the health effects of $PM_{2.5}$ (including ultrafine particles)

Prepared by:

Central Toxicology Laboratory:

P.M. Hext
K.O. Rogers
G.M. Paddle

Reviewed for CONCAWE by:

M. Evans
R. Priston
W. Tordoir

J. Urbanus (Technical Coordinator)

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ABSTRACT

A review has been prepared of the health effects of exposure to $PM_{2.5}$ particles (aerodynamic diameter < 2.5 µm), including so-called ultrafine particles (aerodynamic diameter < 0.1 µm). The report covers briefly sources, composition and characteristics of $PM_{2.5}$. It covers in more detail the dosimetry of inhaled particles, and toxicological and epidemiological investigations. A series of conclusions is presented.

KEYWORDS

Particulate matter, $PM_{2.5}$, ultrafine particles, health effects, toxicology, epidemiology.

CONTENTS

SUMMARY

The aim of this report is to review the health effects of exposure to $PM_{2.5}$ particles (particulates with an aerodynamic diameter of < 2.5 µm), including also ultrafine particles (particles of size < 0.1 µm). Subjects reviewed were:

- Sources, composition and characteristics of $PM_{2.5}$ particles.
- Dosimetry of inhaled particles in normal individuals and those with respiratory health impairment.
- Studies in experimental animals on:
  - insoluble particles, including characteristics which may contribute to adverse effects.
  - soluble and acidic particles including components and aggregates, including potential effects on the normal functions that the lungs perform.
  - models of cardiorespiratory disease and emphysema.
  - effects of ambient or experimental air pollutant mixtures.
- Human occupational exposure to particles of relevance to $PM_{2.5.}$
- Human volunteer studies in normal and asthmatic subjects to investigate the effects of acidic aerosols on normal pulmonary function.
- Epidemiology of $PM_{2.5}$.

The major conclusions drawn from the data reviewed were:

- Dosimetric consideration of inhaled $PM_{2.5}$ suggests that asymmetric deposition patterns in some individuals with obstructive lung diseases might result in localised doses from near ambient concentrations that might enhance the already existing conditions.
- Particles of low solubility pose a limited risk to health but animal experiments imply that trace metals and adsorbed components associated with some particle types may enhance pulmonary responses.
- Many of the experimental studies have been conducted at high concentrations and used the rat as experimental species. It is now evident that the rat lung may over-respond to the presence of particles in the lung, especially at high doses, and thus results in this species and their extrapolation to man may need to be interpreted with caution.
- Ambient acidic particles probably pose the greatest risk to health and there is a suggestion from epidemiological studies that acidity is an important aspect of air pollution with respect to respiratory symptoms.
- There is no effect of concern on pulmonary function in normal healthy individuals at concentrations of acidic aerosols as high as 1000 $\mu g/m^3$. Effects that may have biological significance may occur at concentrations below 100 $\mu g/m^3$ in the most sensitive asthmatic individuals.

- There is evidence to suggest that acidic particles may enhance in a synergistic manner the effects of gaseous components of air pollution such as O_3, adding support to the view that health effects associated with episodic increases in urban airborne pollutants arise from an additive or synergistic combination of exposure to both the particulate phase and the gaseous phase.
- UF particles (particles < 0.1 µm diameter) may pose a greater health risk due to higher particle numbers and deposition efficiencies in the lung and greater biological reaction potential, but further studies or evidence will be required for a full evaluation to be made.
- There is a limited number of epidemiology studies that have specifically addressed $PM_{2.5}$. These appear to provide limited evidence of an association between $PM_{2.5}$ levels and acute and chronic mortality available at present. However, this is not convincing for several reasons including study design, lack of robust correlation between environmental data and reported exposed population and inability of identifying or selecting out one individual harmful component ($PM_{2.5}$) from an ambient mixture of a number of potentially harmful components.

The overall pattern that emerges is that $PM_{2.5}$, at normal ambient levels or those seen during episodic pollutant increases, poses limited risk, if any, to normal healthy subjects. Individuals suffering already from cardiorespiratory disease or pre-disposed to other respiratory diseases such as asthma may be at risk of developing adverse responses to exposure to increased ambient levels of $PM_{2.5}$ but more robust evidence is required to substantiate this.

1. INTRODUCTION

The potential adverse health effects of particulate air pollution has been a major focus of attention for many years. A substantial number of publications have appeared in the scientific and popular literature demonstrating correlations between particulate air pollution levels in regions and cities and a number of adverse health effects, including the number of hospital admissions from cardiac and pulmonary disease, increased asthmatic symptoms and mortalities i.e. in those individuals who are already health-impaired. Atmospheric concentrations of the particulate pollutants have been measured by a range of methods and generally expressed in terms of either total suspended particulate (TSP) or the size fraction that is capable of penetrating to the thoracic and alveolar regions of the lung. The latter is commonly known as the PM_{10} fraction (particle size less than 10 µm) and has been considered until recently to be the major fraction of airborne particulate responsible for the reported health effects. The majority of publications contributing to the above have used this fraction for correlating measured airborne particulates with health effects.

The concentrations of these particles are generally well below 50 µg/m^3 but excursions up to several hundred µg/m^3 can occur over short (minutes) or longer (hours) periods depending on climatic conditions and time of year. These are considerably lower than past or current occupational exposure levels to particulate matter in instances where no acute adverse health effects have been associated with exposure. Nonetheless, the increasing concerns regarding the apparent relationship of adverse health effects with particulate air pollution, taking into account that the origin of a proportion of this in city environments especially is derived from automotive exhaust emissions and hence raises the potential for a trend to increasing levels, has led to a number of regulatory authorities and other common interest groups to review in considerable detail many or all factors related to this issue. During these processes and as a result of further considerations of the relationship between the size of particles, their deposition and retention characteristics in the respiratory tract and their ability to induce adverse effects in the lungs of man and animals, the fine fraction of PM_{10}, referred to as $PM_{2.5}$ (particles of size < 2.5 µm), has been considered to represent a higher risk for health. This is understandable since $PM_{2.5}$ represents the highly respirable fraction of a particulate atmosphere and as such will penetrate predominantly to and deposit within the deep alveolar regions of the lung. This fraction also includes the ultrafine fraction of airborne particulate matter. The term ultrafine is used frequently to define particles in the sub-micron range that are less than 0.1 µm (100 nm) primary diameter and this definition of ultrafine particles will be adopted here and used throughout this report. Such particles form part of the normal size spectrum of particulate air pollutants, as will be shown, and scientific concern has been raised that these particles especially may induce health effects at very low concentrations. The information available for this latter size range is considered separately where appropriate. To avoid any confusion between the two size ranges, the term **$PM_{2.5}$** will be used throughout this report as a definition where all particulate matter below 2.5 µm is being considered without further sub-division, but where the fraction below 0.1 µm is considered specifically, the term **UF** will be used.

This report reviews information which has been published up to the end of 1997 on $PM_{2.5}$ particles in the context of their potential to induce adverse health effects in experimental animals and man.

2. AIR QUALITY STANDARDS

A number of countries or international organisations have reviewed environmental particulate matter with the aim of establishing air quality standards. In the past these focused predominantly on PM_{10} or TSP but led to the recognition that $PM_{2.5}$ may be the major fraction responsible for the apparent relationship between airborne particles and adverse health effects. Additionally, this fraction is related more to human activity, industrialisation etc. than natural sources and is therefore potentially more amenable to control measures. This led to the European Union (EU), to include $PM_{2.5}$ in addition to PM_{10} within the draft Directive (EU, 1997) which aimed to establish limits for gaseous and particulate air pollutants. The final Directive, issued in 1999 (EU, 1999) no longer included $PM_{2.5}$ values in the legislation. However, Member States are required to also sample and provide information on $PM_{2.5}$ and the action plans to reduce PM_{10} to the limit values given should include a concomitant reduction in the $PM_{2.5}$ fraction. This legislation, on PM_{10} at this stage, calls for a 2 stage approach to adoption of limit values of PM_{10} based on 24 hour or annual average concentrations, the timing and levels to be achieved being shown in **Table 1**.

The United States Environmental Protection Agency (U.S. EPA) issued new National Ambient Air Quality Standards (NAAQS) for both PM_{10} and $PM_{2.5}$ under the Clean Air Act (U.S. EPA, 1997). These standards set 24 hour and annual average concentrations for PM_{10} of 150 and 50 $\mu g/m^3$ respectively and for $PM_{2.5}$ of 65 and 15 $\mu g/m^3$ respectively. However, the latter is currently held in abeyance due to US Court proceedings on this issue.

3. SOURCES, COMPOSITION AND CHARACTERISTICS OF $PM_{2.5}$ PARTICLES

This section is not intended to be a comprehensive review of the source, composition or fate of $PM_{2.5}$. Extensive reviews of all of these aspects are available (e.g. QUARG, 1996) and the intention here is to provide an overview only.

3.1. SOURCES

$PM_{2.5}$ represents the fine fraction of the commonly measured PM_{10} fraction of ambient aerosols and as such is composed primarily of particles that are formed *de novo* rather than arising from comminution of larger pre-formed particles such as mineral dust. Particles comprising the $PM_{2.5}$ fraction are generally termed primary or secondary. Primary particles are those emitted directly into the atmosphere as a particle or vapour that condenses to form a particle without chemical reaction. Such particles that fall within the $PM_{2.5}$ fraction are usually generated by combustion or derived from condensation of vapours arising from combustion or high temperature processes.

Secondary particles arise from chemical reaction of gas-gas, gas-liquid or liquid-liquid reactions which result in the formation of solid low volatile liquid aerosol particles. For example, sulphur dioxide will convert to sulphuric acid droplets that may subsequently react with ammonia to form particulate sulphates.

3.2. COMPOSITION

Environmentally, the chemical composition of $PM_{2.5}$ will vary according to country, region (low or high population), season and industrialisation. For a typical UK urban $PM_{2.5}$ fraction (**Figure 1**, adapted from QUARG 1996), the largest proportion is composed of particles derived from combustion processes (carbonaceous matter) with sulphates comprising the next most abundant fraction. Nitrates, ammonium and chlorides represent smaller proportions. Trace metals may also be present at very low concentration and are not included into **Figure 1**.

Experimentally, where particulate atmospheres are generated under controlled laboratory conditions, the composition is usually homogeneous with respect to the particulate of interest, for example sulphuric acid or diesel exhaust, and such atmospheres are described in the subsequent chapters on experimental exposures in humans and animals.

3.3. SIZE CHARACTERISTICS

The size distribution profile of ambient aerosols has been characterised by many groups and can be considered generally as trimodal. This is illustrated in **Figure 2**, (data from Wilson *et al*. 1977) where it can be seen that there are two distinct distributions in the fraction encompassed by $PM_{2.5}$, one having a median at 0.21 µm and the other 0.018 µm. These medians may vary, thus Berico *et al*. (1997) recently reported similar data for the Bologna urban area where the two equivalent fine distribution modes had median diameters in the ranges 0.3-0.8 and 0.04-0.2 µm. Such differences will reflect different regions etc. in which the samples are collected but also other environmental parameters such as humidity. The latter may readily influence the size of hygroscopic aerosol particles such as those composed of

sulphates. Nonetheless, it is important to recognise that there are distinct size distributions within $PM_{2.5}$, one of which constitutes the UF fraction.

Further consideration of the physical characteristics of particles that constitute the full spectrum of sizes that come under $PM_{2.5}$ are also necessary. This arises from the fact that the concentration of this, and most other particulate fractions, is expressed generally in term of mass per unit volume, e.g. $\mu g/m^3$. However, the number of particles contained within a given mass can vary substantially according to particle size, as does surface area, as shown in **Table 2** for representative sizes within the PM_{10} and $PM_{2.5}$ fractions. These latter parameters are considered to be more important than gravimetric units in term of potential effects in the lung and will be considered where appropriate in subsequent sections.

In conjunction with size, another characteristic of particles that is important with respect to potential health effects is their behaviour in air, since this governs their residence time and distribution in the ambient air and also deposition within the respiratory tract. The behaviour of particles in air is described by their aerodynamic size which encompasses a description of their settling velocity, i.e. the rate at which they will sediment in still air. The latter is important since it provides information on how long or how easily particles can remain suspended in air and will influence distribution between environmental compartments. **Table 2** illustrates settling velocities for the particle sizes within PM_{10} and $PM_{2.5}$, demonstrating that the particle sizes that constitute $PM_{2.5}$ can remain airborne almost indefinitely in modest air turbulence. Such characteristics account for the similarity of indoor particulate size distribution to outdoor $PM_{2.5}$.

The relationship between aerodynamic particle size and deposition characteristics within the respiratory tract of humans and experimental animals is addressed in **Section 4**.

4. DOSIMETRY OF INHALED PARTICLES

All phenomena arising from the interaction of endogenous or exogenous materials with components of living organisms are related to dose and the same will apply to exposure to ambient air pollutants. While dose is normally considered to be the quantity of material that reaches a target, the effectiveness of the dose and potential duration of effect are functions of both retention and clearance of the material. The aim of this section is to:

- describe the factors influencing the received dose of inhaled particulate matter
- describe retention and clearance of inhaled particles in the healthy individual
- consider whether changes in these parameters in health-impaired individuals may contribute to or explain their apparently greater sensitivity to changes in $PM_{2.5}$ concentrations.

4.1. DOSIMETRY OF INHALED PARTICLES IN HEALTHY INDIVIDUALS

Atmospheric particulate concentrations are generally expressed as mass/volume units e.g. mg/m^3 or µg/l and these are frequently interpreted as the dose to the individual. This is clearly untrue since dose is an expression of the weight administered per body weight unit, e.g. mg/kg. While dose is easily assessed orally or dermally, by inhalation it is more complex and is the amount depositing with time in one or more regions of the respiratory tract. This is a complex function of the characteristics of the inhaled material and the physical and physiological characteristics of the exposed human or animal. These characteristics are listed below:

Particle characteristics

- Size
- Shape
- Solubility
- Chemical composition

Respiratory tract characteristics

- Anatomy/morphometry
- Respiratory physiology parameters
- Health status

All will influence the site and proportional deposition of inhaled particles, and hence dose, and may need to be considered when:

- assessing and comparing the effects of airborne contaminants on the respiratory tract and health of exposed populations or individuals
- attempting to correlate data from exposed humans with studies conducted in experimental animals.

Underlying all of these are the mechanisms by which particles deposit within the respiratory tract which will have the major influence on deposited dose with respect to both quantity and region. Subsequent to deposition, the retention and clearance of the deposited material will influence the extent (duration and severity) of any subsequent adverse responses in the respiratory tract. Deposition, retention and clearance are addressed in the following sections.

4.1.1. Deposition

4.1.1.1. Deposition mechanisms and models

A brief description of deposition mechanisms within the respiratory tract of man and animals in relation to size and region and influencing factors is given in Appendix 1, and a plot of the relationship of particle size and regional deposition efficiency in the normal adult is shown in **Figure 3** (based on ICRP, 1994). When the deposition mechanism and major regions of the respiratory tract where these operate are compared with the schematic distribution of particles within $PM_{2.5}$ (**Figure 2**), it can be seen that the majority will deposit at high efficiency in the terminal bronchiolar and alveolar regions. When considering PM_{10} it can be seen from **Figure 2** that a proportion of this fraction is composed of coarse particles that will deposit predominantly in the upper airways (nasopharyngeal, tracheal and bronchial regions).

