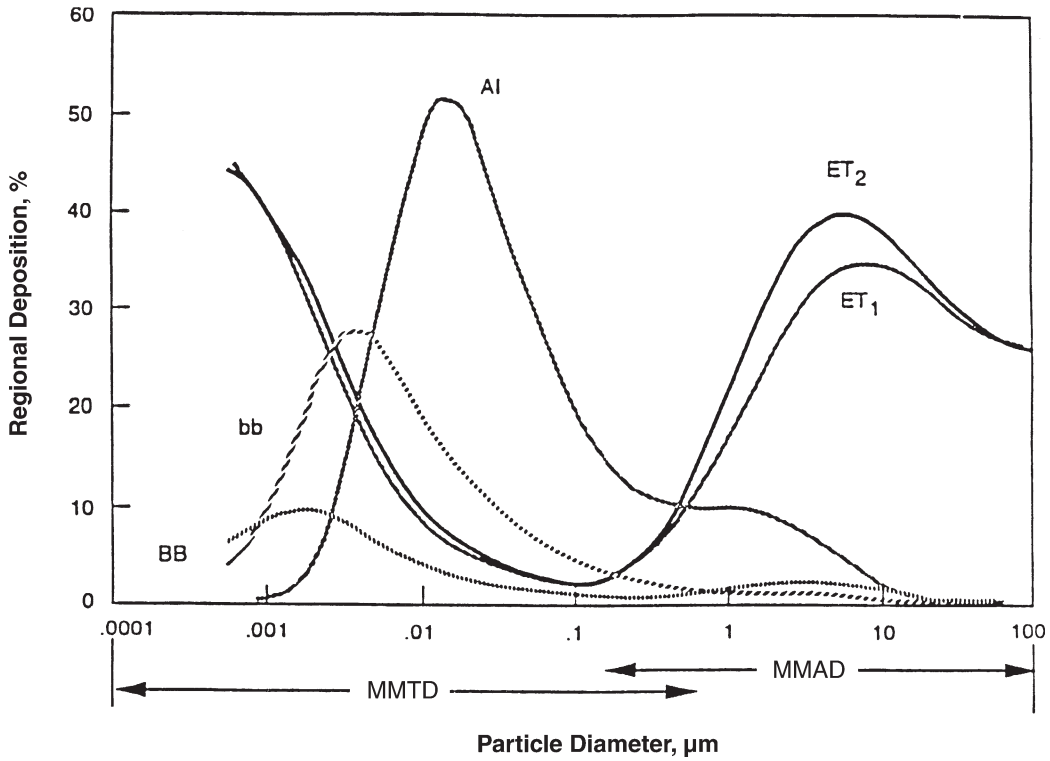
10.9.2 Deposition, Clearance and Fate

The deposition, clearance and fate of inhaled particles in the human airway have been extensively studied using low doses of a wide variety of relatively inert particles. Studies in experimental animals have tended to use very high doses to examine the effects of dose on the mechanisms of the deposition of the particles. A considerable effort has been made to model the deposition and clearance of particles within the respiratory tract.

Deposition

Deposition studies examine the rate and extent to which particles are delivered at the site of toxic action. Detailed reviews of the deposition of inhaled particles have been published by the US EPA (1982a) and more recently by Dodgson and McCallum (1994) and ICRP (1994). When considering the potential health impacts resulting from the inhalation of particulate matter, the regional deposition must be considered rather than just total deposition, owing to the very different clearance times within the regions and the different potential target tissues. The experimental data and modelling efforts are very well developed for deposition of airborne particulate matter, and the information base for humans has been summarized in Figure 10.1.

Figure 10.1 Deposition of inhaled particles in the human respiratory tract during nose-breathing, showing the high alveolar deposition for ultrafine particles of ~20 nm diameter in the AI region of the lung.



AI= alveolar-interstitial (including respiratory bronchioles, alveolar ducts and alveolar sacs); BB= bronche; bb=bronchioli; ET_1=anterior nasal; ET_2=posterior nasal; pharynx and larynx; MMAD=mass median aerodynamic diameter; MMTD=mass median thermodynamic diameter (adapted from ICRP,1994)

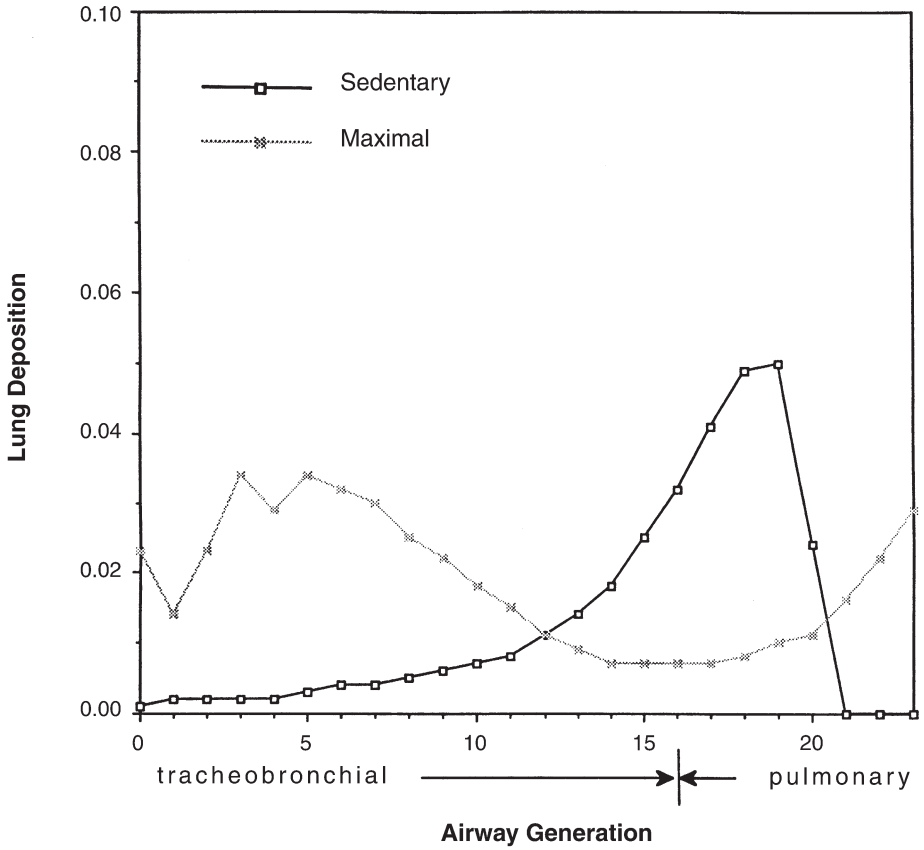
In the extrathoracic region in humans, virtually all particles >10 µm in diameter, when inhaled through the nose, are deposited in the nasal region, whereas during mouth breathing this drops to approximately 65% (US EPA, 1982a). Owing to the bypass of the nasal cavity during oral breathing, up to 10% deposition of particles up to 15 µm in diameter can occur in the tracheobronchial region (Miller et al., 1979). As impaction is the primary mechanism of deposition in the extrathoracic region, the deposition curve is highly dependent on flow rates.

In the tracheobronchial region, only particles <10 µm in diameter (bb and BB in Figure 10.1) are deposited; however, as noted above, during mouth breathing, particles up to 15 µm in diameter can deposit in this region.

The deposition of particles in the pulmonary region in humans is the most critical regarding the health effects associated with particulate matter. These generalized deposition fractions have also been shown to change as a function of breathing patterns. In a series of studies at a constant flow rate of 250 ml/s, increasing the residency time between two and eight seconds resulted in a decrease in the particle size showing the greatest probability of depositing in the lung as well as a decrease in the total deposition fraction (Heyder et al., 1980, 1986). It was also shown that the particle size of maximal deposition for the peak at approximately 3.5 µm diameter, as well as the absolute deposition fraction at the peak, decrease as flow rate increases (Heyder et al., 1980, 1986).

An example of the effect of changes in respiratory mechanics (through exercise) on the overall regional deposition of particles throughout the airway can be seen in Figure 10.2, where the trachea = generation 1; terminal bronchioles = generation 16; and alveoli = generation 23 (Martonen et al., 1992). The model predicts a significant shift in maximum deposition sites from the alveolar region of the respiratory tract towards the conducting airways when switching from sedentary to maximal physical activity in a human inhaling 2 µm-sized particles. The deposition patterns

Figure 10. 2 Model prediction of change in deposition of inhaled 2 µm diameter particles in human airways when physical activity is increased (Adapted from Martonen et al., 1992)



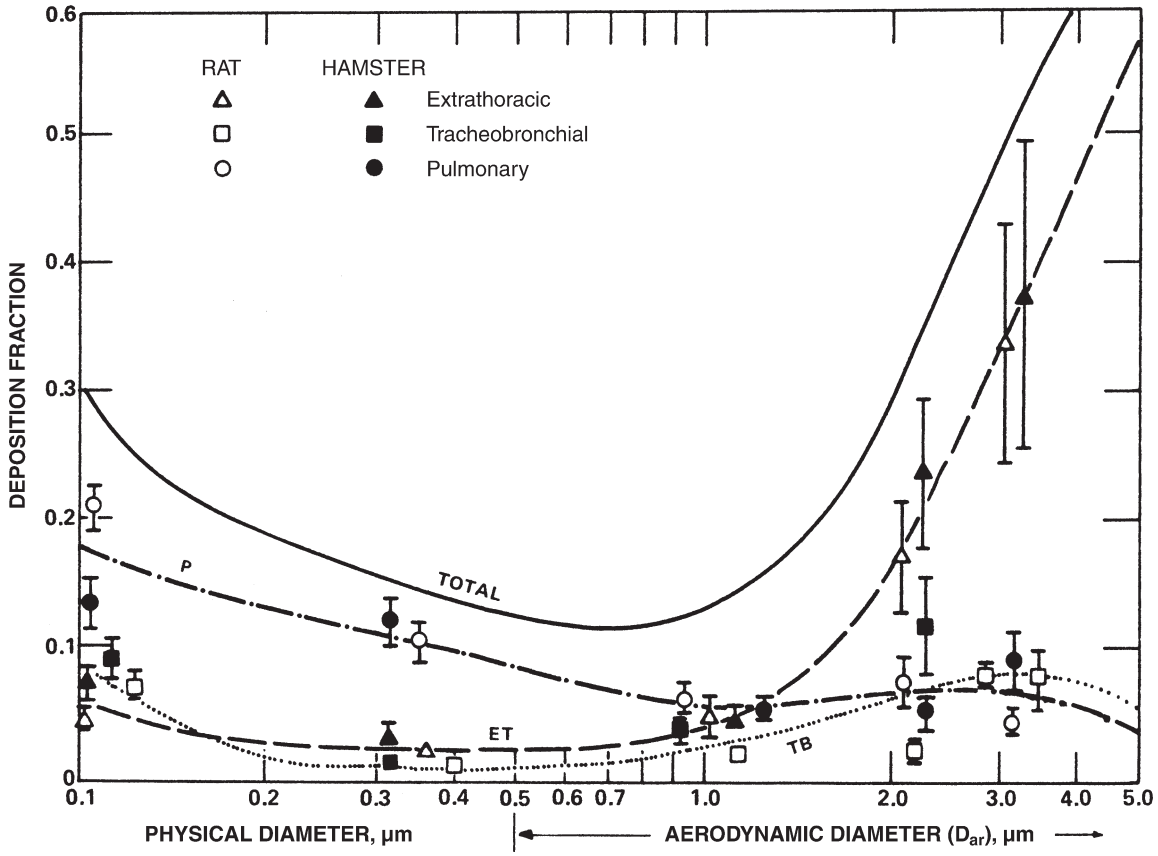
for intermediate levels of exercise are similar, with the pulmonary deposition occurring more deeply in the lung and an increasing amount of deposition in the tracheobronchial regions.

Modelling simulations have predicted significant age-dependent changes in the relative deposition of particles within the airway. Calculations based on diesel exhaust particles (0.2 µm) showed increased deposition in the nasopharyngeal region for children, compared with adults; maximal deposition was in the respiratory bronchiole region (airway 17) for newborns, whereas it was at the alveolar region (airway 20) for adults and children two years of age and older (Yu and Xu, 1987). Becquemin et al. (1987) measured total deposition of 1 or 2 µm polystyrene particles in children (<8 years and >8 and <15 years) and adults. The children, particularly those <8 years of age, showed higher deposition for spontaneous and quiet controlled breathing but showed lower deposition during exercise, with higher deposition in all cases for 2 µm versus 1 µm particles. Specific regional deposition was not examined.

Deposition in experimental animals is important both for understanding the toxicity of particles in animals and for extrapolating such studies to humans. Figure 10.3 summarizes the regional deposition data from Raabe et al. (1977) for rats and hamsters exposed to fused alumino-silicate spheres. It is important to note that particles >5 µm in diameter do not reach the tracheobronchial region and therefore would not be considered “inhalable” for rats or hamsters.

The effect of exercise on particle deposition in animals has also been studied. Exercising and resting rats were exposed for 30 minutes to ultrafine $^{67}Ga_2O_3$ particle aggregates (0.1 µm, 1.7 mg/m^3). Exercising rats had a lower lung deposition efficiency; however, as these rats also had higher inhaled volumes over the exposure period, resulting in more particles being inhaled, the final particle lung burdens in exercising and resting animals were not different (Hesseltine et al., 1986). Increased lung burden was demonstrated when nighttime and daytime particle depositions of gallium oxide aerosol were compared in rats. Greater deposition was found to occur during

Figure 10.3 Deposition of inhaled monodispersed aerosols of fused aluminosilicate spheres in small rodents showing the deposition in the extrathoracic (ET) region, the tracheobronchial (TB) region, the pulmonary (P) region, and the total respiratory tract based upon Raabe et al., (1977). (From EPA 1982)



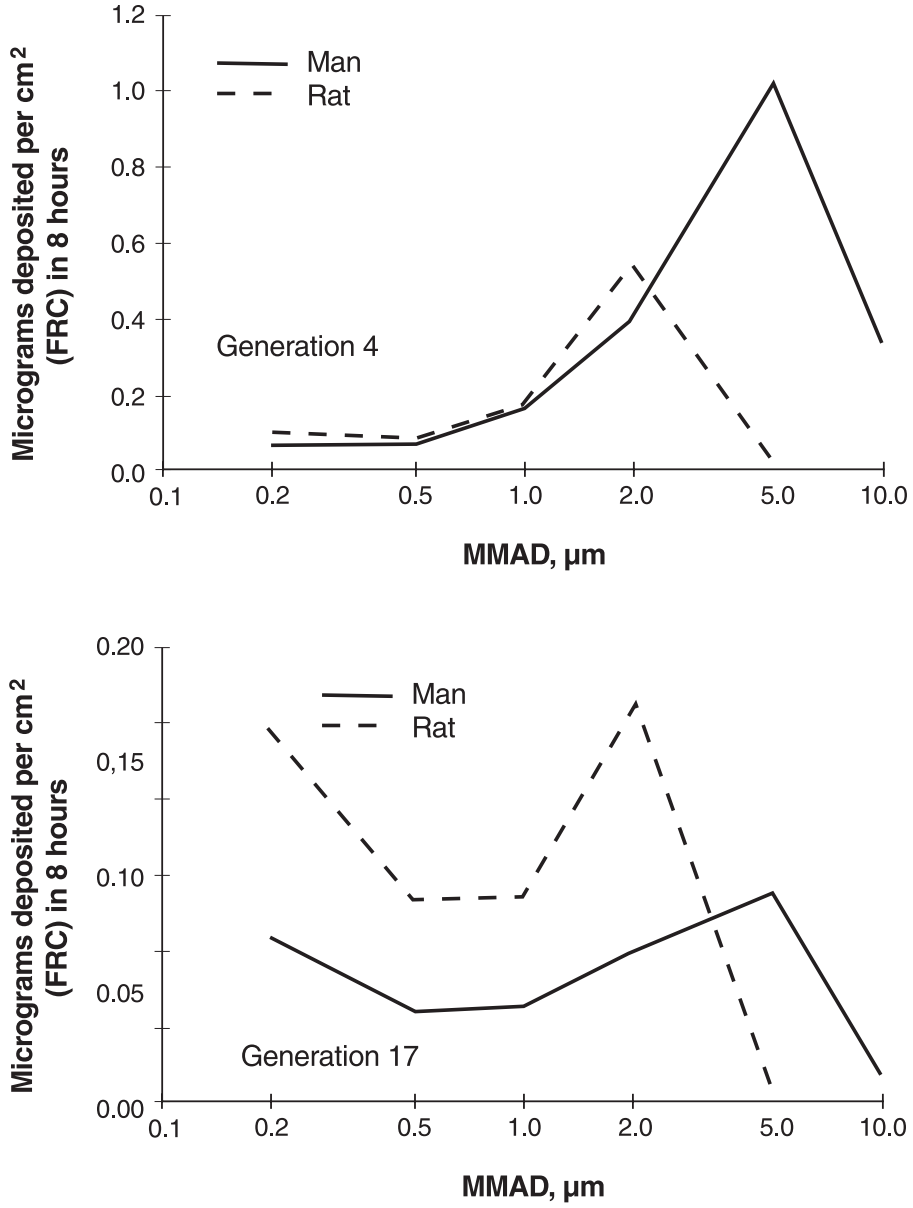
the nighttime exposures, which the investigators postulated may reflect an increased ventilation during the nocturnal activity common to rodents (Hesseltine et al., 1985). In exercising and resting hamsters exposed for 45 minutes to latex particles (0.9 µm, 4959 particles/cm^3), the exercising hamsters had significantly higher particle deposition in their noses, whereas the deposition in the alveolar region was unaffected by exercise compared with resting hamsters (Zeltner et al., 1991).