A number of mathematical models have been developed, using experimental human and animal data, to describe the regional deposited dose of inhaled particles based on the above considerations of deposition mechanism, particle size, respiratory tract morphometry, species, age and sex. These include the empirical model developed by the United States EPA for derivation of Inhalation Reference Concentrations (RfC; U.S. EPA, 1994) and that of the Task Group for Lung Dynamics to the International Commission for Radiological Protection (ICRP, 1994). Computer models have been derived in conjunction with both reports, the advantage to the latter is that it will incorporate particles having diffusive diameters, i.e. representing the UF proportion of $PM_{2.5}$.

Deposition patterns and efficiencies may change in health impaired individuals with further consequences on an individual's health status and this is addressed in a subsequent part of this Section. Breathing mode can also affect deposition efficiency, normal nose breathing giving the pattern shown in **Figure 3** but mouth breathing, due either to work pattern or nasal passage disease or infection, by-passes the filtering action in the nose and can result in enhanced alveolar deposition (**Figure 4**; based on ICRP, 1994).

Age has little effect on lung deposition except in the young (>10 years), where the finer fraction of $PM_{2.5}$ (<0.2 µm) and the UF fraction show increases of up to 12% approximately in deposition efficiency in the lower regions of the respiratory tract (ICRP, 1994).

Considerations of regional deposition and mechanisms up to this point have assumed no changes occur to the inhaled particles on entering the respiratory tract. This is certainly true of insoluble particles but those composed of salts such as sulphates are frequently hygroscopic, absorbing water as they enter the high humidity of the respiratory tract and growing in size as a consequence. This is a confounding factor in assessment of regional deposition since it can result in increasing deposition of particles in the some regions of the respiratory tract, with a

tendency for this to be enhanced in the upper regions for particles representing the upper size ranges of $PM_{2.5}$. However, those comprising the two finer distributions within $PM_{2.5}$ (**Figure 2**) will still penetrate to the alveolar regions but as a result of particle growth will deposit with a greater efficiency than indicated by **Figure 3**. This can be seen in **Figure 5** (based on ICRP, 1994) where alveolar deposition patterns in humans are compared for hygroscopic and non-hygroscopic aerosols.

It is important to recognise the limitations of the ICRP model. While this is based on human data, it is data normalised to represent a population and there are invariably inter-individual variations that may influence deposition patterns. More importantly it is based on healthy individuals and a number of respiratory diseases or conditions may influence considerably deposition in health-impaired individuals. This is considered in **Section 4.2**. Despite the limitations of the ICRP model, and similar limitations apply to others, it is accepted as being the most accurate model available to help in deposition modelling and has been used by Snipes *et al*. (1997) for modelling the deposition of two tri-modal environmental aerosols.

4.1.1.2. Studies on the deposition of $PM_{2.5}$ size particles in the healthy human

Several studies have investigated deposition in relation to breathing pattern and airway morphology, important parameters that may change as a result of developing airway disease and hence have implications on deposition of $PM_{2.5}$ in health-impaired individuals.

Studies on deposition of 2.6 µm particles in normal subjects breathing normally indicate that inter-subject variability in the deposited fraction is a function of breathing pattern (Bennett *et al*., 1987). This depends most strongly on the time period of the breath and is influenced more by the inspiratory than the expiratory period. In contrast, work by Heyder *et al*., (1982, 1988) using monodisperse aerosols of 1-7 µm diameter, also in normal subjects breathing normally, suggest that the peripheral and proximal morphology of the airways has the greater influence for the two larger particle sizes, individuals with wide airways having lower deposited fractions than those with narrow airways. This relationship is lost for 1 µm particles, representing the finer fractions of $PM_{2.5}$ since these particles will remain airborne for longer time periods. Reducing the breathing rate and hence extending the breath time was considered to re-establish this relationship. While there are some conflicting views on what physiological or morphological relationships influence predominantly deposition in the respiratory tract, these studies imply that changes in either as a result of disease may affect in an adverse manner the fraction of airborne particulates depositing in the peripheral lung regions.

Since the elderly appear to be at greater risk from the effect of particulate air pollutants, Bennett *et al*., (1996) compared the deposition of 2 µm carnauba wax particles in healthy adults, of both sexes, ranging in age between 18 and 80. They concluded that during resting, spontaneous breathing, the deposition and hence dose of these particles is independent of age. Total deposition may be greater in males due to a greater minute ventilation but there is little difference if the deposition rate is normalised to lung surface area. Deposition was considered to be influenced predominantly by the breathing pattern of the subjects, as referenced above (Bennett *et al*., 1987). The specific airway resistance of individuals was also shown to correlate with deposition of 2 µm particles suggesting, as expected, that some particles of this size deposit in the conducting airways. At smaller particles sizes, e.g. 1 µm, this correlate is not apparent (Kim *et al*., 1988), consistent with the majority of particles of this size or less depositing predominantly in the alveolar regions.

In the context of dose depositing in various regions of the lung, it is important to note that the larger size fraction of $PM_{2.5}$ particles will show some degree of asymmetric deposition at upper airway bifurcations due to impaction (see Appendix 1) operating as a major mechanism of deposition in these regions.

A number of groups have measured total deposition patterns of UF particles in humans or deposition patterns of UF particles, including radon daughters, in casts of the human respiratory tract. Schiller et al., (1988) assessed total deposition patterns of UF silver particles ranging in size from 0.005 to 0.08 µm for a variety of breathing patterns in 4 individuals during steady state mouth- and nose-breathing. Since such particles deposit predominantly by diffusional particle transport, total deposition for mouth breathing increased from approximately 40% to 90% with decreasing particle size over the range tested. Increasing mean residence time within the lung increased total deposition by approximately 20% of the larger size particles tested but had little effect at the lower sizes. For particles larger than about 0.02 µm nose-breathing and mouth breathing showed similar total deposition. In the smaller size ranges total deposition with nose breathing was fractionally greater than with mouth breathing. These data are in close agreement with an earlier study by Wilson et al. (1985) who showed total deposition efficiencies of 0.71 ± 0.07, 0.62 ± 0.06 and 0.53 ± 0.05 for UF particles of bis (2-ethylhexyl) sebacate of sizes 0.024, 0.043 and 0.075 µm respectively.

Cohen et al., (1990) measured deposition of 0.2, 0.15 and 0.04 µm diameter technetium-99m labelled ferric oxide particles (as surrogate for radon daughter particle deposition) in a human central airway cast. As with the studies in normal human subjects the smallest particles showed greatest deposition but, more importantly in the context of potential dose of UF particles received by tissues, the deposition at airway bifurcations was greater than along the airway lengths. This would not normally be expected from the mechanisms responsible for deposition of particles in this size range but the authors attribute this to asymmetric and turbulent flow, as opposed to laminar flow, in these regions of the airways. Further similar results were reported by Cohen (1996) using technetium-99m labelled ferric oxide or sodium chloride labelled particles of 0.05, 0.1, 0.18 and 0.4 µm to assess deposition in casts of major and minor airway branches. Kinsara et al. (1995) also showed asymmetric deposition in airway casts with molecular phase radon progeny.

4.1.2. Retention and clearance of deposited particles

Once particles have deposited within the respiratory tract, their fate may have a major influence on the development or progression of health effects. Soluble particles depositing in any region are likely to interact rapidly with the tissues and induced responses are likely to be rapid and possibly short lived (hours to days). Insoluble particles depositing in the upper regions, encompassing the trachea, bronchi and terminal bronchioles are cleared relatively rapidly i.e. within 1-2 days, in the normal healthy individual by mucociliary mechanisms. Again, if these do induce a tissue mediated response it will be short lived unless clearance is impaired due to underlying disease or other inhibitory cause of mucociliary clearance. A small proportion may enter the epithelium of these upper airways (Churg *et al*., 1990). Their fate and influence on any existing or developing lung condition is unknown.

Insoluble particles penetrating to the alveolar regions will be cleared considerably slower. Clearance from these regions is mediated predominantly by alveolar macrophages. These free ranging cells phagocytose the deposited particles and over an extended time period (days to months) will slowly migrate to terminal

bronchioles where they are then cleared rapidly by the mucociliary escalator. High lung burdens of insoluble particles can result in impairment or even cessation of this normal clearance mechanism. This is evident in humans exposed occupationally in the past to dusts such as coal and in animal experiments using high concentration exposures (see **Section 5** on animal studies). Such impairment over a period of time may result in the development of pneumoconiotic responses. Another consequence of such loading is that some particle laden macrophages migrate through the lymph system and accumulate within the lung-associated lymph glands. While the relevance of overt overloading of the lung to health effects of ambient aerosols is questionable unless a pre-existing condition already exists, investigations of the effect of particle loading and retention in the lung in both animals and man have revealed a number of interesting facts concerning particles of sizes composing the two main distributions within $PM_{2.5}$.

Most information pertaining to the retention of particles by the normal lung has been derived from animal studies. Ferin *et al*. (1992) showed that when UF titanium dioxide (TiO_2) particles (mean size approximately 0.02 µm) were compared with "fine" particles (mean size approximately 0.25 µm), by instillation as equal masses into the lungs of rats either as a single dose or repeated over a 12 week period, they translocated into the pulmonary interstitium to a greater extent and were hence retained for longer time periods. This was demonstrated also by inhalation, a more pertinent method of administration (Oberdörster *et al*., 1994). The latter demonstrated also that clearance of the UF particles was appreciably slower than for the fine particles, an effect not unexpected if material is being retained within the interstitium. Another implication from the results of these studies is that UF particles depositing in the alveolar regions, albeit initially in the form of agglomerates, by-pass more easily the normal macrophage phagocytosis clearance and readily translocate into the epithelium. These results and the accompanying tissues responses may have implications for health of inhaled UF particles.

Animal studies, described in more detail in **Section 5**, have shown that a proportion of inhaled particles will readily enter and be retained in the alveolar epithelium, especially those composing the UF proportion, i.e. of sizes < 0.1 µm. While little is known about the migration of fine particles into the lung parenchyma of humans and subsequent retention, Churg and Brauer (1997) used analytical electron microscopy to count, size and identify particles in autopsy samples of the lungs of a limited range of residents of Vancouver. The particles identified fell into two broad categories, silica/silicates having geometric mean diameters of 0.49 µm (GSD 2.2) and metals of geometric mean diameter of 0.17 µm (GSD 2.0). Particles of < 0.1 µm diameter constituted less than 5% of the total. These authors concluded from these results that human lung can effectively retain the fine particles composing $PM_{2.5}$. They expressed surprise that "ultrafine" particles comprised only a small percent of those found and could not rule out completely that this may have been an artefact of sample preparation. If the result was representative, then this points to limited retention of UF particles by the human lung. It is evident that UF particles are present in macrophages obtained by bronchoalveolar lavage from people from a range of occupations (Godleski *et al*. (1995), indicating that such clearance can follow the normal macrophage mediated pathways. However, the extremely small size means that UF particles may well pass through the lung epithelium into the blood stream, or alternatively, the very high surface area of even a relatively insoluble particle means that dissolution in biological tissue may be rapid. The lack of appreciable numbers of UF particles being retained (Churg and Brauer, 1997) does not detract from the possible acute effects that might be induced following a short term high exposure scenario.

4.2. DOSIMETRY IN HEALTH IMPAIRED INDIVIDUALS

As described in the preceding sections, the majority of data generated to determine regional lung dosimetry, particle disposition, retention and clearance has been conducted in healthy individuals using controlled breathing. However, health concerns and correlation of these with exposure to ambient aerosols are generally confined to those individuals who comprise the more susceptible populations, such as children or the aged, or suffer already from an underlying disease. A proportion (or all) of the latter may either exhibit a greater response to a given dose of deposited particulate or the disease may influence deposition such that the deposited dose is increased. The latter might be expected with respiratory diseases such as asthma, bronchitis and chronic obstructive pulmonary disease (COPD) since it is known that in individuals suffering from these the normal physiological parameters may change. This has been confirmed by a number of studies investigating deposition of inhaled aerosols in normal subjects and those suffering from obstructive airway disease.

4.2.1. Deposition

Studies in normal healthy adult individuals show an apparent lack of any appreciable effect of age on the deposition of particles in the lung. However, if increased risk from airborne particulate matter is associated with the elderly, as suggested by epidemiological studies, then the presence of an underlying respiratory condition which may enhance lung deposition might predispose these individuals to the development of adverse symptoms. Changes in breathing pattern occur in patients with obstructive pulmonary diseases (Tobin *et al*., 1983) and together with the associated morphometric changes in the airways the deposition of inhaled particles would be expected to be enhanced in both the conducting airways and the alveolar regions. Increased deposition in the conducting airways will occur by virtue of the fact that deposition in these airways is mainly by inertial impaction and hence an increase in velocity results in an increase in deposition. Increased deposition on the alveolar regions will arise from increased penetration of particles to these regions resulting from reduced airway volume, uneven airflow and increased residence time in the deep lung. The latter is a consequence of a reduction in upper airway residence time resulting from the higher velocities in narrowed airways.

Kim and Kang (1997) showed that particle deposition in the lung increases in subjects with airway obstruction in proportion to the severity of the obstruction. They measured lung deposition of 1 µm sebacate particles in normal subjects and subjects having varying levels of airway obstructions: smokers, smokers with airway disease, asthmatics and patients with COPD. The measured deposited fractions were 16, 49, 59 and 103% greater respectively when compared with the normal subjects. These showed a good correlate with lung function measurements (FEV_1 and FEV_{25-75}) which the authors interpret as showing that deposition increases proportionally with increasing level of airway obstruction. This study was conducted using a standardised breathing pattern for both normal subjects and those with obstructive airway disease (500 ml tidal volume, 30 breaths per minute). The authors consider that with expected reduction in breathing rate at rest in patients with COPD, there would be greater deposition than observed under the experimental test conditions. This is confirmed by the work of Bennett *et al*. (1997) who compared deposition of 2 µm carnauba wax aerosols in resting normal subjects and subject suffering from COPD. The latter showed increased resting minute ventilation with a resultant three fold increase in deposition. Asymmetric patterns of

ventilation and hence deposition develop with increasing severity of experimental lung obstruction (Kim *et al*., 1989) or obstructive lung disease (Taplin *et al*., 1977; Olséni *et al*., 1994). These will result in some regions of the lung receiving a considerably greater dose of deposited particulate, which together with the already enhanced deposition in such subjects, and in association with excursions in ambient concentrations, may lead to localised lung burdens that are capable of inducing inflammatory effects. If, as mentioned previously, it is the particle number or surface area that is the influencing parameter for particle induced health effects, a moderate increase in dose in terms of mass may be equivalent to a substantial change in these other particle parameters.

4.2.2. Retention and clearance

Pre-existing disease, pharmacological agents and many other factors can affect considerably the fast mucociliary-mediated clearance of particles. However, there is a lack of data on clearance of particles from the deep regions of the lung in health impaired individuals. It would be expected that impairment of the normal functioning of the lung would encompass also the clearance mechanisms for these regions, resulting in more prolonged retention and hence increasing the potential for initiation or prolongation of any particle-mediated effects.