The fractional regional deposition efficiency alone does not necessarily reflect the actual dose received by a given region in the respiratory tract. Instead, the actual amount (dose) deposited per unit surface area of a given airway generation may be of much greater relevance for respiratory tract dosimetry. Figure 10.4 shows two examples of a deposited surface area dose in individual generations of the lower respiratory tract of rats and humans (Oberdörster et al., 1994b). Whereas for larger particles a greater surface area dose in the upper generations is achieved in humans, the opposite is true for the smaller particles (<~3 µm) depositing in the transitional zone of the lung (generation 17) (i.e., a greater surface area dose is reached here in the rat). These models predict significant differences in the deposited dose between specific airway generations in rats and humans. This difference can have significant implications for observed effects and further complicates the extrapolation of animal studies to humans.

Clearance and Fate

The mechanisms by which particles are cleared from each of the three regions of the airway discussed above are quite different. In the extrathoracic region, clearance occurs by mechanical processes; in the nasal area, by blowing, wiping or sneezing; and in the more anterior regions, either through swallowing (in mucus) or by expectorating. Clearance from the extrathoracic region may take up to one or more days in humans (Proctor and Wagner, 1965).

Figure 10.4 Model prediction of mass deposition rate per unit airway surface in generation 4 and generation 17 of the lower respiratory tract in rats and humans, as a function of inhaled particle size. Significant differences are apparent (inhaled concentration: 1 mg/m^3; nasal breathing at rest) (Oberdörster et al., 1994b).



In the conducting airways of the tracheobronchial region, the most prominent mechanism for elimination of particles is via the action of the mucociliary escalator into the gastrointestinal tract. This is a very fast clearance pathway, which is mainly completed within 24 hours after deposition of particles in this region. The half-time of clearance in humans ranges from half an hour in the larger airways to five hours in the smaller airways (US EPA, 1982a). However, there is some evidence of a long-term component to tracheobronchial retention (Stahlhofen et al., 1986a, 1986b; Smaldone et al., 1988); potential mechanisms for this long-term phase could be phagocytosis and subsequent retention by airway macrophages in the conducting airways or retention of particles in the sol phase of the mucus layer on top of the epithelial cells (Gehr et al., 1990; Lehnert et al., 1992).

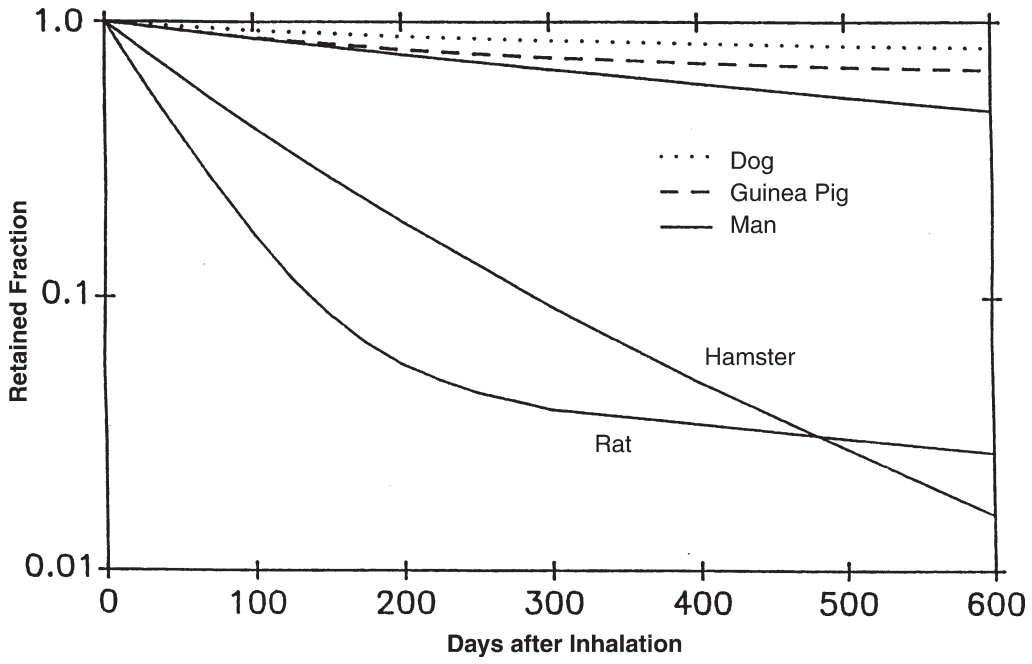
In the pulmonary region, insoluble particles are rapidly phagocytized by alveolar macrophages, or they may also enter the interstitium via endocytosis by alveolar epithelial cells, specifically type I cells. Under conditions of lung-particle overload, type II cells have also been implicated in the endocytosis and subsequent translocation of particles into the interstitium (Lehnert et al., 1992). Ultrafine particles (<~50 nm) have a much greater propensity to penetrate into the pulmonary interstitium and escape phagocytosis by alveolar macrophages (Ferin et al., 1991).

Following phagocytosis, the macrophages can migrate such that they are cleared via mucociliary flow; however, particle-laden alveolar macrophages are found in the lung up to many hundreds of days post-exposure, indicating that some macrophages do not migrate and that particles are re-ingested by other generations of alveolar macrophages.

Although mechanisms of clearance are very similar among different species, significant differences have been observed with respect to the pulmonary long-term retention of highly insoluble particles in different species, as summarized in Figure 10.5. Lung clearance of these particles in rats and hamsters is much faster than in dogs, guinea pigs and humans. Mice are also similar to rats in this regard. For the bulk of the deposited particles in the alveolar region, the clearance rate in the rat is ~1% per day, equivalent to a retention half-time of ~70 days. In contrast, the human pulmonary retention half-time for highly insoluble particles is ~500 days.

The change of clearance rate with time after cessation of exposure has been determined by Bailey et al. (1985) for humans. Based on their studies with radiolabelled fused alumino-silicate particles (1 and 4 µm in diameter) in humans, they found clearance to have two phases, with half-times of tens of days (intermediate phase) and several hundred days (slow phase). Clearance times were unaffected by particle size. Particle size did play an important role in alveolar macrophage-mediated lung clearance in rats instilled with ~10 µm polystyrene microspheres. Although they were phagocytized, they were not removed from the lungs of rats after a prolonged post-depositional period (Oberdörster et al., 1992b).

Figure 10.5 Long-term lung clearance of highly insoluble particles determined in different species by pulmonary retention measurements over several hundred days. (Adapted from Bailey et al., 1985b, 1989; Snipes et al., 1989)



There are a number of potential processes whereby particles are cleared via translocation through the interstitium. Phagocytosis by interstitial macrophages can occur, resulting in release of mediators and also transport of phagocytized particles in lymphatic channels to regional lymph nodes (Lehnert et al., 1992). Translocation of a small fraction of particles from lymph nodes into the blood compartment and then into any organ of the body may occur, as is suggested by results of studies with fibres that were found to appear in post-nodal lymph (Oberdörster, 1988) and by results with high lung burdens of titanium dioxide particles (Lee et al., 1985b) appearing in spleen and liver.

A recent report by Adamson et al. (1992) also indicates that transport of particle-containing interstitial macrophages back into the alveolar region may occur during a controlled inflammatory response. Particle size can play a decisive role in the ability of particles to translocate from the alveoli to the regional lymph nodes that receive lymphatic drainage from the lung. For example, Snipes and Clem (1981) found that 3 and 7 µm polystyrene particles instilled into the lungs of rats passed to the mediastinal lymph nodes, whereas larger (9 µm diameter) particles did not.

Rat studies have shown that a high interstitial particle load can result in an accumulation of particles in the bronchus-associated lymphatic tissue (BALT), which would be important for immune responses in the human lung (Ferin and Oberdörster, 1992). BALT has only recently been found to occur at significant levels in human lung (Pabst and Gehrke, 1990).

The retention and, accordingly, the effects of inhaled particles may also be influenced by the level of body activity. Exercise done by hamsters after they were acutely exposed to a colloidal gold aerosol (0.005-0.02 µm in diameter) has been reported to enhance the clearance of the particles from their lungs (Sweeney et al., 1990). Conversely, Collier et al. (1988) found that resting and exercising rats inhaling ^{57}Co-labelled fused alumino-silicate particles (~1-2 µm in diameter) showed no difference in the lung clearance of these particles.