4.3. SUMMARY

- A number of mathematical models have been developed, using experimental human and animal data, to describe the regional deposited dose in healthy humans of inhaled particles based on the deposition mechanism, particle size, respiratory tract morphometry, species, age and sex.
- Although the elderly appear to be at greater risk from the effect of particulate air pollutants, comparison of the deposition of $PM_{2.5}$ in healthy adults, of both sexes, ranging in age between 18 and 80, showed that during resting, spontaneous breathing, the deposition and hence dose of these particles was independent of age.
- Animal studies have shown deep lung clearance of deposited particles to be slow and clearance of UF particles may be prolonged further due to passage into the lung epithelium.
- Pre-existing lung diseases with associated changes in breathing patterns etc. can alter deposition patterns and quantities, resulting possibly in localised regions of enhanced deposition.

5. EXPERIMENTAL ANIMAL STUDIES

Experimental animals are used commonly to assess the potential health effects to man of new materials being placed on the market or to investigate effects seen in humans as a result of occupational or environmental exposures. While a lot can be learned from clinical symptoms seen during exposure etc. and from controlled studies in human subjects, animal studies have many advantages, including close control over experimental conditions, good and adequate controls, shorter lifespan to enable studies to be conducted over a good proportion of the lifespan where appropriate and the use of physical, biochemical and pathological techniques that cannot be used in man except after natural death. The high profile over many years of the potential effects of occupational or environmental exposure to particulates has resulted in a large number of studies being conducted in animals which are directly related to or of potential relevance to the effects of $PM_{2.5}$ particles.

When investigating the effects of inhaled particles in the most common experimental species (rat, mouse, guinea pig), the characteristics of their respiratory tracts are such that particles that penetrate to the alveolar regions will be equivalent to $PM_{2.5}$. Thus the majority of animal studies have used aerosols within the size range of interest here. However, the majority of animal studies have been designed to demonstrate distinct toxicological end-points, often in the context of occupational exposure, and as such have used high atmospheric concentrations to achieve these aims. The relevance of these studies is questionable with respect to potential health effects in the general population where exposures are generally to atmospheric concentrations well below 50 $\mu g/m^3$. For these reasons, the majority of animal studies reviewed here were those conducted to investigate atmospheric concentrations of particulates that fall within a spectrum considered to be relevant to general population health effects, but where specific issues are considered, reference may be made to studies testing high particulate concentrations.

In the majority of studies the rat has been the experimental animal of choice. It is worthy to note that there is increasing evidence that the rat exhibits heightened inflammatory type responses to inhaled particles. This is considered in the subsequent sections but this fact needs to be considered in the context of many of the inflammatory type lung responses that workers are using as models of the adverse effects of inhaled particles.

The aim of this section is to review what data exists that might help in understanding the proposed link between increased ambient particulate levels and health effects in humans. To achieve this in the context of the considerable amount of data available and the various mechanisms proposed for the adverse health effects induced by ambient particulates, this is broken down into the following sections:

- Insoluble particles
  - mechanisms or factors contributing to particle effects
  - contribution of adsorbed components to particle effects
- Soluble and acidic particles
  - effects on pulmonary function
  - effects on lung morphology
  - effects on pulmonary clearance
- Effects of particles on resistance to disease

- Health-impaired animal models and the effect of exposure to particles
- Effects of a combination of air pollutants on experimental animals

5.1. INSOLUBLE PARTICLES

The major source of the insoluble particle fraction of ambient $PM_{2.5}$ is combustion, these particles being predominantly carbonaceous. Diesel exhaust particulates can be considered representative of these particles and a considerable number of experimental studies have been conducted on freshly generated diesel exhaust. As mentioned above, because these were based on the classical design of toxicity studies, concentrations were invariably well in excess of those encountered in the environment. Nonetheless, there are possible implications from the results of these studies and others on insoluble particulates, that may be pertinent to responses in the lung of health-impaired individuals where asymmetric deposition may produce localised “hot-spots” of deposited particles (see **Section 4**, Dosimetry of Inhaled Particles).

A full review of diesel exhaust emissions, including effects on experimental animals, has been published recently (IPCS, 1996). The primary size of the particulate phase of diesel exhaust is below 0.1 µm since their source is a combustion process. However, since they are produced at high local concentrations, they undergo aggregation rapidly such that the majority have already produced many large aggregates upon emission from the exhaust (Bérubé et al., 1999).

Early studies on the effects of diesel exhaust on experimental animals were designed to assess changes that might be extrapolated to any potential health effects in persons exposed either occupationally or environmentally. Particulate concentrations were generally well in excess of 1 mg/m^3 for extended time periods and resulted in some changes in lung function and pathology, as for example in rats exposed to 3.5 and 7.0 mg/m^3 for up to 2 years, where pathological changes accompanied by lung physiology changes were seen after 1 year exposure (Mauderley *et al*., 1988).

Findings of concern in this study and consistent with those in other studies on both diesel exhaust and other particulates were the apparent enhanced inflammatory type responses of the rat lung to the prolonged presence of particulate matter. While these were seen generally at high concentrations, more recent studies with UF particulates (Heirich *et al.* 1995; Oberdörster et al., 1994) have shown effects at concentrations lower than those inducing similar effects in rats exposed to larger sized, but nonetheless respirable, particles.

The emerging picture when comparing long term exposure of experimental animals to a range of insoluble particulate materials, and by making comparisons with occupationally exposed humans, is that the progression of pulmonary changes (physiological and pathological) with time are similar in most species and man with the exception of the rat. When compared with other experimental species, rats consistently exhibit greater inflammatory, hyperplastic and fibrotic responses to a similar lung burden of particulates (**Table 3**). For example, in two studies which compared the effects in lungs of rats and monkeys after 24 months exposure to high concentrations of petroleum coke (Klonne *et al*., 1987) or shale particles (MacFarland *et al*., 1982), the response in the monkey lung was minimal whereas pulmonary inflammation developed in the rat. This pattern of differences in response is relatively consistent across other studies where different species have been compared. The responses seen in the rat are considered to be rat-specific and not attributable to

differences such as deposition patterns, respiratory physiology or lung clearance, all of which will vary between different species. It is also important to recognise in the context of this report that adverse lung responses were observed only at high exposure levels which resulted in impaired lung clearance in the rat and the resultant substantial increases in lung particulate burdens. Lung burdens below those producing clearance impairment were not associated with adverse effects and would be more relevant to ambient particulate concentrations. However, it is important to recognise this potential over-reaction of the rat lung when considering mechanistic type studies considered to be relevant to $PM_{2.5}$ effects but in which enhanced particulate lung burdens are used to ensure promulgation of effect within a practical time-scale.

5.1.1. Fate of ultrafine particles

While the lung response to high particulate burdens is of little relevance to the normal healthy individual exposed only to ambient concentrations of particulate, possible implications for health-impaired individuals cannot be dismissed where asymmetric deposition can lead to foci of deposited material in the lung (see **Section 4**). Of possible relevance here are differences observed with UF particles in animal studies at considerably lower atmospheric concentrations than those inducing changes with particles in the size range 0.1 - 2.5 µm, which together with more recent investigations on UF particles, may have implications for such individuals. Ferin *et al*. (1992), comparing an ultrafine sample of titanium dioxide (UF-TiO_2; primary particle size approximately 20 nm) with a normal commercial pigment grade (PG-TiO_2; particle size approximately 250 nm) showed that at equivalent masses the UF-TiO_2 entered the pulmonary interstitium to a larger extent than the PG-TiO_2. This resulted in an enhanced inflammatory response associated with translocation of particles into the interstitium and prolongation of lung clearance. Furthermore, under the experimental conditions used in most studies the UF particles are generally aggregated. The ability to translocate into the epithelium with resultant inflammatory response is related to the extent to which these will deaggregate into primary particles once deposited (Oberdörster, 1996). This is considered to account for the differences reported by Oberdörster *et al*. (1995) between pulmonary translocation of ultrafine carbon black particles and UF-TiO_2 particles.

With evidence linking the aggregation and deaggregation of UF particles to the subsequent lung responses, it is readily hypothesised that the enhanced deposition pattern of non-aggregated primary UF particles (singlets, comprising the smallest particle distribution in **Figure 2**) together with the expected rapid translocation into the epithelium, would result in an aggressive tissue response to exposure to relatively low concentrations of singlets. For most inhalation studies in experimental animals generation conditions either predispose the direct generation of aggregates or the lifetime of singlets is too short, in relation to effect concentrations, for these to penetrate to the lung before aggregation occurs. However, under very specific conditions, not pertinent to environmental particles as considered below, UF singlets with sufficient lifetime can be generated and have shown, as predicted, exceptionally high toxicity. This discovery arose from the extensive research that was conducted to understand the extreme toxicity of the combustion fumes of PTFE. When generated in a temperature range of 450-800 °C with re-circulation of the generated fume through the furnace, the 30 minute LC_{50} can be as low as 0.045 mg/l (Levin *et al*., 1982), in contrast to that when PTFE is combusted under less stringent conditions, where the equivalent LC_{50} is in excess of 5 mg/l and attributable to the production of the toxic gases hydrogen fluoride and carbonyl

fluoride. Warheit *et al*. (1990) and Lee and Seidel (1991), amongst other groups, demonstrated that the lung changes observed in rats exposed to PTFE fume showed marked alveolar and interstitial oedema, haemorrhage, alveolar capillary endothelial damage and alveolar capillary neutrophilia, all consistent with effects at the alveolar and terminal bronchiolar level. This “supertoxicity” was attributed to the particulate generated and maintained in a singlet state, once aggregation had occurred, or particles were collected and administered intratracheally into rats, a substantial reduction in the toxicity was observed. Oberdörster *et al*. (1995) repeated these studies with similar results, the authors ascribing the enhanced toxicity to rapid translocation into the alveolar epithelium.

There is no doubt from the studies described above that UF particles are capable of inducing enhanced pulmonary response in animals that can be likened to UF induced diseases in man such as metal or polymer fume fever. Aggregation and/or deaggregation can have a marked influence on the induction of such effects. As mentioned previously, when considering the dosimetry of the inhaled particles, the evidence for any correlate with effects observed is probably related more closely to particle numbers or surface area than gravimetric measurements.

It should be noted that while UF induced diseases in man exist, they are usually associated with exposure to high concentrations (in the mg/m^3 range) of freshly generated fume, some such as zinc also having inherent biological activity. Such fumes will undergo rapid aggregation to reduce their potency. The same is true for environmental particulates. One of the major sources of primary particles of UF size in the environment is combustion but as already mentioned, the majority of particles have already undergone substantial aggregation before they are emitted from the exhaust and from results of toxicological studies will not pose any exceptional hazard compared with other ambient particles.

5.1.2. Mechanisms or factors contributing to particle effects

In recent years a number of groups have started to investigate the mechanism(s) by which insoluble particles may induce the pulmonary responses observed in experimental animals. Much of this has been targeted at high lung burdens but may have some relevance to ambient particle effects provided that the results are viewed with the caution mentioned previously regarding the relative responses in different animal species, especially the rat. The generally accepted view at present is that high particulate lung burden, either general or very localised, may initiate inflammatory responses with the potential for subsequent effects on lung physiology and pathology. It is clear that apart from size, the surface chemistry of insoluble particles is likely to play a role in the induction of biological effects. Additionally, or alternatively, ambient particles are likely to have other materials adsorbed to the surface, some of which may be biologically active. Studies investigating the role of the surface chemistry or adsorbed materials are reviewed in the following sections.

5.1.2.1. Surface chemistry - free radical production

In the context of ambient particulates, Li *et al*. (1997), during an assessment of the pro-inflammatory effect in the lung of rats of collected PM_{10} (collected in Edinburgh, UK), considered the potential contribution of the UF fraction by comparing results with those seen using fine and UF carbon black (particle sizes 0.2-0.5 µm and 0.02 µm respectively). Particles were administered by intratracheal instillation at rates of 50-125 µg/rat PM_{10} and 125 µg/rat for the carbon black samples. Analysis of bronchoalveolar lavage fluid taken 6 hours after instillation showed marked

inflammatory response with PM_{10}, with a greater response following UF carbon black but limited response following the fine carbon black. The authors use these results in conjunction with additional measurements indicative of free radical production by the PM_{10} sample to suggest that this activity resides in the UF fraction of PM_{10} and the UF fraction was responsible for the majority of the results observed.

The surface free radical activity of a PM_{10} sample (possibly the same or similar to that used above) was also demonstrated *in vitro* by Gilmour *et al*. (1997) using an assay based on strand breakage of plasmid DNA. The authors attribute much of the activity to the $PM_{2.5}$ fraction of the sample tested although they could not rule out the influence of solubilised material due to the technique of fractionating the collected sample. In the same study they compared the free radical activity of UF-TiO_2 and pigment grade TiO_2 (PG-TiO_2). Both demonstrated activity, the UF-TiO_2 having considerably greater activity than PG-TiO_2. The authors could not explain this difference on the basis of relative surface areas. This is not surprising since commercial TiO_2 is often surface-coated with other materials such as silicon and binders while the UF-TiO_2 used by virtually all investigators is Degussa P25, a specially prepared ultrafine material with high surface oxidant-catalytic activity (personal communication from TiO_2 manufacturers). These differences in themselves add further to the evidence that the surface properties of essentially insoluble particles will influence their biological activity and hence potential health effects.

5.1.2.2. Surface chemistry - role of metals

Li *et al*. (1997), Gilmour *et al*. (1997) and Donaldson *et al*. (1997) proposed that it is the iron (Fe) on the surface of PM_{10} particles (by inference the UF fraction) that is responsible for free radical activity that in turn invokes the subsequently observed inflammatory responses. There is no doubt that PM_{10} and $PM_{2.5}$ contain a varying proportion of Fe and other trace metals arising from industrial emissions and since these often arise from condensation of vapour they will often also condense onto the surface of inert particles. Many trace metals are biologically active and hence, if deposited in the lung, could conceivably initiate or influence subsequent biological responses.

Pritchard *et al*. (1996) investigated the ability of a range of collected particulate pollutants containing several metals (including Cr, Fe and Ni) to:

- generate oxidants
- induce inflammatory responses and airway hyperreactivity after intratracheal instillation into rats
- influence bacterial infection and mortality in mice.

Effects on all these parameters reflected the metal concentrations. The particle sizes of the dusts tested ranged between 2.6 and 5 µm MMAD, sufficiently close to $PM_{2.5}$ to demonstrate that particles that fall strictly within $PM_{2.5}$ will have similar potential for pulmonary effects.

Further investigations for a potential link between the metal content of particles and the pulmonary response come from studies by Costa and Dreher (1997), Dreher *et al*, (1997) and Kodavanti *et al*. (1997). Costa and Dreher (1997) tested a hypothesis that the health effects of ambient particulate matter arise from the transition metal content. Samples of particulate matter were collected from three emission sources (fly ashes resulting from burning of residual oil, domestic oil and coal) and four

ambient samplers situated in the USA, Canada and Europe. Particle sizes ranged between 1.78 and 4.17 µm but the high GSD for the majority indicated that a large proportion of even the largest was within $PM_{2.5}$. Samples were administered to rats by intratracheal instillation in equi-mass or equi-metal doses to address directly the influence of the particles or metals on acute lung injury and inflammation. At 24 and/or 96 hours following instillation, bronchoalveolar lavage fluid was analysed for cell content and protein, albumin and LDH activity. Results indicated that the lung dose of bioavailable transition metal and not particle mass was the primary determinate for the observed acute inflammatory response for both the combustion sources and ambient samples.