Clearance mechanisms and pathways for soluble particle compounds involve diffusional transport via the tight junctions of the airway epithelium into underlying structures, including capillaries of the blood and lymph circulation. Pinocytotic processes (i.e., the cells engulf the particles within membrane vesicles) across the alveolar epithelium have also been described. Once in the circulatory system, transport to storage and excretory organs occurs as the final stage of the lung clearance process. Solubilization of particle compounds in the acidic milieu of the alveolar macrophage phagolysosome can also contribute significantly to dissolution of these particles. Additional details of lung clearance of inhaled particulate matter materials are described in several review articles (e.g., Schlesinger, 1985; Oberdörster, 1988; Snipes et al., 1989).

10.9.3 Conclusion

Quantitative extrapolation of results from animal studies to humans for purposes of risk assessment requires not only consideration of the differences in anatomical, morphological and functional aspects of the different respiratory tracts, but also detailed dosimetric adjustments to account for exposure-dose-response relationships obtained from animal studies. Dosimetric adjustments that must be made to appropriately extrapolate animal studies to humans would include adjustments for differences in particle deposition efficiencies in the respiratory tract and differences in retention and clearance kinetics. Deposition efficiencies of inhaled particles in the various regions of the respiratory tract differ between laboratory animals and humans. For example, respirable particles for humans can be larger than 5 µm, whereas for rats those particles are no longer respirable. Once the particles are deposited in the respiratory tract, they will be removed by specific clearance mechanisms. The main removal mechanisms for highly insoluble particles consist of the mucociliary escalator in the conducting airways and of phagocytosis by alveolar macrophages in the alveolar region. The particle removal rates vary considerably among species – e.g., the rat has a pulmonary retention half-time for highly insoluble particles of ~70 days, whereas in humans the retention half-time is in the order of 500 days. These species' differences need to be taken into account when interpreting or extrapolating results from animal inhalation studies.

An example of comparative dosimetry for particle exposure is illustrated by Miller et al. (1995). Their findings show that, while deposition of particles on a mass per unit alveolar surface area is not different between humans and rats, dose metrics based upon particle number per various anatomical parameters (ventilatory unit, alveolus, or alveolar macrophage) exhibit marked differences between rats and humans, particularly for particles 0.1-0.3 µm in size. Based on

the calculations per ventilatory unit or per alveolus, humans receive much greater numbers of particles than do rats when exposed to the same concentration and size of particles. The trend of differences between humans and rats is even more pronounced for the individuals with compromised lungs (smokers, asthmatics and patients with chronic obstructive pulmonary disease) compared with normal subjects. Therefore, rats exposed to 1000 to 1500 μg/m^3 of particles may actually have received the levels of particles equivalent to 120-150 μg/m^3 in humans.

On the other hand, a purely dosimetric adjustment for extrapolation from experimental animals to humans will not be sufficient, as it is based on the unproven assumption that the same dose in the respiratory tracts of experimental animals and of humans will result in the same effect. Most likely, this is not the case. Even between rodent species, responses vary considerably; for example, pulmonary inflammatory and fibrogenic effects after inhalation of highly insoluble particles are most readily induced in rats, much less in mice and least in hamsters. Similarly, particle-induced lung tumours were seen only in rats but not in mice and hamsters. However, these particle-induced responses were seen only at inhaled concentrations of particles that led to very high lung burdens during chronic exposures (i.e., they are not relevant for extrapolation to humans).

Given these limitations, the animal toxicology studies should be interpreted in terms of how such studies might lead to an understanding of the mechanisms that lead to the causal path for the effects reported in the human epidemiological studies.

10.10 MECHANISTIC EVALUATION

The animal studies clearly show effects on the lungs resulting from the inhalation of particulate matter, effects that can be attributed to a particle effect rather than to the inherent chemical toxicity of the particle. These effects include decreased lung function, as measured by a number of parameters; decreased (or in some cases increased) particle clearance from the lung; increased alveolar macrophage numbers and activity (although the more cytotoxic particles can reduce both alveolar macrophage numbers and activity); decreased antimicrobial defence capacity (although this may be related to the cytotoxicity of the particle); biochemical changes associated with activation of alveolar macrophages or fibrosis; and morphological changes, including collagen formation and fibrosis, lipoproteinosis and epithelial hyperplasia. Cardiovascular abnormalities have also been observed after acute exposure to particulate matter. There is also evidence that prolonged exposure to high levels of particles can lead to lung cancer in rats.

A significant concern with all these studies is that the particle concentrations required to generate the toxic effects are well above those reported in the ambient environment. Many studies required particle levels that exceeded the clearance capacity of the lung to such an extent that clearance essentially shut down – the so-called “particle overload” (Morrow and Mermelstein, 1988; Morrow, 1994).

What these studies have demonstrated is that within the range of particles ≤10 μm in diameter (i.e., PM_{10}), the smaller particles seem to have more pronounced effects. This would not be unexpected if the total particle load was seen in terms of the number of particles, rather than particle mass. As shown in Figure 10.6, the urban ambient aerosol is represented by a trimodal particle size distribution, with an ultrafine, a fine and a coarse mode. Essentially all of the particles in these three different modes are respirable and would conform to the PM_{10} definition. However, some more recent epidemiological studies have suggested that the fine fraction of the PM_{10} particles (e.g., $PM_{2.5}$) correlates better with observed adverse effects.

Table 10.1 compares the number of particles per cubic centimetre of air for the same mass concentrations, for differently sized particles. As can be seen from this table, a particle with a diameter of 2.5 μm (i.e., conforming to the $PM_{2.5}$ definition) contributes only 1 particle/cm^3 to a total mass concentration of 10 μg/m^3, whereas it takes 2.4 million particles of 20 nm diameter (i.e., ultrafine particles) to achieve the same mass concentration. In addition, particle surface area is also considerably increased for the fine and ultrafine particles.

As for mechanisms, it has been suggested that the ultrafine particles, by virtue of their numbers and level of toxicity, could present a potential hypothesis to explain the effects reported at low total particle mass levels. Unfortunately, ambient monitoring of the ultrafine particle mode of the urban aerosol is much more difficult, and, therefore, few data are available.

The exact mechanism of the increased toxicity of ultrafine particles is not known. However, several factors appear to be important. One is that their

Figure 10. 6 Particle volume distribution of ambient aerosol measurement by Wilson et al., (1977) in road tests. A typical trimodal distribution showing a coarse, an accumulation (fine), and a nuclei (ultrafine) mode is shown with geometric mean diameter of 4.9 µm, and 0.21 µm and 18 nm, respectively. (SC=geometric standard deviation). (Adapted from Wilson et al., 1977)

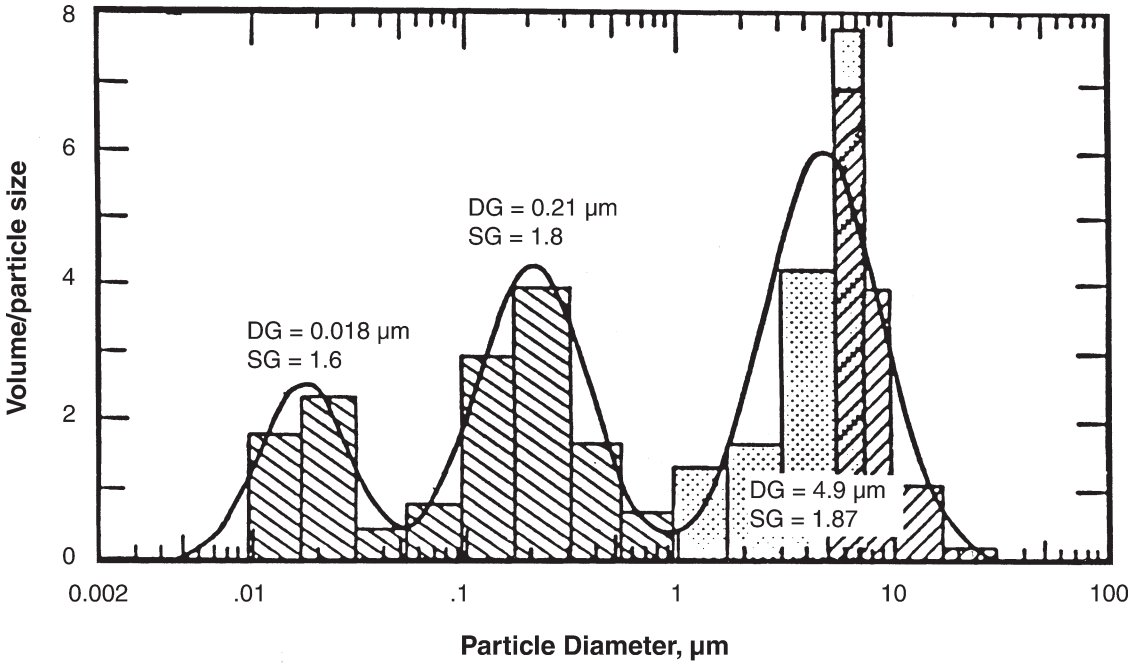


Table 10.1 Numbers and Surface Areas of Monodisperse Particles of Unit Density of Different Sizes at a Mass Concentration of 10 µg/m^3 (Oberdörster et al., 1995)

Particle diameter (µm)	Particle no./cm^3 air	Surface area (µm^2/cm^3 air)
0.02	2 400 000	3 016
0.1	19 100	600
0.5	153	120
1.0	19	60
2.5	1.2	24

considerable surface area represents a larger area for interaction with biologically active sites; the surface area of retained particles of different sizes, including ultrafines, was found to correlate with the pulmonary inflammatory response (Oberdörster et al., 1992). Another factor is that such ultrafine particles are not as readily phagocytized by alveolar macrophages, and therefore they are more likely to react with alveolar epithelial cells and be translocated to the interstitium (Ferin and Oberdörster, 1992). Finally, it appears that inhalation of ultrafine particles as singlet particles, especially when freshly generated, is of significance and leads to greater effects than inhalation of aggregates of ultrafine particles (Oberdörster et al., 1994a). The highly reactive surfaces of these particles apparently lead to severe damage of the alveolar-epithelial barrier, causing acute pulmonary edema and inflammation, although a pathogenic mechanism is still to be elucidated.