Dreher *et al* (1997) administered residual oil fly ash (ROFA; particle size approximately 2 µm) to rats by intratracheal instillation which resulted in an inflammatory response. A leachate of ROFA, containing predominantly Fe, Ni, Ca, V, Mg and sulphate produced a similar lung injury. Depletion of Fe, Ni and V from the leachate abrogated the response and correspondingly the leached ROFA particles produced only minimal pulmonary effects. A surrogate transition metal solution containing Fe, V and Ni largely reproduced the lung injury induced by ROFA. Metal interactions and pH were found to influence the severity and kinetics of lung injury induced by ROFA and soluble transition metals. Kodavanti *et al*. (1997) extended these investigations to show that ROFA, the metal leachate and individual metals (Ni, V and Fe) induced a number of proinflammatory cytokine genes and that it was the persistence of the induction rather than the degree that was more closely associated with histological changes induced by ROFA and associated metals.

Further studies on the possible role of metals in ambient particles in induction of an inflammatory response was reported by Carter *et al*. (1997). They exposed human bronchial epithelial cells to ROFA *in vitro* and measured the release of inflammatory cytokine proteins (IL-8, IL-6 and TNF-α) and expression of the mRNA for these proteins. ROFA exposure induced both proteins and mRNA. Cytokine production was inhibited by the inclusion of either the metal chelator desferoxamine or free radical scavenger dimethylthiourea. In addition, vanadium containing compounds, but not iron or nickel sulphates, mimicked the effects of ROFA. Based on these results, the authors considered that the metals present in ROFA may be responsible for production and release of inflammatory mediators by the respiratory tract epithelium and suggest that these mediators contribute to the effects of particulate air pollutants.

While the studies reported provide some evidence for the role of surface metals in the induction of lung disease by ambient-type particles, caution must be exercised at present in fully accepting such a hypothesis. The doses used in the animal studies were large, 5 mg in the case of the study by Pritchard *et al*. (1996) and were introduced in most cases by intratracheal instillation. The latter introduces a bolus of material in suspension into the lung which does not distribute evenly, such as occurs with inhalation, and represents a rapid introduction of a massive quantity of material. Such quantities introduced within a very short time would be expected to induce an inflammatory response which may be mediated further by additional components such as surface metals. Furthermore, the use of the rat which appears to over-respond to pulmonary insult, must also be considered when evaluating these data.

5.1.3. Contribution of adsorbed components to particle effects

Insoluble particles can act as carriers for adsorbed vapours or soluble compounds such as salts or acids. Although the latter are addressed as distinct particulates in **Section 5.2**, there is evidence that acid-coated particles can exert considerably greater responses than pure aerosols of the acid. Amdur and Chen (1989) exposed guinea pigs for 3 hr per day for 5 days to zinc oxide (ZnO) aerosols (particle size < 1 µm) coated with a surface layer of sulphuric acid. Decrements in lung volume, pulmonary diffusing capacity and indications of pulmonary inflammation were observed at sulphuric acid concentrations of 20 µg/m^3. Chen *et al*. (1992) using similar aerosols, measured induction of airway hyperresposiveness in guinea pigs. This was induced by exposure to a concentration of 20 µg/m^3 sulphuric acid (0.87 mg/m^3 ZnO) for 1 hour. The same degree of response required a concentration of 200 µg/m^3 of pure sulphuric acid aerosol.

The ability of the particle to carry acid rather than a salt appears to influence the potential potency for inducing pulmonary responses. Chen *et al*. (1990) showed that coal fly ash (particle size 0.21 µm) produced in a laboratory furnace from Illinois coal statistically significantly reduced total lung capacity, vital capacity and CO diffusing capacity in guinea pigs immediately, and at 2 and 8 hours post exposure. In contrast, Montana lignite fly ash had little effect. The authors consider these differences to be due to some of the sulphate associated with both types of fly ash to be present partly as sulphuric acid on the Illinois particles but neutralised on the Montana particles by the high alkali content.

Carbon black particles were used by Hemenway *et al* (1996) as surrogate for environmental carbon particles generated from combustion processes to assess the ability to form H_2SO_4 from SO_2 and hence act as a potential carrier for this into the lungs. Co-generation of 10 mg/m^3 carbon black with 10 ppm SO_2 at relative humidities up to 60% resulted in a constant level of sulphate associated with the particles (4 µg SO_4^{2-} /mg carbon black) whereas at 85% humidity this increased more than threefold to 13.7 µg SO_4^{2-} /mg carbon black. Consequently, using alveolar macrophage phagocytosis as an indicator of potential adverse effect of the inhaled particles in mice, the authors showed that only at the high humidity were biological effects evident and concluded from the results that fine carbon particles can be an effective vector for delivery of toxic amounts of SO_4^{2-} to the periphery of the lung under conditions of elevated relative humidity.

Carbon black particles were also used by Jakab and Hemenway (1993) for co-exposure of mice with acrolein vapour, which is normally absorbed as a vapour in the upper respiratory tract regions. Exposure for 4 hours/day for 4 days to 10 mg/m^3 carbon black and 2.5 ppm acrolein resulted in suppression over 11 days of alveolar macrophage phagocytosis and lipopolysaccharide induced TNF-α (an inflammatory cytokine) production in co-exposed animals only. Additional studies in which mice were co-exposed to carbon black (10 mg/m^3) and acrolein (5 ppm) for 4 hours /day for 2, 4, 6 or 8 days showed similar effects on macrophage phagocytosis for 4 days exposure but an apparent adaptation with continued exposure. These data demonstrate further the potential for materials adsorbed onto particulates to be transported to regions of the lungs where they might not normally reach and possibly induce effects not associated generally with the core particle.

5.1.4. Summary

- The majority of toxicological studies on $PM_{2.5}$ type particles have been in rats and using high doses. Comparison with other species indicate that the rat over-responds to the persistent presence of particles in the lung.
- UF particles produce a more pronounced pulmonary response when compared with $PM_{2.5}$ size particles on an equivalent weight/volume atmospheric concentration basis, probably due to differences in translocation within the lung, with additional influences of the state of aggregation of the particles when inhaled and the ease with which disaggregation may occur once deposited.
- The surface chemistry of particles (free radical production capability, presence of metals) and adsorbed components such as H_2SO_4 may influence the pulmonary response to deposited particles.

5.2. SOLUBLE AND ACIDIC PARTICLES

The origin and formation of aerosols composed of soluble salts and acids (**Section 3**) means that these fall within the fine fractions, i.e. $PM_{2.5}$, of ambient aerosols. As such, the majority of studies conducted in the past on these aerosols and reviewed under PM_{10} are equally pertinent to considerations of $PM_{2.5}$. These have investigated changes associated with particles sizes and changes induced in pulmonary physiological parameters, biochemistry and morphology. These are reviewed in the following sections.

5.2.1. Effect of particle size on pulmonary responses

One important characteristic of these aerosols that appears to influence many responses is particle size. The finer the particle size the greater the irritancy, for example, at equivalent concentrations of H_2SO_4, 0.3 µm aerosols were more irritant, as measured by pulmonary resistance in guinea pigs, than particles of 1 µm diameter and 2.5 µm diameter (Amdur *et al*., 1978a; Amdur, 1974; **Figure 6**). Particle size also influences acute mortality but in an inverse relationship to that seen for irritancy. Thus Wolff *et al*., (1977) demonstrated the LC_{50} of 0.4 µm MMAD sulphuric acid aerosols prepared from sulphur trioxide reacting with water vapour to be in excess of 109 mg/m^3 for guinea pigs whereas a small increase to 0.8 µm MMAD resulted in an LC_{50} of 30 mg/m^3. Deaths were attributable to laryngospasm or bronchospasm which accounts for the size relationship since the larger particles will deposit to a greater extent in these sensitive regions. Finer particles will penetrate to the lower respiratory tract where additional damage occurs prior to death. Laryngeal and bronchial spasm is a common response in the guinea pig exposed to irritant materials. While much of the work on acid aerosols has been conducted in the guinea pig, studies with other experimental species indicate that the guinea pig may be over-sensitive to such aerosols and results should be interpreted with caution if extrapolating to other species or man.

While the studies of Amdur (1974) and Amdur *et al*. (1978a) provide a good correlation of concentration related effects in association with particle size, Silbaugh *et al*. (1981a) showed that at constant particle size with differing concentrations, on an individual animal basis there may be considerable inter-animal variation, with some animals being “responders”, i.e. demonstrate effects, and other being “non-responders”. These differences were related possibly to the individual animals

baseline airway characteristics prior to exposure, which has inferences on the potential sensitivity of individual humans to exposure to irritant particles.

5.2.2. Effects on pulmonary function

A number of studies have investigated the effects of acidic aerosols on other pulmonary mechanical functions. Examples are given in **Table 6** which show that changes are measurable only at relatively high concentrations, generally in excess of 1000 µg/m^3. The need for similarly high concentrations to elicit responses are seen in studies designed to measure airway responsiveness in association with exposure to H_2SO_4. These studies were conducted because changes in airway responsiveness, particularly to a hyperreactive state, are typical of a number of conditions associated with pulmonary disease such as asthma, bronchitis and COPD.

Silbaugh *et al*. (1981b) exposed guinea pigs for 1 hour to H_2SO_4 concentrations ranging from 4000 to 40000 µg/m^3 (particle size 1 µm) and measured histamine challenge responses. Only animals showing a constrictive response during exposure to the acid aerosol at concentrations ≥ 19000 µg/m^3 showed a similar response to challenge, suggesting that airway constriction may have been a prerequisite for the development of hyperreactivity. In contrast, Chen *et al*. (1992), using 0.06 µm aerosols of H_2SO_4 found bronchial hyporeactivity in guinea pigs exposed for 1 hour to 200 µg/m^3 but with no change in baseline pulmonary resistance. Similarly, Kobayashi and Shinozaki (1993) found hyperresponsiveness of the airways of guinea pigs exposed for 24 hours/day for 14 days to 3200 µg/m^3 H_2SO_4 (particle size 0.54 µm), with no effect at 1000 µg/m^3. It is worthy to note that the hyperresponsiveness was preceded after 3 days exposure by hyporesponsiveness and that after 30 days the airways had returned to normal. This recovery was not evident in rabbits exposed to 250 µg/m^3 H_2SO_4 aerosol (particle size 0.3 µm) for 1 hour/day, 5 days/week for up to 12 months. Hyperresponsiveness was seen at 4 months, had increase by 8 months but then stabilised since it was similar at 12 months. Again, there was no change in baseline resistance.

The preceding studies demonstrate that repeated exposure to acid aerosols can lead to induction of airway hyperresponsiveness but as with pulmonary mechanics the concentrations required to elicit such changes are often in excess of 1000 µg/m^3. Although the mechanisms responsible for induction of this state are not fully understood, one possibility involves increased sensitivity to mediators affecting airway smooth muscle control. Stengel *et al*. (1993) showed an enhanced sensitivity of H_2SO_4 exposed guinea pigs to the neuropeptide Substance P that effects bronchial muscle tone. El-Fawal and Schlesinger (1994) showed that exposure of rabbits to concentrations of H_2SO_4 ≥ 75 µg/m^3 produced increased responsiveness in subsequently isolated bronchial and tracheal rings when exposed *in vitro* to both histamine or acetylcholine.

The latter studies showed effects at concentrations as low as 76 µg/m^3 which are approaching ambient levels to which individuals can be exposed. This raises the possibility that the effects and potential mechanisms may be of some relevance to human exposures but direct extrapolation must be made with caution since these studies combined *in vivo* exposure with *in vitro* estimations of effects.

5.2.3. Effects on pulmonary clearance

5.2.3.1. Mucociliary clearance

Pulmonary clearance mechanisms play an important role in the removal and neutralisation of inhaled particulate matter, including infective agents, and any exposure-related changes to this process may have serious consequences on the health status of the individual. Mucociliary clearance operates in the tracheobronchial tree down to the respiratory bronchioles and macrophage mediated clearance in the alveolar and non-ciliated bronchiolar regions. Studies assessing the effect of a single 1 hour exposure of rabbits to acid aerosols (particle size 0.3 µm) ranging in concentration from 100-2200 µg/m^3 showed increased clearance at the lower concentrations with decreasing clearance at the higher (Schlesinger *et al*., 1984). Repeated exposure for 1 hour/day, 5 days/week for 4 weeks to 250-500 µg/m^3 resulted also in increased bronchial clearance rates during a 2 week post exposure holding period (Schlesinger *et al*., 1983). In contrast, Gearhart and Schlesinger (1988) and Schlesinger *et al*. (1992) report reductions in clearance rates with prolonged exposures to 250 µg/m^3 and 125 µg/m^3 H_2SO_4 respectively for up to 12 months duration, although the latter observed an initial increase followed by a persistent decrease. These differences may be explained by the increasing numbers of epithelial secretory cells reported in these studies together with a probable trend towards a more viscous mucous secretion.

5.2.3.2. Cell mediated clearance

Macrophage mediated alveolar clearance was retarded in rabbits exposed to 500 µg/m^3 H_2SO_4 for 2 hours/day for 14 days (Schlesinger and Gearhart, 1987) but enhanced in rabbits exposed to 250 µg/m^3 H_2SO_4 for 1 hour/day, 5 days/week for up to 240 days (Schlesinger and Gearhart, 1986). These represent relatively simplistic evaluation of the effect of acid aerosols on macrophage function since it is likely that the aerosols will affect a number of the macrophage functional properties. For example, Chen *et al*. (1992) showed a depression of intracellular pH in macrophages recovered from guinea pigs exposed to 300 µg/m^3 H_2SO_4 following exposure for 3 hours to aerosols of 0.3 and 0.04 µm particle sizes. This depression was seen also after 4 days exposure to the UF (0.04 µm) particles but not to the fine (0.3 µm). Thus once again the particle size and probably particle number has a marked influence on the effect observed and probably duration. Production of inflammatory mediators by macrophages may also be affected by exposure which could have implications on subsequent tissue responses, although again, where studies have been conducted there are conflicting reports of responses. Zelikoff and Schlesinger (1992) exposed rabbits to 50-500 µg H_2SO_4 for 2 hours and measured release from alveolar macrophages of the inflammatory mediators TNF and superoxide radical. Suppression was observed at concentrations ≥ 75 µg/m^3. Similar effects were seen when the exposure duration was extended to 4 days and higher concentrations (Zelikoff *et al*. 1994). In comparison with these responses, Chen *et al*. (1992) report increased release of TNF from macrophages obtained from guinea pigs exposed to 300 µg/m^3 H_2SO_4 for a single 3 hour exposure or 4 daily exposures, with hydrogen peroxide production also increased after the 4 daily exposures. The results of these studies together with a number of other differences between apparently similar studies may be attributable both to particle size and also interspecies differences.

5.2.4. Effects on lung morphology

Many studies have investigated the morphological effects of exposure to acid aerosols but as for physiological investigations, the majority have exposed animals to concentrations well in excess of those considered of relevance to humans exposed to ambient aerosols and these will not be considered here. More appropriate exposures in the range of 70-250 µg/m^3 have been conducted. Gearhart and Schlesinger (1989) exposed rabbits to 250 µg/m^3 H_2SO_4 (particle size 0.3 µm) for 1 hour/day, 5 days/week for 4, 8 and 12 months with some animals given a 3 month recovery at the end of the 12 month exposures. Results at all time points showed a shift towards a greater frequency of smaller airways (narrowed airways; a finding in humans associated with exposure to irritant tobacco smoke and an early change relevant to clinical small airway disease) but a return to normal following the recovery period. These authors also showed increased numbers of secretory cells in these airways and a return to normal was not seen at the end of the 3 month recovery period. In another study, Schlesinger *et al*. (1992) exposed rabbits for 2 hour/day, 5 day/week for up to 12 months to 125 µg/m^3 H_2SO_4 aerosol (particle size 0.3 µm). No bronchial inflammation was seen but there was again an increase in secretory cells numbers in the small airways at 12 months.