No firm conclusions can be drawn from the results of the numerous animal toxicology studies described in this chapter to answer the question of which particle type and size is most likely to cause the adverse effects observed in human epidemiological studies, described in Chapter 12. With respect to increased morbidity in children due to respiratory tract symptoms (e.g., Dassen et al., 1986; Burnett et al., 1995), it is plausible that acidic aerosols (i.e., sulphates) could be a major causative factor. Results from animal studies, almost exclusively in guinea pigs, have unequivocally shown that these aerosols lead to increased bronchial reactivity that may be of particular importance for asthmatic individuals, although pure sulphate aerosols had to be administered at >100 µg/m^3 to cause these effects (Chen et al., 1992b). The mean concentrations of ambient sulphates measured across Canada from 1984 to 1993

ranged from 1.6 to 6 µg/m^3 (Dann, 1994), and increased bronchial reactivity at these levels has not been observed in animal studies. However, Chen et al. (1992a) showed that sulphuric acid exposure levels as low as 20 and 30 µg/m^3, when the acid was coated onto carrier particles, caused increased hypersensitivity in guinea pigs. The carrier particles were generated as ultrafine particles (50 nm), which had a high specific surface area (Table 10.1) and, therefore, could well function as carriers for adsorbed compounds. As the co-administered ultrafine carrier particles were at a concentration of ~1-2 mg/m^3, they were in all likelihood aggregated. Still, an additional effect of these particles cannot be excluded; moreover, these high levels of particle air pollution do not occur in the ambient environment.

Thus, although the particle carrier hypothesis appears to be reasonable, the much higher experimental concentrations compared with relevant ambient exposure levels make it very difficult to use such data for direct extrapolation to humans. Regardless, it appears that the fine-particle phase is more important than the larger particles for these types of effects.

The role of surface-complexed iron (Fe^{3+}) on particles for inflammatory responses in the respiratory tract should also be considered (Ghio et al., 1992; Ghio and Hatch 1993; Costa et al., 1994). An increase in reactive oxygen species via the Fenton and Haber-Weiss reaction may potentiate the inflammation. However, concentrations of iron needed to cause the increased inflammatory response as well as the necessary concentrations of inhaled particles are not likely to be encountered at normal ambient particle levels.

The epidemiological finding that ambient particulate matter air concentrations below 100 µg/m^3 are associated with increased mortality (Section 12.2) cannot be directly explained by animal studies as far as PM_{10} and $PM_{2.5}$ particles are concerned. Except for the ultrafine fume particles, none of the other particle types and sizes used in animal inhalation studies cause such acute dramatic effects, including high mortality at relatively low concentrations. However, one comparative dosimetric analysis conducted by Miller et al. (1995) indicates that, based on the calculations per ventilatory unit or per alveolus, humans receive much greater numbers of particles than do rats when exposed to the same concentration of PM. The trend of differences between humans and rats is even more pronounced for the individuals with compromised lungs (smokers, asthmatics and patients with chronic obstructive pulmonary disease) compared with normal subjects. Therefore, rats exposed to 1000 to 1500 µg/m^3 of particles may actually have received the levels of particles equivalent to 120-150 µg/m^3 in humans.

The mechanism for particle-induced cardiovascular response is not yet clear. It may involve the oxidation of low density lipoprotein by reactive oxygen species accompanying particulate matter pollution. Oxidized low density lipoprotein has been found to cause endothelial damage, the proliferation of smooth-muscle cells, monocyte-endothelial interactions, platelet aggregation, and inhibition of endothelial-derived relaxation of vascular smooth muscles (Morel et al., 1983; Ocana, 1989; Berliner et al., 1990; Cushing et al., 1990; Ezaki et al., 1994; Ikeda et al., 1995; Ichinose et al., 1995). Thus diesel exhaust particle-induced oxidization of low density lipoprotein may play a pathological role in inducing atherosclerosis and coronary vasospasm.

With respect to an association of particulate matter air pollution with increased lung cancer (Section 11.4.6), results of animal studies are again not very informative. Animal inhalation studies aimed at investigating carcinogenic potentials of inhaled particles have shown that high inhaled concentrations of diverse particles such as titanium dioxide, carbon black and diesel exhaust cause lung tumours in rats. Initially, it was thought that diesel exhaust-induced lung tumours were due to the existence of PAHs adsorbed onto the diesel particles (Heinrich et al., 1986a); however, subsequently performed studies with carbon black showed that these particles without adsorbed PAHs were as effective in inducing lung tumours (Heinrich et al., 1992; Pott et al., 1994).

Thus, although particles in general have been shown to induce lung tumours in rats, inhaled concentrations were in the milligram per cubic metre range, far beyond current ambient environmental levels. Indeed, these high experimental exposure concentrations have been shown to lead to particle overload-related reactions in the lung, which include chronic inflammatory and fibrotic responses (Morrow and Mermelstein, 1988; Morrow, 1994). The term “overload” indicates that the alveolar macrophages have been overwhelmed to such an extent that their ability to clear particles out of the lung becomes impaired. This impairment of clearance in turn results in increasing amounts of particles being retained in the lung, with subsequent translocation to interstitial sites and respective effects on alveolar, epithelial and interstitial cells. It has even been shown that DNA

adducts are induced by high levels of carbon black as well as diesel soot in the lungs of rats, although the quality of the adducts may differ between the two particle types (Bond et al., 1990). Mechanistically, it is suggested that the chronic inflammatory response occurring in the lung from high particle load leads to the release of many mediators – i.e., cytokines, growth factors, enzymes and oxygen-derived radicals – from alveolar macrophages and possibly other lung cells (Driscoll et al., 1990; Morrow, 1994). These mediators in turn induce increased epithelial cell proliferative responses and adduct formation, thereby increasing the possibility of mutations becoming manifest, and they may eventually result in tumorigenesis.

This "overload" concept, and its implications with respect to the interpretation of results from studies conducted at high particle loads, is similar to the maximum tolerated dose (MTD) concept in cancer bioassay protocols. Without going into the specific details of the MTD concept, the basic premise is that in many cancer bioassay protocols, including the National Toxicology Program (NTP), the high-dose group in the study should be dosed at a level that induces some level of toxicity. Concerns have been raised in the scientific community that such dose levels may not be relevant, as they may exceed the normal ability of the test animals to detoxify the material or cause tissue damage that may lead to secondary processes that would not be seen at lower, more environmentally relevant, dose levels. Many scientific papers have been written on the MTD issue, too many to cite adequately; however, an excellent critique written by Haseman (1985) provides a basic overview of the issues.

Thus, although the hypothetical, mechanistic scenario derived from the animal studies may be very plausible, these high-load chronic inhalation studies performed in experimental animals may have no relevance for low-level ambient exposures.

Complex interactions between differing constituents of air contaminants have been examined in animal studies only to a limited degree, mostly focussing on one particle and one gas-phase compound only. As shown in Section 10.9, such combined exposures resulted in mixed responses, showing either no effect of the combination or some synergism with respect to certain endpoints, but overall the results are equivocal. However, realistic environmental conditions are far more complex than those re-created in the laboratory, and the respective effects of exposures to such mixtures are not well predictable. In addition, it should be kept in mind that susceptible groups within the population may react much more sensitively to a given inhaled particle/gas mixture. Furthermore, a compromised respiratory system will also be much more sensitive to the adverse effects of inhaled pollutants. Unfortunately, most of the studies performed today have been concerned with the deposition and clearance efficiencies in such conditions, rather than with health endpoints *per se.*

10.11 CONCLUSIONS

The data available from experimental animal studies indicate that the particle types found in the ambient atmosphere that are most likely to induce acute adverse effects include the fine particle mode, including acidic sulphates, possibly occurring as a coating on fine or even ultrafine carrier particles. These effects include decreased lung function, decreased (or in some cases increased) particle clearance from airways, altered alveolar macrophage numbers and functions, modified immunological function, cytotoxicity and histological changes, and cardiographic abnormalities. There is also evidence that prolonged exposure to high levels of particles can lead to lung cancer in rats. Bronchial hypersensitivity to non-specific stimuli and increased morbidity from cardio-respiratory symptoms would most likely occur in animals pre-disposed to cardio-respiratory diseases.

It appears that the ultrafine particle mode may be of significant toxicological importance. On the other hand, the coarse particle mode is less likely to induce acute adverse responses, although these larger particles may well contribute to such effects. The particle deposition data clearly indicate that TSP is not an appropriate measure of particulate matter as it relates to human health effects, as only particles <10 µm in diameter (and possibly up to 15 µm in diameter during mouth breathing) penetrate to the lungs. The evidence from the animal toxicology studies indicates that the toxic effects of airborne particles are more likely to be associated with the fine mode of particulate matter air pollution (i.e., $PM_{2.5}$, as opposed to PM_{10}), including acidic sulphates, possibly occurring as a coating on fine or even ultrafine carrier particles.

The interaction between ozone and sulphuric acid evidently is one in which the damaging effects of the ozone in the centriacinar region of the lung are enhanced by sulphuric acid aerosols.

11 HUMAN HEALTH EFFECTS – CLINICAL STUDIES

A series of recent reports have shown that variation in ambient PM_{10} concentrations, even at current levels, is positively associated with the variation in daily cardiopulmonary mortality and total mortality, excluding accidental and suicide deaths (Bascom, 1994). These associations have been shown in different communities, including Philadelphia, St. Louis, the Utah Valley and Santa Clara County, California of the US, and in Ontario, Canada, with widely differing particle composition and climates. Despite these epidemiological findings for acute and chronic adverse health effects from air pollution associated with respirable particles, there are as yet few complementary data from animal toxicology studies and controlled human exposures to respirable particles. In this chapter, a review of controlled human exposure studies (clinical studies) relevant to understanding the effects of inhaled particulate matter at current concentrations is presented, based on a contract report prepared for Health Canada (Utell and Samet, 1994).

11.1 CLINICAL STUDIES

Carefully controlled, quantitative studies of exposed humans offer a complementary approach to epidemiological investigations (Utell et al., 1993). Human clinical studies utilize laboratory atmospheric conditions considered relevant to ambient pollutant concentrations and document the health effects that result from breathing known concentrations of pollutants. Advantage is taken of the highly controlled environment to identify responses to individual pollutants and characterize an exposure-response relationship. In addition, such a controlled environment provides the opportunity to examine interactions among pollutants or with other environmental variables, such as exercise, humidity or temperature. Insofar as individuals with acute and chronic respiratory diseases can participate in exposure protocols, potentially susceptible populations may also be studied. This approach also has its limitations. For practical and ethical reasons, studies must be limited to small groups, which may not represent larger populations. Exposure must also be limited to short durations and to concentrations of pollutants expected to produce mild and transient responses. Exposures are often limited to a single pollutant, or to a very limited pollutant mix that never replicates the complex mixture to which populations are actually exposed. An endpoint assessment will invariably include pulmonary function which may only indicate physiological reflex rather than tissue injury. Furthermore, transient responses in clinical studies have never been validated as predictors of more chronic and persistent effects.

An important feature of controlled clinical studies is the opportunity to examine both healthy volunteers and individuals with underlying cardiopulmonary diseases who are likely to be particularly vulnerable to particulate pollution. Subjects are typically characterized by age, gender, race and lung function. Normal volunteers are characterized by the absence of allergies, often documented by skin testing and lack of hyperreactive airways as assessed by inhalation challenge tests. Within the rubric of healthy volunteers (absence of allergies and normal lung function), adolescent, elderly normal and “smoker” subgroups have been studied in various protocols. Healthy volunteers are typically able to perform vigorous exercise for extended periods and are usually able to tolerate the more invasive investigative techniques such as bronchoalveolar lavage (BAL).

Other “susceptible” subpopulations recruited to participate in clinical studies include asthmatics, individuals with allergies or acute upper respiratory infections and subjects with chronic obstructive pulmonary disease (COPD) or coronary artery disease. For research and clinical purposes, asthmatics can be characterized by their responsiveness to methacholine or carbachol, presence or absence of allergy (skin tests or IgE levels), use of medications, severity of symptoms and degree of airway obstruction assessed by pulmonary function tests. Severely obstructed asthmatics have rarely participated in controlled clinical studies. In Table 11.1 some potential links between susceptible groups and morbidity effects are outlined.

Table 11.1 Potential links between susceptible groups and morbidity effects

Population	Adverse health effects
COPD and asthma	worsening of V/Q
— exacerbation	increased airway reactivity
— pulmonary edema	increased work of breathing
— respiratory infection	increased risk of respiratory infection
individuals with respiratory infections	
— exacerbation	worsening of V/Q
— hypoxemia	hypoxia
individuals with cardiac disease	
— arrhythmia	worsening of V/Q
— pulmonary edema	hypoxia
	pulmonary hypertension

Abbreviations: COPD = chronic obstructive pulmonary disease
V/Q = ventilation-perfusion ratio

Exercise done on either a treadmill or a bicycle ergometer is generally an important component of exposure studies. Exercise enhances the pollutant dose both by increasing ventilation and by causing a switch from nasal to oral breathing. As described in the proceeding chapter, oral breathing effectively bypasses the nose which would otherwise filter out particles and gases, therefore increasing the delivery of particles to the lower respiratory tract. In addition, the effect of exercise on airway drying may enhance the response to pollutants. Minute ventilation during exposure is clearly an important determinant of the magnitude of change in selected physiological measures. Less clear is the influence of continuous versus intermittent exercise on pulmonary function responses. The impact on pulmonary function of exercising at different intensities during pollutant exposures has been reviewed by Horvath (1985).

Finally, the double-blind protocol, which uses a cross-over design, with a sodium chloride control exposure for the aerosol and a clean air control exposure for the gaseous pollutant, is optimal. In such experiments, the investigator, the technician and the subject are unaware of the exposure conditions, that is, whether the exposure on a single day involves a pollutant or control substance. Such a strategy eliminates potential observer bias and avoids relaying clues to volunteers regarding anticipated responses. This is especially important when studying asthmatics, as a significant number of asthmatics may tend to respond to psychological stimuli with bronchoconstriction (Spector et al., 1976).

11.2 SOLUBLE PARTICLES – ACIDIC AND SULPHATE

As described thoroughly in Chapter 3, particulate air pollution is a complex mix of chemical species, of both natural and anthropogenic origin. Of this mixture, clinical studies have tended to examine only the latter category (anthropogenic particles), especially inorganic nitrates and sulphates, since these have played a prominent role in “acid rain.” These studies were reviewed previously by Utell (1985) and more recently by the US EPA’s Acid Aerosols Issue Paper (US EPA, 1989).

In the atmosphere, the major acid sulphate species include $(NH_4)_2SO_4$, NH_4HSO_4 and H_2SO_4. In the laboratory, pulmonary responses of normal and asthmatic subjects have been assessed following exposure to each of these acids. In this section, a summary of recent controlled human exposure studies involving acidic sulphates is presented. Nitrate particles are not considered in this review, as they have not been found to invoke symptoms or functional decrements (US EPA, 1989), or cellular and biochemical responses in airway lavage fluids (Aris et al., 1993) in clinical studies at concentrations

below 1000 µg/m^3. The few reports of controlled exposures to diesel, carbon black or other particles will also be reviewed. Studies in individuals with underlying lung disease (i.e., "susceptible subpopulations") are emphasized, recognizing that responses are typically defined by changes in mechanics; however, findings from healthy volunteers, especially alterations in defence mechanisms or cellular responses, are also considered, if relevant.

11.2.1 Studies of Healthy Volunteers

Controlled experimental exposures have been used to examine the respiratory effects resulting from the inhalation of acidic aerosols, with measured outcomes limited primarily to symptoms and airway function. Although exposures to concentrations of H_2SO_4 aerosols below 1000 µg/m^3 generally do not alter airway function of normal subjects (Utell et al., 1984), Utell et al. (1983a) observed an increase in throat irritation and airway reactivity to carbachol inhalation in normal volunteers 24 hours after exposure to 450 µg/m^3. No change in airway reactivity was detected immediately after exposure. Effects of H_2SO_4 on slowing of mucociliary clearance in small airways begin at concentrations as low as 100 µg/m^3; the duration of the slowing increases with hours of exposure (Spektor et al., 1989).