Morphometric analysis of lungs of rats exposed to ammonium sulphate or ammonium nitrate aerosols (equivalent respectively to 70 µg/m^3 sulphate or 350 µg/m^3 nitrate and particle sizes 0.2-0.5 µm and 0.4-0.8 µm) for 4 hours/day, 4 days/week for 8 weeks, showed moderate to substantial changes in alveolar nuclear density, alveolar chord length, septal thickness and cross sectional area (Kleinmann *et al*., 1995). Increase lung permeability and depressed macrophage function in cells isolated by bronchoalveolar lavage were also found.

5.2.5. Relationship of acidity to toxicity

The majority of studies investigating the effect of acidic aerosols on lung function, morphology and clearance have used H_2SO_4. A comparison of the irritant potential for other sulphate aerosols (Amdur *et al*., 1978b) provided a ranking with H_2SO_4 being considerably more irritant than other sulphates (**Table 4**). This supports the view that H_2SO_4 is the major irritant in ambient acidic aerosols and contributes the greatest to any perceived health effect. It also implies that other salts would result in reduced pulmonary effects compared with those induced by H_2SO_4 at equivalent concentrations.

Where others salts such NH_4HSO_4 have been used the reduced activity has been considered to be related to the H^+ concentrations. Schlesinger and Chen (1994) provide evidence that the difference observed with NH_4HSO_4 is due probably to the degree of neutralisation of the acid aerosols by respiratory tract ammonia since more total H^+ remains available from inhaled H_2SO_4 than from inhaled NH_4HSO_4 when atmospheres have the same total H^+ concentration. Thus, the greater potency of H_2SO_4 aerosols compared with NH_4HSO_4 is likely to be due to a greater neutralisation of the latter after entering the respiratory tract.

5.2.6. Summary

- Particle size of acid aerosols may influence irritancy, with finer particles being more irritant than coarse.
- Pulmonary function changes can be induced by exposure to acidic aerosols but high concentrations (> 1000 µg/m^3) are generally required to elicit responses.
- Exposure to acidic aerosols generally induces an increase in mucociliary clearance but with prolonged exposure morphological and mucus changes may result in decreased clearance.
- Cell mediated clearance may be affected by exposure to acidic aerosols but results are conflicting between an enhancement or retardation. Effects have been reported at concentrations as low as 125 µg/m^3 H_2SO_4 with chronic exposure.
- Morphological changes in the respiratory tract may occur with prolonged exposure to acidic aerosols at concentrations as low as 125 µg/m^3 H_2SO_4.
- The effects of acidic aerosols are probably due to the H^+ concentrations.

5.3. EFFECTS OF PARTICLES ON RESISTANCE TO DISEASE

Infectious diseases of the respiratory tract are considered as one of the factors that can pre-dispose human individuals to the effects of inhaled materials. Since pulmonary infections may also be more prevalent in the elderly and infirm, the potentially more sensitive population, the potential for inhaled particles that fall within $PM_{2.5}$ to exacerbate any underlying infection by affecting the pulmonary microbial defence mechanisms could have serious health consequences. Since experimental animals are usually healthy and disease free, the majority of animal studies designed to assess this possibility have used challenge with an infective agent in association with exposure to particulates.

Jakab (1992) co-exposed mice to 3.5 mg/m^3 carbon black and 2.5 ppm formaldehyde, considered to represent an “ambient pollutant mixture”, to determine if the latter adsorbed onto the particles would affect intrapulmonary killing of *Staphylococcus aureus*. No differences were observed between control and treated mice. In a more extensive investigation of different defence mechanisms, Jakab (1993) exposed mice to 10 mg/m^3 carbon black (particle size 2.45 µm) for 4 hours/day for 4 days and challenged these with the infective agents *Staphylococcus aureus, Proteus mirabilis or Listeris monocytogenes*. The defence mechanism against *Staphylococcus aureus* is alveolar macrophage dependent, that against *Proteus mirabilis* is dependent on both the alveolar macrophages and neutrophils while that against *Listeris monocytogenes* involves specific acquired immunity. An outline of the results of these studies and others investigating the effects of both insoluble particles and acid aerosols on microbial infectivity are shown in **Table 6**. These show clearly that even at concentrations considerably higher than those experienced during air pollution episodes, the particulate material did not reduce the resistance of the host to bacterial infection.

It is important to note that the majority of these types of studies were conducted in a murine model. Zelikoff *et al*., (1994) exposed rabbits to 0.5, 0.75 or 1.0 mg/m^3 H_2SO_4 for 2 hours/day for 4 days and measured intracellular killing of *Staphylococcus aureus* by alveolar macrophages obtained by bronchoalveolar

lavage 24 hours after the last exposure. This was reduced at the two higher concentrations and bacterial uptake was reduced at the highest concentration only. These results imply that repeated exposure to H_2SO_4 may reduce host resistance to bacteria in the rabbit in contrast to other species but the relevance to man is uncertain.

In a study in which dogs were exposed continuously to a mixture of sulphur related air pollutants, containing both gaseous and particulate phase components, for 290 days (Heyder *et al*., 1992), a reduction of macrophage phagocytic activity and decreased formation was interpreted as a decline in the bacterial defence capacity (Maier *et al*., 1992). In the absence of any observable health effects in these animals that might result from reduction of this capacity of the lung, any decline is considered to be of limited, if any, biological significance.

In summary, there are no convincing adverse effects on bacterial host-defence mechanisms in animals as a result of exposure to acidic aerosols.

5.4. HEALTH-IMPAIRED ANIMAL MODELS AND EFFECT OF EXPOSURE TO PARTICLES

A limited number of animal studies have been conducted in animals where a model of the health-impaired human has been developed to determine if this state potentiates the effect of inhaled particulates.

5.4.1. Cardiorespiratory disease model

COPD has been identified as the pre-existing condition most often associated with reports of air-pollution related deaths and common components of this condition are pulmonary hypertension and right heart enlargement. These can be induced in rats by subcutaneous administration of the pyrrolizidine alkaloid monocrotaline (Costa *et al*., 1994). However, it must be recognised that monocrotaline induced pulmonary hypertension in the rat is not a true model of the disease in humans. The pathological reactions in the rat differ from those found in the human disease (Heath, 1992). While the disease in man and the rat share similar features such as medial hypertrophy of small pulmonary arteries with crenation of elastic laminae and fibrinoid necrosis, in man there is a major component of muscle cell migration and intimal proliferation. This indicates that it is a disorder of cell growth as well as disease a of vasoconstriction and that the rat is therefore a poor model for the disease in man.

The monocrotaline induced hypertension rat model was used by Costa and Dreher (1997) in association with the studies described earlier on transition metals, to show that the response to intratracheal instillation of ROFA (particle size approximately 2 µm) was enhanced appreciably by the pre-existing disease state such that mortalities occurred. These appeared to be related to altered cardiac function although it was unclear whether this was associated with direct cardiac injury or was secondary to pulmonary failure. Electrocardiac measurements showed that ROFA alone induced a mild arrhythmia over the 96 hour period when acute pulmonary inflammation occurred but the monocrotaline pre-treated animals exhibited substantially more and worsened dysrhythmias. Cardiac alterations progressed with time and correlated with the time of death. The apparent correlation of these effects with the transition metal content of ROFA was demonstrated by testing particles with

virtually no biologically available metals (Mt St. Helens volcanic ash) which proved innocuous in this animal model.

The monocrotaline-treated rat was used also by Killingsworth *et al*. (1997) to explore further potential mechanisms responsible for air-pollution induced mortalities in people. Monocrotaline-treated rats and saline controls were exposed by inhalation to 580 µg/m^3 fuel oil ash (particle size 2.06 µm) or filtered indoor air for three daily exposure of 6 hours duration. The day following the last exposure right ventricular systolic pressure was measured, histopathology evaluated and assessments made of cellular and molecular parameters indicative of an inflammatory response. No mortalities occurred in control animals or monocrotaline dosed animals exposed to air only but 42% of those with monocrotaline-induced lung disease and exposed to fuel oil ash died. Monocrotaline alone induced a number of markers of inflammation, e.g. neutrophils in bronchoalveolar lavage fluid and rodent proinflammatory chemokines in lung and heart, but fly ash enhanced the response. Right ventricular systolic pressure was unchanged in monocrotaline-treated rats during exposure to fly ash, indicating that the deaths were not due to an acute rise in pulmonary artery pressure. The results of the studies by Costa and Dreher (1997) and Killingsworth *et al*., (1997) confirm that fly ash can induce an inflammatory response in the lung of control animals and that it is capable of enhancing inflammation induced by monocrotaline. The marked incidence of mortalities in animals with pre-existing cardiorespiratory disease is considered by some investigators to reflect the apparent similar susceptibility for those people who have similar pre-existing conditions.

It is important to consider the results of these studies in the context of the earlier comment on the relevance of the monocrotaline rat model to cardiovascular disease in man. In the absence of any other appropriate model it can be considered to demonstrate increased susceptibility of the already severely compromised rat to the additional insult of exposure to particulates.

5.4.2. Asthma

Asthma, another condition considered to pre-dispose individuals to the effects of inhaled $PM_{2.5}$, has been modelled with limited success in animal models but there are no data available to evaluate if this experimentally induced effect influences effects induced by inhalation of materials comprising $PM_{2.5}$.

5.4.3. Emphysema

Emphysema is a common disease in humans and a similar condition can be induced experimentally in animals by exposure to elastase. This has been used by several investigators to assess the effect of $PM_{2.5}$ on a number of physiological and biological parameters.

Loscutoff *et al*. (1985) exposed rats and guinea pigs with elastase-induced emphysema to $(NH_4)_2SO_4$ (1,000 µg/m^3, particle size 0.4 µm) or NH_4NO_3 (1,000 µg/m^3, particle size 0.6 µm) for 6 hours/day, 5 days/week for 20 days in order to determine whether repeated exposures would alter pulmonary function compared to saline-treated controls. Results indicated that the specific induced disease state did not enhance the effect of acidic sulphate aerosols in altering pulmonary function. Similar findings were reported by Lewis *et al*. (1973) who exposed dogs whose lungs were impaired by exposure to NO_2 to H_2SO_4 (889 µg/m^3) for 21 hours/day for 620 days.

Mauderly *et al*. (1990) exposed young rats having elastase-induced emphysema to whole diesel exhaust (3.5 mg particulate/m^3) for 24 months (7 hours/day, 5 days/week) and examined number of parameters, including pulmonary function (e.g., respiratory pattern, lung compliance), bronchoalveolar lavage fluid components (e.g., enzymes, protein, collagen), histopathology, and morphometry. There was no evidence that the diseased lungs were more susceptible to the diesel exhaust than were normal lungs. In fact, in some cases, there seemed to be a reduced effect of the diesel exhaust in the emphysematous lungs. This could be due, in part, to a reduced lung burden in the diseased lungs, resulting from differences in deposition and/or clearance compared to normal lungs.

Raub *et al*., (1985) exposed rats with elastase-induced emphysema to Mt. St. Helens volcanic ash (9.4 mg/m^3, particle size 0.65 µm) for 2 hours/day for 5 days with and without 2.7 mg/m^3 SO_2. The effects on pulmonary mechanics in these rats were similar to those noted in normal animals exposed to the same atmosphere.

It should be recognised that this model of emphysema, as with the monocrotaline rat model for cardiorespiratory disease, is not identical to that seen in man. The rat model is accompanied by toxicologically significant inflammatory reactions that are not seen as part of the disease in man. A more relevant animal model of emphysema might be the tight-skin mouse that develops the disease spontaneously due to a genetic defect. While the disease is well characterised in this animal, no studies have been conducted to investigate the effect of exposure to particulates on disease progression, lung clearance or development of further lung disease.

5.4.4. Summary

- A limited number of studies have investigated the effects of exposure to $PM_{2.5}$ in animal models considered to be representative of cardiorespiratory disease or emphysema.
- Pre-existing “cardio-respiratory” disease in the rat enhanced the response to exposure to some particles, such responses showing an apparent correlate with the chemistry of the particles.
- Pre-existing “emphysema” did not influence the pulmonary response to insoluble or acidic particles.
- It must be clearly recognised that both disease states are induced artificially in animals and are not truly equivalent to the disease as presented in humans, hence any extrapolation to effects in humans based on these models is speculative.

5.5. EFFECTS OF COMBINATIONS OF AIR POLLUTANTS ON EXPERIMENTAL ANIMALS

Some experimental studies have assessed the effects on animals of direct exposure to ambient particulate pollutants in the environment or to mixtures considered to be representative, in part, to those to which the general population might be exposed environmentally. Results need to be interpreted with caution since although each exposure atmosphere contained particulates, other gaseous pollutants were also present and may modify additively or synergistically the effects observed, or induce effects in regions other than those expected from particulate exposure alone. It is also possible that the particulate acted as carrier for some or all of the gaseous pollutants, as addressed previously.

5.5.1. Exposure to ambient pollutants

Saldiva *et al*. (1992) housed rats for 6 months in the centre of São Paulo and compared pulmonary function, mucociliary clearance and mucus rheology, airway histochemistry, bronchoalveolar lavage components and epithelial ultrastructure with animals housed in a rural area considered to represent clean conditions. In those exposed in São Paulo, where particulate, O_3, SO_2 and CO were monitored, secretory cell hyperplasia, ultrastructural ciliary alterations, more viscous mucus and impairment of mucociliary clearance was observed. Nasal resistance was also increased, as was the number of inflammatory cells recovered in the bronchoalveolar lavage fluid. While this study demonstrates an effect of the pollutants in exposed rats, it cannot differentiate between the particulate and gaseous phases and hence cannot ascribe these effects to particulate alone. Indeed, the majority of the symptoms reported are more consistent with gaseous phase effects in the rat. It should be remembered also that the rat appears to be more responsive to inhaled particulates than other species and the same may also apply to irritant gases such as O_3 and SO_2. Thus caution may be required in extrapolating these studies to potential human exposures.

5.5.2. Exposure to experimental pollutant mixtures

The GSF-national centre for Environment and Health laboratories, Germany, have conducted continuous exposures of dogs for 290 days to sulphur related air-pollutants (Heyder *et al*., 1992; Maier *et al*., 1992; Kreyling *et al*., 1992; Schulz *et al*., 1992; Takenaka *et al*., 1992). Atmospheres were generated from sodium metabisulphite solution, the resultant atmospheres contained both gaseous sulphur dioxide (approximately 26%) and 0.3 mg/m^3 particulate (approximately 63%; particle size 0.6 µm). To establish a baseline the dogs were first held under clean air conditions for 400 days. Effects observed after exposure included decrease in lung compliance, a supposed decline in the bacterial defence capacity as indicated by reduced macrophage phagocytic activity and decreased formation of oxygen radicals (see **Section 5.3**), reduced particle clearance, hyperplastic change in the nasal passages, disturbed tracheal mucociliary clearance, early peripheral airspace enlargement. These studies were extended subsequently to include H^+ ions in the atmospheres with exposure for 13 months (Heyder *et al*., 1997). Dogs were exposed 16.6 hours/day to either to neutral sulphite atmosphere as used before (1.54 mg/m^3 particulate concentration; particle size 1.02 µm) or acid sulphate aerosol (concentration 5.66 mg/m^3; particle size 1.08 µm). Parameters measured are shown in **Table 8** which compares the effect of the neutral and acid aerosols. These show that some of the effects produced by the neutral aerosol were either less pronounced, not detectable or even reversed when particle-associated H^+ were also delivered to the lungs, indicating antagonism of effects rather than additive or synergistic. However, the proximal alveolar region responded to the acidic aerosols compared with the neutral aerosols, showing an elevation of alkaline phosphatase concentration in the epithelial lining fluid and proliferation of the type II epithelial cells.