Only one study has been done to examine the effects of H_2SO_4 aerosols on host defence mechanisms at the alveolar level (Frampton et al., 1992). Non-smoking volunteers were exposed for two hours to an aerosol of approximately 1000 µg/m^3 H_2SO_4 or to a control substance (sodium chloride, NaCl), with intermittent exercise, in a random double-blind fashion. BAL was done 18 hours after exposure to detect evidence of an inflammatory response, change in alveolar cell subpopulations or changes in alveolar macrophage function, all components of host defence. Compared to exposure to NaCl, exposure to H_2SO_4 did not increase numbers of polymorphonuclear leucocytes (PMN) in BAL fluid. The percentage of T lymphocytes decreased in association with H_2SO_4 exposure, but the decrement was not statistically significant. Antibody-mediated cytotoxicity of alveolar macrophages increased in association with H_2SO_4 exposure. Significant changes were not found in release of superoxide anion or inactivation of influenza virus *in vitro*. The investigators concluded that brief exposures to H_2SO_4 aerosols at 1000 µg/m^3 did not cause an influx of inflammatory cells into the alveolar space. No evidence was found for alteration in alveolar antimicrobial defence 18 hours after exposure.

11.2.2 Studies of Asthmatic Volunteers

As with SO_2, asthmatic subjects have been found most susceptible to the effects of acidic aerosol exposure, although different laboratories have found differing threshold exposure concentrations. Generally, however, studies of adult asthmatics have failed to show alterations in lung function at levels below 200 µg/m^3. Utell et al. (1983b) exposed asthmatics to H_2SO_4, NH_4HSO_4, sodium sulphate hydrogen ($NaHSO_4$) and a control NaCl aerosol at concentrations of 100, 450 and 1000 µg/m^3. Following exposures to the 450 and 1000 µg/m^3 aerosols for 16 minutes, specific airway conductance decreased in relation to the acidity of the aerosol, supporting the hypothesis that airway effects are related to acidity rather than to the sulphate ion. More prolonged exposures to H_2SO_4 aerosols have also been performed. With 10-minute exercise periods every 30 minutes, a two-hour exposure to 100 µg/m^3 H_2SO_4 aerosol resulted in a small reduction in flow rates. Response to H_2SO_4 was highest after the first 45 minutes, then airway function recovered, returning towards the baseline, indicating that the effect did not seem progressive over time. Respiratory ammonia was identified as a factor influencing response to H_2SO_4 aerosols. Utell et al. (1989) enhanced airway responses to inhalation of 350 µg/m^3 aerosols in exercising asthmatics by reducing the level of endogenous respiratory ammonia. This provided further evidence that aerosol acidity significantly affected bronchoconstriction caused by inhalation of sulphate aerosols.

Adolescent asthmatics appear to be more sensitive than adult asthmatics to the effects of acidic aerosols. Functional decrements have been observed in adolescent asthmatics at levels as low as 35 µg/m^3 (MMAD 0.6 µm) for 40-90 minutes (Koenig et al., 1989; Hanley et al., 1992; Koenig et al., 1992). The low range of H_2SO_4 concentration is around the peak values measured during the summer months in the eastern United States and southern Canada. The apparent difference in sensitivity of adult and adolescent asthmatics may also be due to selection bias, differences in aerosol sizes or exposure protocols. However, adolescent asthmatics respond to concentrations of H_2SO_4 aerosols an order of magnitude lower than those causing a response in normal subjects. Field studies in summer camps of both normal

and asthmatic children reported decrements in pulmonary function during pollution episodes that included increased levels of acidic aerosols (Raizenne et al., 1989). This reinforces the concern that children and adolescents may be particularly susceptible to the effects of acidic atmospheres.

The sensitivity of asthmatics to acidic aerosols has also been observed in elderly subjects aged 60-75 years (Koenig et al., 1993). When elderly normal and asthmatic volunteers inhaled H_2SO_4 or $(NH_4)_2SO_4$ (70 µg/m^3, MMAD 0.6 µm) for 40 minutes with mild exercise, a significant increase in total respiratory resistance was seen in the asthmatic subjects but not in normal subjects. However, compared with younger asthmatic subjects under a similar H_2SO_4 dose regimen (Koenig et al., 1992), elderly asthmatic subjects do not seem more at risk for adverse respiratory effects from inhalation of acid aerosols.

In another publication by Koenig et al. (1990), asthmatic adolescents were preexposed to ozone (0.12 ppm, 45 min) followed by a low level of SO_2 (0.1 ppm, 15 min) with intermittent exercise ($\underline{V}_E$ = 30 L/min). Preexposure to ozone elicited a greater degree of bronchial responsiveness than did pure air followed by SO_2, or two exposures to ozone alone, indicating some synergism between the effects of SO_2 and ozone. Given that both ozone and SO_2 concentrations were at subthreshold levels for the experimental design used, it is tantalizing to speculate that preexposure to ozone would likely potentiate exposures to acid aerosols as well.

Frampton et al. (1995) found that preexposure to H_2SO_4 may potentiate ambient levels of ozone-induced health effects in allergic asthmatic subjects (28 ± 7 years old, n = 30). In this study, each healthy (28 ± 5 years old, n = 30) and asthmatic subject was preexposed to 100 µg/m^3 H_2SO_4 (MMAD 0.66 µm) or NaCl aerosols (MMAD 0.45 µm), for 3 h. One day later (24 hours), the subjects were exposed to 0.08, 0.12, or 0.18 ppm ozone for 3 h. During each exposure, subjects performed intermittent exercise ($\underline{V}_E$ = 33 - 40 L/min). In healthy subjects, there was no convincing evidence for symptomatic or physiologic effects of exposure to either NaCl or H_2SO_4 aerosols or ozone alone, and there was no clear evidence for an effect of aerosol preexposure on the ozone response. In asthmatic patients, ozone (starting from 0.08 ppm), NaCl and H_2SO_4 aerosols independently caused decrements of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1); yet the severity of effects induced by NaCl and H_2SO_4 aerosols were not significantly different. H_2SO_4 aerosol altered the pattern of spirometric response to ozone in comparison with NaCl preexposure and appeared to enhance the small mean decrements in FVC and FEV_1 that occurred in response to 0.18 ppm ozone.

Concurrent exposure of healthy and asthmatic adults to low levels of ozone (0.12 ppm) plus sulphuric acid aerosols (100 µg/m^3) did not cause substantial changes in lung function and symptoms than exposure to ozone alone, in either asthmatic or healthy subjects (Linn et al., 1994). In a very recent study conducted by Linn et al. (1997), current exposure of children with allergy or asthma (n = 26) to pollutants (containing H_2SO_4 aerosol 100 µg/m^3, ozone 0.1 ppm, and SO_2 0.1 ppm) for 4 hours had a significantly positive association with symptoms, while in healthy children that association was not significant. The pollutant mixture did not significantly alter pulmonary function parameters in either groups (Linn et al., 1997).

11.2.3 Volunteers with COPD

Based on both pathophysiologic considerations and epidemiological observations, subjects with COPD are considered potentially at high risk for mortality and morbidity from particles. To determine whether low-level H_2SO_4 aerosol inhalation induced alterations in lung function in volunteers with COPD, older subjects (mean age = 62 years) were exposed to 90 µg/m^3 H_2SO_4 aerosols in an environmental chamber for two hours (Morrow et al., 1994). Subjects were defined by dyspnea on exertion, obstructive airway disease (FEV_1 = 1.4 L or 53% of predicted, and FEV_1/FVC = 0.56) and a lack of response to bronchodilators. In contrast to findings in asthmatic patients, the volunteers with COPD showed no greater decrements in pulmonary mechanics in response to H_2SO_4 than to the control aerosol, NaCl. Subjects with COPD, presumably the most vulnerable subpopulation, showed virtually no change in flow rates, even with periods of intermittent mild exercise during their exposure periods. Therefore, in the only study evaluating responses to specific components of particulate matter, enhanced responsiveness to acid could not be identified. This cannot be taken to mean that they might not respond to other forms of particulate matter.

11.2.4 Summary of Results on Soluble Particles

The findings of these studies provide evidence that normal individuals experience few or no adverse effects on airway function or on defence or immunological mechanisms, at relatively high concentrations of acidic particulate pollution compared to ambient levels. However, acidity has been shown to affect the slowing of mucociliary clearance at concentrations as low as 100 ug/m^3. Asthmatic patients may experience adverse effects on airway function in clinical studies at concentrations equivalent to relatively high ambient levels. These effects are more pronounced in adolescents and children, at concentrations experienced on occasion in ambient air (~ 35 µg/m^3 H_2SO_4 for 40 min). Collectively, these data demonstrate that asthmatics do manifest significant, although small, reductions in their performance during moderate physical activity after inhaling acidic aerosol concentrations close to high ambient levels. For asthmatics, the irritant potency of sulphate aerosols appears related to acidity *per se*. Inhalation of the more acidic sulphates produces the most significant bronchoconstriction. Exposure to less acidic sulphate aerosols causes no significant change in lung function. Other findings suggest that the amount of titratable acidity in an aerosol is a determinant of pulmonary effects (Fine et al., 1987). In any case, the decrements in lung function are relatively small after exposure to acid particles and are not progressive, and they are mitigated by ammonia neutralization. There exists evidence that preexposure to H_2SO_4 may potentiate ambient levels of ozone-induced symptoms and pulmonary function decrements in asthmatic subjects, and that a concurrent exposure of asthmatic children to both pollutants may also potentiate symptoms, but not pulmonary function decrements. The individuals with the most severe obstructive disease, namely COPD, do not appear to respond to acid particle inhalation with bronchoconstriction or symptoms thereof.

11.3 INSOLUBLE PARTICLES

11.3.1 Deposition and Clearance Studies

Inhalation of insoluble particles has been used primarily as a tracer to examine deposition and long-term clearance kinetics from the human lung, not for a determination of inherent toxicity. In a study reported by Bailey et al. (1982), subjects inhaled 10 breaths of 25 µL of fused alumino-silicate particles in ethanol. Monodisperse 1.2 µm- and 3.9 µm-diameter particles were labelled with radioactive tracers. Clearance was followed for more than 200 days. Approximately 8% of 1.2 µm and 40% of 3.9 µm particles cleared within six days. Overall retention of the remaining material showed half-times averaging 320 days, with considerable inter-subject variation. Approximately 10 other studies examining long-term clearance rates in humans have been conducted with particles such as iron oxide, manganese oxide and polystyrene; clearance half-times have generally been faster than 320 days (Bailey et al., 1982). No effects on symptoms or lung function changes were noted in these studies, presumably because of the low particle concentrations and very short exposure times.

Studies of deposition of diethyl hexyl sebacate particles of 0.02 to 0.4 µm has been examined in subjects with obstructive and restrictive lung diseases (Anderson et al., 1990). Particle loads were achieved after 4-10 breaths, and inhalation of the experimental aerosols probably required less than one minute. The data show that deposition of these ultrafine particles was increased in subjects with obstructive lung disease when compared with healthy subjects, while it was unchanged in subjects with restrictive lung disease. The lung function tests were not done. The absence of clinical responses was undoubtedly related to limited exposure (4-10 breaths) of the aerosol as well as small sample sizes.

11.3.2 Effects on the Respiratory Tract

Few studies have been done on insoluble particles and their effects on the human respiratory tract. However, in one recent controlled exposure study, healthy and asthmatic volunteers inhaled respirable carbon particles (Anderson et al., 1992). Four exposure scenarios were defined: (1) clean air; (2) 0.5 µm H_2SO_4 aerosol at 100 µg/m^3; (3) 0.5 µm carbon aerosol at 250 µg/m^3; and (4) 250 µg/m^3 carbon plus 100 µg/m^3 H_2SO_4 aerosol generated from fuming H_2SO_4. Electron microscopy showed that nearly all the acid in group 4 became attached to carbon particle surfaces and that most particles remained in the submicrometre range. All exposures were for one hour, with alternate 10-minute periods of exercise and rest. Respiratory symptoms and lung function were measured before, during and after exposure, whereas bronchial reactivity to methacholine was measured post-exposure. Analyses were done for effects of H_2SO_4 or carbon, separate or interactive, on all outcome measures. The investigators con-

cluded that for both healthy and asthmatic subgroups, the associations between mean changes in lung function measures and the experimental pollutants were small and of no clinical significance. In persons without lung disease, forced expiratory function measurements did not show any significant variations associated with carbon or H_2SO_4. Likewise, the changes in lung function for the asthmatic group as a whole did not suggest a significant adverse effect of the pollutants, separately or combined, in comparison with the effect of clean air. However, one asthmatic subject showed severe clinical manifestations and substantial decrements in FEV_1 and FVC following carbon and acid treatments. Acid-coated carbon particles induced much greater adverse health responses than did acid or carbon black alone in this asthmatic subject, who might represent a vulnerable subgroup.

Finally, Sandstrom and Rudell (1991) examined the bronchoalveolar inflammatory response to inhalation of diesel exhaust. Diesel exhaust from an idling diesel engine was diluted with air and introduced into an exposure chamber. Median concentrations in the breathing zone were 3×10^6 particles/cm^3, 3.7 ppm nitric oxide (NO), 1.6 ppm NO_2, 27 ppm carbon monoxide (CO) and 0.3 mg/m^3 formaldehyde. Exposures were done for one hour and included moderate exercise on a bicycle for 10-minute periods alternating with rest. Lavage was done 18 hours after exposure and demonstrated an increase in neutrophils in the bronchoalveolar but not the bronchial portion of the lavage. Phagocytosis of opsonized yeast cells *in vitro* by macrophages from the BAL was significantly altered. As exposures to pollutants such as SO_2 or NO_2 alone have typically not induced an acute inflammatory response, the investigators speculated that the responses may have been caused by particles or hydrocarbons in the diesel exhaust.

Unfortunately, far too few clinical studies have been done with either carbon or diesel particles to allow meaningful conclusions to be drawn.

11.4 OVERALL CONCLUSIONS FROM CLINICAL STUDIES

Controlled human exposures to acidic and inert particles have not caused significant alterations in respiratory function in healthy individuals at relatively high levels compared to those generally experienced in the environment. The clinical studies identify asthmatics as a susceptible population, but not persons with chronic obstructive pulmonary disease (COPD) or the elderly, at least not for acidic particles, based on a single study. Asthmatics, especially children and adolescents, may experience adverse effects on airway function at concentrations experienced on occasion in ambient air (~ 35 µg/m^3 H_2SO_4 for 40 min).

None of the human clinical studies have used particle generation systems that reflect the complexity of ambient particles. Based on the extremely limited clinical database available on various species of particles, acidic aerosols produce the most significant bronchoconstriction, while the toxicity of sulfate is related to acidity *per se.* The toxicity of nitrates was not considered, since previous work had shown it not to exert effects on lung function at concentrations below 1000 µg/m^3 in clinical studies. Inert particles did not appear to affect lung function in either healthy or asthmatic volunteers in the few studies available. Although very fine particle diesel exhaust affected neutrophil production and macrophage clearance of microorganisms from the lung, the effects cannot be ascribed with certainty to particles, since formaldehyde and other combustion gases were also elevated.

Very little work has been carried out on the effect of particle size specifically on airway mucociliary function. Although limited studies have shown that fine particles (less than 2.5 µm) are cleared from the lung more slowly than larger particles, and that submicrometre (<1.0 µm) particles clear very slowly, taking more than one to two years in a few cases, especially in patients with obstructive lung diseases. A newly published study by Peters et al. (1997) shows that symptoms and decrements of peak expiratory flow in asthmatic subjects (n = 27) were significantly associated with the 5-day mean of the number of ultrafine particles (MMAD 0.01 to 2.5 µm)(Peters et al., 1997).

Evidence of a dose-response relationship, and increased risk of an adverse effect associated with increased exposure, bolsters the argument for causality. Although the ranges of particle concentrations usually exceed those experienced by the general population, little evidence for a dose-response relationship has been documented in the clinical toxicological literature. Even at high particle concentrations in susceptible subpopulations, acidic aerosols have been found to produce only small decrements in lung function.

Overall, the clinical data lend only very limited support to the observations seen in the epidemiology studies, particularly to the observations that high ambient particulate concentrations are associated

with mortality within hours or a few days at most. They do implicate one susceptible subpopulation, asthmatics, who currently comprise five to eight percent of the population, a percentage that has been rising in the past decade in Canada as well as in other western countries. Possible explanations for the discrepancy between clinical and epidemiological data may lie in the following: (1) the experimental subjects can only be exposed to the tested air pollutants for short duration for practical and ethical reasons, while an urban pollution episode usually lasts a few days for general population exposure; a clinical study has shown that doubling the length of exposure to H_2SO_4 exerted greater effect on bronchial mucociliary clearance than did an order of magnitude increase in the concentration of H_2SO_4 (Spektor et al., 1989); (2) ethically investigating responses in those people most likely to be affected by air pollutants is almost impossible; (3) the pulmonary function parameters that are most often used in clinical studies may not be sensitive enough to indicate particle-induced adverse health effects; (4) artificial particles used in exposure chambers may not reflect the potential synergistic effects of particulate matter and aerosol mixtures; (5) in most human studies, the sizes of aerosols used are above 0.5 µm. Since nanometre-sized ultrafine particles have been found in animal studies to induce acute pulmonary inflammation and death at very low concentrations, and they are present in ambient air, ultrafine particles may be a good candidate to provoke acute alveolar inflammation with release of mediators capable, in susceptible individuals, of causing cardio-respiratory responses.