The effects of exposure to a mixture of acid aerosols (H_2SO_4) and O_3 were investigated by Kimmel *et al*. (1997). They exposed rats for 4 hours/day for two days to 500 µg/m^3 of 0.3 µm (fine) or 0.06 µm (UF) H_2SO_4 aerosols separately or in combination with 0.6 ppm O_3 and measured morphometrically the volume percentage of lung parenchyma containing markedly to severely injured alveolar septae. There were no differences between the UF or fine acid exposure groups and the control group for any of the morphologic endpoints while O_3 alone produced

an increase over controls. Volume percentage of markedly to severely injured tissue was increased in the UF, but not fine, animals exposed to mixed atmospheres when compared with the O_3-only exposed group. In addition, a synergistic interaction between O_3 and UF, but not fine, H_2SO_4 was apparent for this endpoint. Bromodeoxyuridine cell labelling index in the periacinar region was greater in the rats exposed to the fine H_2SO_4 and O_3 mixture than that in rats exposed to O_3 alone, and a synergistic interaction between O_3 and fine H_2SO_4 was found for this end point. None of the exposures produced any changes in ventilatory parameters. These results show a lack of effect of fine or UF H_2SO_4 particles at 500 $\mu g/m^3$ but in combination with O_3 there can be an exacerbation of effects which is influenced by aerosol droplet size.

Synergy between H_2SO_4 or $(NH_4)_2SO_4$ and O_3 on pulmonary biochemical parameters (lavage protein concentration, total lung protein, collagen synthesis) has also been demonstrated by Last (1989) who concludes that the combination for this synergy to occur is one of a relatively insoluble oxidant gas and a respirable acidic or acidogenic aerosol such that appreciable deposition occurs in the centriacinar region of the lung. The synergy arises from the acid aerosol potentiating the damaging effect of O_3 in this region and not vice-versa. A similar conclusion was made by Kleinman *et al*. (1989) investigating effects in the rat of acid aerosols in combination with O_3 or NO_2. In the case of the latter, nasal passage lesions were seen also and attributed to the acid aerosol.

A synergistic effect of acute experimental exposure to airborne urban particles with co-exposure to O_3 was shown by Vincent *et al*. (1997). The particulate material was collected from the environment of Ottawa, filtered and re-suspended in air for exposure of rats for 4 hours to either 5 mg/m^3 or 50 mg/m^3, alone or in combination with 0.8 ppm O_3. The MMAD of the particles was 4.6 µm but with a GSD of 3.2 the resultant atmosphere was composed of approximately 30% $PM_{2.5}$ and any effects in the pulmonary regions can be attributed to this fraction. 32 hours after exposure cell proliferation was measured as an assessment of lung effects. The particles alone did not induce a proliferative response while O_3 alone resulted in a proliferation rate of 0.42 ± 0.16 in the bronchioles and 0.57 ± 0.21 in the parenchyma. This was increased to 3.31 ± 0.31 and 4.45 ± 0.51 in the bronchioles and 0.99 ± 0.18 and 1.47 ± 0.18 in the parenchyma with co-exposure to 5 or 50 mg/m^3 respectively of particulate. Changes were most notable in the epithelia of terminal bronchioles and alveolar ducts and did not distribute to the distal parenchyma, a pattern of response consistent with the regional effect of O_3 and deposition pattern of particles in the rat lung.

The above studies provide for a number of mixed exposure scenarios which may have relevance to human exposure. One is a combination of acidic aerosols with relatively insoluble gases such as O_3 or NO_2. Here the acid aerosol, which alone exerts an effect at relatively high concentration, can potentiate the damaging effect of the gases in the deep regions of the lung whilst alone or in combination the aerosol produced nasal passage effects. Another scenario is that of inert particles in combination with acidic components. In this case the particle may contain acidic components, as with ambient $PM_{2.5}$ or may carry adsorbed gases, which in turn may convert to acids, to the deep lung where the adsorbed and possibly modified component enhances tissue responses. Nasal passage effects reported in conjunction with these scenarios is likely to result from absorption of the soluble acid gas by these regions, possibly in combination with acid-coated particles. Exposure to ambient pollutants is likely to be to a combination of all these scenarios with effects possible in both the upper and lower regions of the respiratory tract, as seen by Saldiva *et al*. (1992).

It is evident from the above that while acidic aerosols alone can produce adverse effects in the respiratory tract the concentrations at which this occurs are in excess of those that might be encountered in the environment. However, when in combination with common gaseous pollutants, these aerosols can potentiate the inflammatory responses induced by the gaseous phase components. Adsorption of soluble gaseous components onto relatively inert aerosol particles can also potentiate effects in the parenchymal regions. When the gaseous phase is composed of relatively insoluble oxidants the development of nasal lesions can be attributed to the acidic aerosols, since these alone are capable of inducing such effects. Where a soluble gas is present, such as SO_2, this can produce lesions in these regions.

With respect to expected deposition patterns of particles and regional effects observed in some of the examples above, the nasal/upper respiratory tract effects are consistent with exposure to a gaseous atmospheric component such as SO_2, which will be absorbed almost totally by these regions and will not normally penetrate to the distal regions. Therefore, effects in the latter can be attributed predominantly to the particulate phase in the GSF studies and possibly the study by Saldiva *et al*. (1992) although in this case O_3 and NO_2 could have also produced effects since these exert effects in the distal regions rather than upper regions of the respiratory tract due to their lower water solubility, as seen in the study by Kimmel *et al*. (1997).

Some of the studies described show the potential for ambient air particulate material (which will contain a high proportion of $PM_{2.5}$ in a city) and particulate in a mixed experimental atmosphere to exert effects in the normal animal. This can also be extrapolated to possible similar effects in normal or especially health impaired human individuals exposed to such mixtures environmentally. In addition, the study by Kimmel (1997) using relatively high concentrations of H_2SO_4 aerosols suggests that the gaseous phase may have a role to play by acting as an initiator of an effect with particles then exacerbating the response.

5.5.3. Summary

- Some experimental studies have assessed the effects on animals of direct exposure to ambient particulate pollutants in the environment or to mixtures considered to be representative, in part, to those to which the general population might be exposed environmentally.
- Ambient pollutants induced changes indicative of inflammation in the respiratory tract of rats but it was not possible to differentiate between the particulate or gaseous phases.
- Studies using experimentally generated mixtures of particles and gases suggest that the particulate phase potentiates the effects induced by gaseous phase components such as O_3 and SO_2.

6. EFFECTS OF $PM_{2.5}$ PARTICLES IN HUMANS

Information on the effects of inhaled particulates in humans come from several sources, including epidemiology, occupational exposure, smoking and human volunteer studies. Epidemiology addresses population or sub-population effects in a retrospective manner and is considered separately in **Section 7**. Smoking is a voluntary exposure to high concentrations of mixtures of particles, condensates and gases. While environmental tobacco smoke may contribute to ambient pollutants, smoking *per se* can result in marked health effects due to the high concentrations and characteristics of smoke components and is considered to be of limited relevance to the effect of ambient particulates except possibly for inducing respiratory disease in some individuals and hence pre-disposing them to the development of further effects of ambient pollutants. For the preceding reasons smoking will not be considered further in this review.

The aim of this section is to review:

- some of the occupational exposure data on particulate material relevant to $PM_{2.5}$
- human volunteer studies on insoluble particles
- human volunteer studies on the effects of acidic aerosols on a number of parameters of lung function
- human volunteer studies on mixed particulate/gaseous atmospheres

6.1. OCCUPATIONAL EXPOSURE

Occupational exposure can be considered as retrospective in general and tends to be at least an order of magnitude higher than environmental exposure. It may represent a possible information source with appropriate reservations. A point of importance regarding occupational exposure is that many of the workplace particulates represent the upper particle sizes encompassed by $PM_{2.5}$ which may have less potential for inducing lung effects at low concentration due to deposition and retention patterns and particle numbers or surface area, together with the fact that the majority of exposed workers are likely to represent the more healthy proportion of the general populace.

Occupational exposure to UF particles may occur. One source of such exposure that is also of environmental concern and represents a bridge between occupational and environmental exposure to UF particles is diesel exhaust. Fumes arising from heated metals or polymers represent another potential source of occupational exposure to UF particles.

6.1.1. Diesel exhaust

The particulate phase of diesel exhaust is frequently the major component of urban ambient aerosols (the carbonaceous matter fraction shown in **Figure 2**) and there is widespread occupational exposure. Concerns over the possible health effects of exposure to diesel exhaust have led to a substantial investigation of the effects on workers and to the conduct of a number of animal studies (see **Section 5**). **Table 8** presents findings from a number of studies in which workers or clinical subjects were assessed for subjective or physiological effects of diesel exhaust. This table is

illustrative rather than exhaustive and shows that in general, symptoms of exposure to high concentrations include irritation to the mucous membranes, eyes and respiratory tract and neuropsychological effects such as nausea, headache and vomiting. It is more than probable that many of these symptoms are due to the vapour phase of diesel exhaust, since it is important to recognise that combustion particles represent only a fraction of raw diesel exhaust and that many gaseous components are present at relatively high concentrations. Thus exposure to diesel exhaust should be considered as a mixed exposure.

6.1.1.1. Acute exposure to diesel exhaust

Excluding neuropsychological effects, there is little evidence for short term exposures to diesel exhaust to affect pulmonary function in workers in a wide range of industries in which exposures can occur or subjects exposed under controlled conditions to diesel exhaust (**Table 8**). For example, Ames *et al*. (1982) measured the pulmonary function of 60 coal miners who worked in diesel-engine equipped mines and compared these with 90 coal miners not exposed to diesel. No differences in shift-related pulmonary function were seen between the two groups when smoking habits, age, dust exposure and years in mining were taken into account. Purdham *et al*. (1987) found no statistically significant shift-related changes in FVC or FEV_1 in workers on ferries transporting diesel trucks and other vehicles when compared with appropriate controls and no statistically significant change was seen in spirometry measured over a shift in 232 workers in four diesel bus garages (Gamble *et al*., 1987a). In contrast, Ulfvarson *et al*. (1987) reported statistically significant decrements in FVC and FEV_1 (approximately 8% in FVC, 7% in FEV_1) from exposure to diesel exhaust from trucks across a shift in 23 ferry workers who had not been exposed previously for 10 days. In a repeat of this study (Ulfvarson and Alexandersson, 1990) a statistically significant 5% decrement in FVC was observed but not in FEV_1. In another group a decrement in FVC due to exposure to diesel exhaust was reduced from 10% to 5% when the trucks were fitted with microfilters on the exhausts. Given that in the latter group the effect of filtration was to reduce the 10% decrement to 5%, similar to that seen in the other group tested who had been exposed to diesel exhaust only, any relevance of these changes in FVC to exposure to diesel exhaust is questionable.

The above reductions in pulmonary function, while statistically significant, do not approach decrements considered normally to be of biological significance and in view of the other comments made above, the absence of effects in other studies and lack of evidence for chronic effects of exposure to diesel exhaust (see below), such changes are not considered to raise concerns for normal individuals. Implications for health impaired individuals is discussed later.

6.1.1.2. Chronic exposure to diesel exhaust

Assessment of chronic occupational diesel exhaust exposure also provides little evidence for an excess of respiratory effects of concern. Thus Gamble *et al*. (1983) assessed a large cohort of salt miners for respiratory symptoms, radiographic changes and pulmonary function. While some parameters (e.g. FEV_1 and FVC) were uniformly lower in the miners compared with other workers above ground, there were no changes in pulmonary function associated with years of exposure or cumulative exposure to particles or gaseous components. A similar lack of effect of diesel exhaust in potash miners was reported by Attfield *et al*. (1982) and by Ames *et al*. (1984) for coal miners.

A number of mortality studies have also been conducted with respect to occupational exposure to diesel exhaust. Such studies were confined to employees in the transport industry, railroad workers, operators of heavy construction equipment and miners. Workers in the transport industry (bus garage employees) were assessed by Edling *et al*. (1987) who showed no increased mortality from cardiovascular disease in general although in an earlier pilot study (Edling and Axelson, 1984) a subgroup of heavily exposed workers had a fourfold increase in the risk of dying from this condition. Rushton *et al*. (1983) evaluated mortality in maintenance workers in London bus garages and showed no increased incidence of non-malignant or malignant disease associated with their employment which would have involved exposure to diesel exhaust. No excess of mortalities associated with exposure to diesel exhaust in potash workers was reported by Waxweiler *et al*. (1973) although the authors recognised that there had probably been insufficient time between introduction of the diesel engines and the time of conduct of the mortality study.

Wong *et al*. (1985) studied heavy equipment operators and found lower than expected mortalities and no association between diesel exhaust exposure and lung cancer. Similar findings regarding lung cancer were reported by Hall and Wynder (1984).

A number of studies have been conducted on railroad workers. Some of the earlier studies did not take into account smoking history as a confounding factor nor duration or level of exposure and results are considered unreliable. Garshick *et al*. (1987, 1988) conducted very extensive mortality studies and considered that account was taken of all potential confounding factors. Their results inferred a small but statistically significant elevated lung cancer risk related to diesel exposure. These papers and the results have been subsequently reviewed by a number of authors, including Morgan *et al*. (1997), who have identified several defects in the cause and effect relationships and concluded that the conclusions made by Garshick *et al*. (1987, 1988) were by no means convincing. Muscat and Wynder (1995) reviewed 14 case-control or cohort studies on the relationship between cancer and exposure to diesel exhaust, including the studies by Garshick *et al*. (1987, 1988) and concluded that using common criteria for determining causal associations, the epidemiological evidence was insufficient to establish diesel engine exhaust as a human carcinogen.

The lack of any convincing relationship between occupational exposure to diesel exhaust and lung cancers supports the view that ambient air particulate matter, containing in many instances a high proportion of diesel exhaust particles, is not carcinogenic. From a similar lack of any convincing relationship between occupational exposure to diesel exhaust and long term pulmonary effects or mortalities it can be concluded that for the normal healthy population ambient particulates containing high proportions of diesel particulate are also unlikely to pose any problems. However, any effect on health impaired individuals cannot yet be excluded for reasons of lack of relevant evidence.

6.1.2. Fumes

Examples where exposure to UF particles results in a rapid pulmonary response include metal fume fever and polymer fume fever. Metal fume fever is an acute respiratory disease characterised by symptoms including dyspnea, cough, chest pains and pneumonitis resulting from the inhalation of freshly generated oxide fumes of metals such as zinc and copper. These symptoms arise from an acute

inflammatory response in the lung which has been investigated recently by Kuschner *et al*. (1997), more detail being given in the Clinical Studies section. Normally, exposures are accidental, are in the mg/m^3 range and arise from welding processes. Under normal working conditions Zn concentrations are appreciably below the Occupational Exposure Limit of 5 mg/m^3 and no acute or chronic effects are observed.

Accidental exposure to freshly generated fume from PTFE produces a similar response known as polymer fume fever which under worst circumstances can lead to mortality. A more detailed appraisal of PTFE fumes is given within the animal exposure chapter (**Section 5.1.1**) since these are considered as potential models for highly dispersed, i.e. non-aggregated UF particles (singlets) in the environment. Their existence in this state experimentally is transient but correlates with a high pulmonary toxicity in animals, aggregation reducing markedly the toxicity observed. The same applies possibly to humans, this being a relatively rare phenomenon with effects being evident only with freshly generated fume maintained predominantly in the singlet state. Many cases are reported in association with smoking, either from PTFE contaminated cigarettes (Cooper and Gazzi, 1994; Albrecht and Bryant, 1987) or where PTFE particles are in the atmosphere (Auclair *et al*., 1983). The high temperature of the ignited cigarette will cause thermal degradation and favour the singlet state which is inhaled rapidly and before aggregation occurs. Although it is possible that gaseous decomposition products contribute to the disease, the substantial work investigating the characteristics of PTFE combustion fumes currently supports the view that particulate in the singlet state is the primary cause. Repeated occurrence of polymer fume fever can result in the development of impaired pulmonary function and obstructive lung disease (Kales and Christiani, 1994).

The occupational lung disease “Shaver’s Disease” results from inhalation of ultrafine bauxite fumes (Gartner, 1952). While this disease is associated with high exposure concentrations for extended periods it does indicate a possible difference of effect of ultrafine particles in that it is characterised by a diffuse interstitial fibrosis. This contrasts with the normal picture of pneumoconiosis induced by inhalation of dusts in which the fibrotic component is generally focal.

6.1.3. Other particles

Many other particles to which occupational exposure can occur, such as carbon black and welding fumes, may also be components of ambient $PM_{2.5}$. The former have been used in animal studies to investigate effects of UF particles in the lung and as a surrogate for the carbon core of diesel particles. Epidemiological investigations have shown limited pulmonary effects resulting from long-term occupational exposure and no adverse cancer risk. Carbon black can also be considered experimentally as a surrogate for the carbon core of particles from other combustion processes. Evidence currently available indicates that the carbon core does not represent a hazard.

There is inadequate information on the potential effects of trace concentrations of other particles that may be present in $PM_{2.5}$. Silica may be a component in some areas but the biological reactivity is governed primarily by the crystalline structure and while there is good occupational data pertaining to lung disease and exposure there is inadequate information regarding any environmental exposure. However, given the potential biological reactivity of some crystalline forms of silica, a possible contribution of silica particles to any effects of ambient $PM_{2.5}$ cannot be dismissed at present in such situations where this mineral may be a constituent of this fraction.

6.1.4. Summary

Information from occupational exposure to particles from diesel exhaust that may compose appreciable proportions of $PM_{2.5}$ indicate that there is no untoward toxicity associated with these and that chemically they do not appear to represent an enhanced risk if present in ambient aerosols.

The studies on fumes provide some evidence for an increased potency of $PM_{2.5}$ and UF particles to induce lung disease by virtue of their size characteristics in comparison with particles of sizes encountered more normally in the occupational environment. If theories derived from animal studies on PTFE fumes are correct, then this potency is related to the state of aggregation of these particles, the singlet state favouring high particle numbers, high pulmonary deposition, rapid translocation into the epithelium, very high surface area for interaction with tissues - all factors considered elsewhere in this report as being of potential importance in the apparent enhancement of pulmonary responses to environmental particles at concentrations not normally considered to represent a potential health problem.

The latter comment may also be applicable to metals. A number of metals, including Fe, Pb, V and Zn, are frequently present at low concentrations in ambient particulates (**Section 3.2**). Many metals are known to be toxic by inhalation from animal studies and the history of occupational exposure to metals, although in the latter case these are now controlled by Exposure Limits to levels generally in excess of those seen with environmental pollutants. Nonetheless, their potential toxicity at relatively low concentrations together with recent investigations that suggest they may have a role in the induction of lung effects (**Section 5.1**) implies that trace amounts may influence particle-lung interactions in humans.

6.2. HUMAN VOLUNTEER STUDIES

Human volunteer studies enable both the subject and the conditions to be carefully controlled, including the test material, atmospheric concentration, particle size, humidity, breathing rate and exposure duration, all potentially variable in uncontrolled conditions. Additionally, the effect on individuals who might be pre-disposed to the effects of the particles can be assessed under full medical supervision within the ethical limits for clinical studies, i.e. exposures must be without potential harm to test subjects, whether healthy or health-compromised.

It must be recognised that several factors limit the utility of human clinical studies. To meet legal and ethical requirements, exposures must be without harm and are typically limited to short-term exposures. The number of individuals tested is generally small and may not be representative of populations at risk, especially since individuals likely to be at greatest risk (i.e., the very young and very old, those with severe obstructive lung disease, or combined heart and lung disease) have not been studied for health and ethical reasons.

The endpoints most commonly measured in human clinical studies are symptoms, pulmonary function and airway responsiveness (i.e. changes in lung function in response to pharmacologic bronchoconstricting agents such as methacholine, carbachol, or histamine) and each subject generally serves as his/her own control to eliminate inter-subject variability.

6.2.1. Studies on insoluble particles

There are limited human volunteer studies on insoluble particulates, the majority of information on the health effects of these particles coming from occupational exposure and epidemiology, with little evidence for exposure to environmental levels of such particles being capable of inducing adverse health effects in normal healthy adults. One volunteer study on inert dusts was that of Andersen *et al*. (1979) who studied nasal mucous flow, airway resistance and subjective responses in 16 young healthy subjects during 5-hour exposures in an environmental chamber to 2, 10 or 25 mg/m^3 inert dust (Rank Xerox toner). The $PM_{2.5}$ fraction of the generated atmospheres was less than 1% particles making this of limited application to $PM_{2.5}$ or UF particle effects. However, it is interesting to note that discomfort was proportional to the dust concentration and this might also be expected with fine particles. Additionally, slight bronchoconstriction occurred and persisted during the following day. Due to the large size of the aerosols tested the relevance of the latter result for $PM_{2.5}$ particles is unclear but it cannot be excluded that high concentrations of inert but fine particles may have some minor effect on lung physiological function in healthy humans with potential for increased effects in health-impaired individuals. Information that might be seen to support this comes from Green *et al*. (1989) who exposed normal subjects to air, formaldehyde, carbon black particles and mixtures of the latter two. Carbon black (of $PM_{2.5}$ size range) induced changes on occasion in FVC and FEV_3 during a 2-hour exposure to a concentration of 0.5 mg/m^3. An effect on peak flow was seen subsequent to the exposure. In contrast, albeit at a concentration of 0.25 mg/m^3, Andersen *et al*. (1992) found no more than equivocal effects of exposure of human subjects (healthy and asthmatic) to carbon black on lung physiological measurements.

6.2.2. Acidic aerosols studies

In contrast with insoluble particles, the ubiquitous presence of soluble acidic aerosols in the environment and continued concerns about their potential adverse effects on health has resulted in a large number of clinical studies designed to investigate the effects of acid aerosols of 2.5 µm MMAD or less in normal or health impaired individuals. In view of this and to review their findings, where adequate numbers exist on specific clinical responses, only the more recent studies have been reviewed and only those considered to contribute to any link between exposure and health effect are considered. Since controlled human studies are of direct importance to establishing any exposure related health effects, experimental parameters of studies conducted over the last ten years and considered here are given in **Table 9**.

Most of the studies considered below have investigated the effect of exposure to acidic aerosols on lung function measurements of FEV_1 and FVC. A clinically significant effect is considered by most occupational health physicians to have occurred where the decrease in either of these parameters was 20% or greater. Anything less may fall within the very broad range for normal humans, even if pre-exposure measurements are being used to provide control parameters for the same individual once exposed. Thus some of the changes reported below may have statistical significance but may not be of clinical significance. Further comment will be made in the summary of the following sections.

6.2.2.1. Pulmonary function studies of sulphuric acid in healthy subjects

Twenty-one healthy subjects, between the ages of 18 and 45 years, were exposed by Avol *et al*. (1988) on separate occasions to air and H_2SO_4 aerosol (particle size 0.85 to 0.91 µm) for 1 hour at each of three concentrations (363, 1128, 1578 µg/m^3). All subjects were exposed to each atmosphere, separated by one week. Three 10-min periods of moderate exercise were included. A slight increase in cough was found at the two highest concentrations of H_2SO_4, but no effects were found on spirometry, specific airway resistance (SR_{aw}), or airway reactivity to methacholine. Frampton *et al*. (1992) exposed 12 healthy non-smokers to aerosols of NaCl (control) or H_2SO_4 (particle size 0.9 µm) at 1175 µg/m^3 for 2 hours in an environmental chamber. Four 10-min exercise periods were included. Mild throat irritation was described by 4 of 12 subjects after acid exposure and 3 of 12 subjects after NaCl exposure. No effects on lung function were found.

A number of other studies (Anderson *et al*., 1992; Koenig *et al*., 1993; Linn *et al*., 1994; Frampton *et al*., 1995) included healthy subjects exposed to H_2SO_4 aerosols at concentrations below 1000 µg/m^3 and none showed meaningful effects on lung function. Anderson *et al*., (1992) studied the responses of 15 healthy subjects exposed for 1 hour in a chamber to air, 100 µg/m^3 H_2SO_4, 200 µg/m^3 carbon black, and carbon black coated with H_2SO_4, (particle size approximately 1 µm). Exposures containing acid were without effects on symptoms, lung function, or airway reactivity. Subjects were more symptomatic and demonstrated greater increases in SR_{aw} after air than after pollutant exposure.

Two studies designed to examine the effects of combined or sequential exposure to acid aerosols and O_3 found no direct effects of exposure to approximately 100 µg/m^3 H_2SO_4 on lung function of healthy subjects, using exposure durations of 3 hours (Frampton *et al*., 1995) or 6.5 hours for two successive days (Linn *et al*., 1994). In addition, Koenig *et al*. (1993) studied eight elderly subjects age 60 to 76 years exposed by mouthpiece to air, H_2SO_4, or ammonium sulphate at approximately 82 µg/m^3 H_2SO_4 for 40 min. No effects were found on spirometry or total respiratory resistance.

These studies demonstrate for young healthy adults, brief exposures to H_2SO_4 at mass concentrations more than an order of magnitude above ambient levels do not alter lung function. Some subjects report increased lower respiratory symptoms, including cough, at 1000 µg/m^3 and higher levels. One study suggests that the elderly do not demonstrate decrements in lung function at low H_2SO_4 exposure levels of approximately 82 µg/m^3.

6.2.2.2. Pulmonary function studies on sulphuric acid in asthmatic subjects

Individuals with asthma often experience bronchoconstriction in response to a variety of stimuli, including exercise, cold dry air, or exposure to smoke and dusts. Considerable individual variability exists in the nature of stimuli that provoke a response, and in the degree of responsiveness. Thus, for clinical studies involving asthmatic subjects, subject selection and sample size are important. Differences among subjects may explain, in part, the widely differing results between laboratories studying effects of acid aerosols. For example, in some studies described below, asthmatic subjects were specifically selected to have exercise-induced bronchoconstriction (Koenig *et al*., 1989, 1992, 1994; Hanley *et al*., 1992), or responsiveness to hypo-osmolar aerosols (Balmes *et al*., 1988). The interval for withholding medications prior to exposure differed among various laboratories and different studies. In addition, the severity of asthma differed among studies; severity

is often difficult to compare because published information describing clinical severity and baseline lung function is often incomplete.

Several studies have suggested that asthmatics are more sensitive than healthy subjects to effects of acid aerosols on lung function. Utell *et al*., (1982) found statistically significant decrements in specific airway conductance (SG_{aw}) in asthmatic subjects exposed by mouthpiece for 16 min to 450 and 1,000 µg/m^3 H_2SO_4 (particle size 0.5 to 1.0 µm). Additionally, exposure to neutralisation products of H_2SO_4 produced smaller decrements in function in proportion to their acidity (H_2SO_4 > NH_4HSO_4> $NaHSO_4$).

Koenig and colleagues studied the responses of adolescents with allergic asthma to H_2SO_4 aerosols with particle sizes in the respirable range, and concentrations only slightly above peak, worst-case ambient levels. In one study (Koenig *et al*., 1983), ten adolescents were exposed to 110 µg/m^3 H_2SO_4 (particle size 0.6 µm) by mouthpiece for 30 min at rest followed by 10 min of exercise. The FEV_1 decreased 8% after exposure to H_2SO_4, and 3% after a similar exposure to NaCl. In another study (Koenig *et al*., 1989), nine allergic adolescents were exposed to 68 µg/m^3 H_2SO_4 (particle size 0.6 µm) for 30 min at rest followed by 10 min of exercise. All subjects had exercise-induced bronchoconstriction. Effects were compared with similar exposures to air, 0.1 ppm SO_2, 68 µg/m^3 H_2SO_4 + 0.1 ppm SO_2, and 0.05 ppm HNO_3. The FEV_1 decreased 6% after exposure to H_2SO_4 alone and 4% after exposure to H_2SO_4 + SO_2, compared to a 2% decrease after air. Increases in total respiratory resistance were not statistically significant. These studies suggest that allergic adolescent asthmatics with exercise-induced bronchoconstriction might be more sensitive to effects of H_2SO_4 than adult asthmatics and that small changes in lung function may be observed at exposure levels below 100 µg/m^3. While the changes reported are relatively small compared with those considered to be of normal clinical significance, the consistency of the findings between studies suggests that they may be of biological significance for people already suffering from a mild form of health impairment such as asthma.

Avol *et al*. (1988) assessed the effects of H_2SO_4 aerosols on 21 adult asthmatics who were exposed to air or approximately 400, 1000, and 1,500 µg/m^3 H_2SO_4 (particle size 0.85 to 0.91 µm) for one hour with intermittent exercise. Healthy subjects used for comparison purposes were described in the previous section. The asthmatic subjects experienced concentration-related increases in lower respiratory symptoms (most notably, cough), with some persistence of symptoms at 24 h. The FEV_1 and FVC showed statistically significant dose-related decrements at the two higher concentrations and SR_{aw} showed a comparable trend. All three showed statistically significant effects of time, function being worse during exposure than pre-exposure, a finding typical of asthmatics that is attributable to exercise-induced bronchoconstriction. These findings are similar to those of Utell, *et al*. (1983a), who found statistically significant effects on SG_{aw} following exposure for 16 minutes at rest to 450 and 1000 µg/m^3 H_2SO_4 and statistically significant effects on FEV_1 at 1,000 µg/m^3 particle size 0.8 µm). Exposures in this case were at rest and for short duration only. As earlier, while the changes reported are relatively small compared with those considered to be of normal clinical significance, the consistency of the findings between studies suggests that they may be of biological significance for people already suffering from a mild form of health impairment such as asthma.

The potential for neutralisation of some of the H^+ in H_2SO_4 aerosols by respiratory tract ammonia and subsequent effect on response to the aerosol is raised in the equivalent animal studies section (**Section 5.2.5**). In recognition of this potential, most of the clinical studies considered already used lemon juice mouth wash or similar acidic treatment to neutralise oral ammonia prior to exposure. Utell *et al*.

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

Avol *et al*. (1990) attempted to replicate the findings of Koenig *et al*. (1989) in adolescent asthmatics using a larger group of subjects. Thirty-two subjects with mild asthma, aged 8 to 16 years, were exposed to 46 and 127 µg/m^3 H_2SO_4 (particle size approximately 0.5 µm) for 30 min at rest followed by 10 min of exercise. Bronchoconstriction occurred after exercise in all atmospheres, with no statistically significant difference between clean air and acid exposures at either concentration. These exposures were conducted in an environmental chamber compared with mouthpiece exposure used in the study by Koenig *et al*. (1989) but subsequent measurements using oral breathing only and a concentration of 134 µg/m^3 H_2SO_4 showed a similar lack of response to the acid aerosol. It is possible that the subjects in the Koenig *et al*. (1989) study, all of whom demonstrated exercise-induced bronchoconstriction during a specific exercise challenge test, represented a more responsive subgroup of adolescent asthmatics. Only 15 of the 32 subjects in the Avol *et al*. (1990) study were known to have exercise-induced bronchoconstriction and in this context results from Hanley *et al*., (1992) suggest that exercise responsiveness is predictive of the potential to demonstrate a response to exposure to H_2SO_4.

Koenig *et al*. (1992) examined the effects of different mouthpiece exposure times to H_2SO_4 (particle size 0.6 µm). Fourteen allergic asthmatic subjects aged 13 to 18, with exercise-induced bronchoconstriction, were exposed to air or 35 and 70 µg/m^3 H_2SO_4 for 45 min and 90 min, on separate occasions. The exposures included alternate 15-min periods of exercise. Decrements in FEV_1 (6%) occurred with the shorter exposure to the lower concentration. Changes following exposure to 70 µg/m^3 and following 90 min exposures were not statistically significant. The authors concluded that duration of exposure did not play a role in the response to H_2SO_4 aerosols. In the absence of a concentration response, the findings at the lower concentration may be due to chance and the study is not considered to demonstrate an effect of H_2SO_4 at these exposure levels.

Anderson *et al*. (1992) included 15 asthmatic adults in a study comparing the effects of exposure for 1 hour to air, 100 µg/m^3 H_2SO_4, 200 µg/m^3 carbon black particles, and acid-coated carbon black (particle size 1.0 µm). Decrements in FEV_1 were observed for all exposures and while analysis of variance for FVC showed a statistically significant interaction of acid, carbon, and time factors the largest decrements occurred with air exposure.

In a study of elderly asthmatics, Koenig *et al*. (1993) exposed nine subjects (60 to 76 years of age) by mouthpiece to air, $(NH_4)_2SO_4$ or 70 µg/m^3 H_2SO_4 (particle size 0.6 µm), with and without lemonade gargle. Exposures were 30 min at rest followed by 10 min of mild exercise. Greater increases in total respiratory resistance occurred following H_2SO_4 without lemonade than following the other atmospheres, with insignificant differences between atmospheres.

In a study which compared the effects of H_2SO_4 exposure in subjects with asthma and COPD, Morrow *et al*. (1994) exposed 17 subjects in an environmental chamber to 90 µg/m^3 H_2SO_4 or NaCl (particle size < 1 µm) for 2 hours with intermittent exercise. Pulmonary function before and after exercise was measured after each of four 10 min exercise periods, and again 24 h after exposure. Decrements in FEV_1 were consistently greater with H_2SO_4, although the difference was statistically significant only following the second exercise period. FEV_1 decreased approximately 18% after H_2SO_4 compared with approximately 14% after NaCl. Reductions in SG_{aw} were statistically significantly different only following the fourth exercise period. No changes were found in symptoms or arterial oxygen saturation, and there were no significant changes in lung function 24 h after exposure.

In summary, a number of studies suggest that while there were no clinically significant effects of inhaled H_2SO_4 aerosols in asthmatic subjects and no consistent pattern of responses, in general they appear to be slightly more sensitive physiologically than healthy subjects to the effects of acid aerosols on lung function, with adolescent asthmatics possibly being more sensitive than adults. However, many subjects tested developed exercise induced asthma and a number of studies indicate that acidic aerosols did not exacerbate this response, raising questions on the relevance of the relatively small increases reported (as compared with clinically significant changes) for some other subjects using similar protocols. It is also worthy to note that where NaCl aerosols were used as a control, these aerosols also caused some decrement in function, in the case of the studies by Morrow *et al*. (1994) to a similar extent to the H_2SO_4. This suggests that asthmatics may respond in a similar manner to a wider range of aerosols than just acidic aerosols.

6.2.2.3. Effects of acid particles on airway responsiveness

Airway responsiveness can be quantitated by measuring changes in expiratory flow or airways resistance in response to inhalation challenge. The challenging agent is typically a non-specific pharmacologic bronchoconstrictor such as methacholine or histamine but other agents include carbamylcholine (carbachol), cold dry air, sulphur dioxide, hypo-osmolar aerosols, or exercise. It is important to note here that while many individuals with airway hyperresponsiveness do not have asthma, virtually all asthmatics have airway hyperresponsiveness, probably reflecting underlying airway inflammation. Changes in clinical status are often accompanied by changes in airway responsiveness. Thus alterations in airway responsiveness may be clinically significant, even in the absence of direct effects on lung function (Godfrey, 1993; Weiss *et al*., 1993).

Utell *et al*. (1983a) observed, in healthy non-smokers, an increase in airway responsiveness to carbachol following exposure to 450 µg/m^3 H_2SO_4 (particle size 0.8 µm). The increase occurred 24 hours, but not immediately, after exposure. In addition, some subjects reported throat irritation between 12 and 24 h after exposure to H_2SO_4. These findings suggested the possibility of delayed effects. These investigators also observed increases in airway responsiveness among asthmatic subjects following exposure to 450 and 1000 µg/m^3, but not 100 µg/m^3 H_2SO_4.

Avol *et al*. (1988) included assessment of airway responsiveness in their studies of healthy and asthmatic subjects exposed to varying concentrations of H_2SO_4. No effects on responsiveness were reported at H_2SO_4 concentrations as high as 2000 µg/m^3. However, airway challenge was performed only at two concentrations which may have been insufficiently effective to detect small changes in airway responsiveness. Using a similar methacholine challenge protocol, Linn *et al*. (1989)

found no change in airway responsiveness of healthy subjects following exposure to 2000 µg/m^3 H_2SO_4 for 1 hour, at particle sizes ranging from 1 to 20 µm. Anderson *et al*. (1992), in their study of responses to 100 µg/m^3 H_2SO_4, 200 µg/m^3 carbon black, and acid coated carbon, found no effects on airway responsiveness in healthy or asthmatic subjects when using a conventional methacholine challenge.

These data suggest that there is little, if any, effect of low concentration acid aerosol exposure on airway responsiveness in healthy or asthmatic subjects.

6.2.2.4. Effects of acid particles on lung clearance mechanisms

Brief exposures to H_2SO_4 aerosols have shown consistent effects on mucociliary clearance. The direction and magnitude of the effect are dependent on the concentration and duration of the exposure, the size of the acid particle, and the size of the tracer particle used for clearance measurements.

A study in healthy non-smokers by Leikauf *et al*. (1981) found that exposure to 110 µg/m^3 H_2SO_4 (particle size approximately 0.5 µm) for 1 hour at rest accelerated bronchial mucociliary clearance of 7.5 µm tracer particles, while a similar exposure to 980 µg/m^3 H_2SO_4 slowed clearance. A second study (Leikauf *et al*., 1984), using a smaller tracer particle (4.2 µm) to assess more peripheral airways, found slowing of clearance with both 108 and 983 µg/m^3 H_2SO_4, in comparison with distilled water aerosol. Spektor *et al*. (1989) extended these studies, exposing, ten healthy subjects to H_2SO_4 (particle size 0.5 µm) or distilled water aerosols for up to 2 hours. Two different tracer administrations were used (tracer particle size 4.2 µm), one before and the other after exposure. Following a 2 hour exposure to 100 µg/m^3 H_2SO_4, clearance half-time tripled compared with control, with reduced clearance rates still evident 3 hours after exposure. These findings suggested that brief, resting exposures to H_2SO_4 at > 100 µg/m^3 accelerate clearance in large bronchi but slow clearance in more peripheral airways in a dose-dependent fashion.

Spektor *et al*. (1985) exposed ten asthmatic subjects to 0, 110, 319, and 911 µg/m^3 H_2SO_4 (particle size 0.5 µm) for 1 hour. Interpretation of the results from the more severe asthmatics was confounded by the non-homogeneous distribution of the tracer aerosol in these subjects but clearance was decreased following the highest concentration of acid exposure in the six subjects with the mildest asthma, responses similar to those of the healthy subjects.

In summary, exposure to acidic aerosols can have effects on lung clearance mechanisms. Whether effects as described above represent adverse effects is unclear but are probably of little implication for healthy humans. However, any prolongation of such effects due to repeated exposure might lead to further reduction in the health status of individuals already health-impaired.

6.2.2.5. Bronchoscopy and airway lavage

Fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) is used frequently in assessment of airway diseases had also been used for sampling the lower airways of humans in clinical studies of oxidant air pollutants. As with animal studies where this technique is used more extensively, the cellular and non-cellular components of the lavage fluid can provide a sensitive measure of inflammation. In addition, the lavaged cells can be assessed subsequently *in vitro* for functional changes important in inflammation and host defence.

Frampton *et al*. (1992) utilised bronchoscopy to evaluate the effects of exposure to acid aerosols. Healthy non-smokers (12 subjects) were exposed to aerosols of NaCl (control) or H_2SO_4 (particle size 0.9 µm) at 1000 µg/m^3 for 2 hours. Four 10-minute exercise periods were included. Fiberoptic bronchoscopy with BAL was performed 18 hours after exposure but revealed no evidence for airway inflammation. Markers for changes in host microbial defence, including lymphocyte subset distribution, antibody-dependent cellular cytotoxicity of alveolar macrophages, and alveolar macrophage inactivation of influenza virus, were not statistically significantly different between H_2SO_4 and NaCl exposures.

Culp *et al*. (1995) determined the composition of mucins recovered during bronchoscopy of the subjects studied by Frampton *et al*. (1992), as well as from some non-exposed subjects. Mucin composition was similar when non-exposed subjects were compared with NaCl-exposed subjects and no differences were found between H_2SO_4 and NaCl exposure. Since bronchoscopy was performed 18 hours after exposure, any transient effects of exposure would not have been detected.

In summary, the very limited amount of information available shows no indication of induction of pulmonary irritancy following single exposures to 1000 µg/m^3 H_2SO_4 or NaCl aerosols.

6.2.3. Summary

A range of studies have investigated the potential of acidic aerosols to affect the normal physiological functioning of the lungs in both healthy and asthmatic subjects. Concentrations ranged from 46-1500 µg/m^3. There is no evidence for a clinically significant effect (considered to be greater than a 20% reduction from the normal) on lung function on both these subject groups. Some studies suggest that asthmatic subjects may be slightly more sensitive physiologically than healthy subjects to the effects of acid aerosols on lung function, with adolescent asthmatics possibly being more sensitive than adults. However, many subjects tested developed exercise induced asthma and a number of studies indicate that acidic aerosols did not exacerbate this response, raising questions on the relevance of the relatively small increases reported. It is also worthy to note that where NaCl aerosols were used as a control, these aerosols also caused some decrement in function, in the case of the studies by Morrow *et al*. (1994) to a similar extent to the H_2SO_4. This suggests that asthmatics may respond in a similar manner to a wider range of aerosols than just acidic aerosols.

While the relevance of the positive responses reported above is questionable with regard to any impairment of health, it is apparent that there is no effect of concern in normal healthy individuals at concentrations as high as 1000 µg/m^3. Effects that may have biological significance may occur at concentrations below 100 µg/m^3 in the most sensitive asthmatic individuals.

There is little, if any, effect of low concentration acid aerosol exposure (100 µg/m^3) on airway responsiveness in healthy or asthmatic subjects.

Exposure to acidic aerosols can have effects on lung clearance mechanisms. Whether effects as described above represent adverse effects is unclear but are probably of little implication for healthy humans. However, any prolongation of such effects due to repeated exposure might lead to further reduction in the health status of individuals already health-impaired.

The very limited amount of information available to date shows no indication of induction of pulmonary irritancy, as indicated by lack of change in the free cell population of the lung, following single exposures to 1000 µg/m^3 H_2SO_4 or NaCl aerosols.

6.3. STUDIES ON MIXED ATMOSPHERES

Exposure to environmental aerosols, especially during pollutant episodes, is rarely to one particulate type and invariably involves co-exposure to gaseous pollutants such as SO_2 and O_3. Many epidemiological studies have attempted to assess the relevant contributions of the particulate and gaseous phases to the reported health effects and have frequently indicated that both may have contributed. To test these considerations a number of controlled human studies have been conducted using exposure to individual gaseous or particulate components and results compared with exposure to the mixture.

Anderson *et al*. (1992) found no effects on lung function following exposure to 200 µg/m^3 carbon black alone, or carbon particles coated with H_2SO_4. Aris *et al*. (1990) found no effects on airways resistance of exposure to mixtures of hydroxymethanesulphonic acid and H_2SO_4. Koenig *et al*. (1989) found that exposure of adolescent asthmatic subjects to 68 µg/m^3 H_2SO_4, with 0.1 ppm SO_2 did not increase the responses seen with H_2SO_4 alone.

In contrast, Linn *et al*. (1994) suggest that exposure to O_3 with H_2SO_4 may enhance the increase in airway responsiveness seen with O_3 exposure alone. Fifteen healthy and 30 asthmatic subjects were exposed to air, 0.12 ppm O_3, 100 µg/m^3 H_2SO_4 (particle size approximately 0.5 µm), and O_3 + H_2SO_4, for 6.5 hours on 2 successive days, with intermittent exercise. Airway responsiveness was measured after each exposure day and compared with baseline measured on a separate day. Exposure to H_2SO_4 alone caused no statistically significant changes in lung function, symptoms, or bronchial reactivity relative to clean air. Exposure to O_3 alone or O_3 + H_2SO_4 caused a progressive, statistically significant ($p < 0.05$) decline in forced expiratory function, smaller on the second day than the first. Bronchial reactivity increased after exposure to O_3 with or without H_2SO_4. Changes in mean lung function and bronchial reactivity with O_3 + H_2SO_4 exposure were modestly larger than changes with O_3 exposure, but the differences were statistically non-significant or marginally significant. A minority of individual asthmatic and non-asthmatic subjects showed substantially greater declines in function with exposure to O_3 + H_2SO_4 relative to O_3 alone. Repeat exposure studies of these subjects after several months again showed an excess response to O_3 + H_2SO_4 on average, but there was no appreciable correlation between the excess responses of individual subjects in the original and repeat studies. The authors concluded that for typical healthy or asthmatic adults heavily exposed to acid summer haze, O_3 is more important than H_2SO_4 as a cause of short-term respiratory irritant effects but it is apparent that H_2SO_4 may enhance somewhat the effects of O_3.

Koenig *et al*. (1994) exposed adolescent asthmatic subjects to air, 0.12 ppm O_3 + 0.3 ppm NO_2, O_3 +NO_2 + 73 µg/m^3 H_2SO_4 (particle size 0.6 µm), and O_3 + NO_2 + 0.05 ppm HNO_3. Exposures were by mouthpiece for 90 min, with intermittent exercise, on two consecutive days. Airway responsiveness was measured by methacholine challenge at screening and on the day following the second exposure. No effects on airway responsiveness were found for any atmosphere. However, challenge following exposure utilised only doses of methacholine well below the level causing statistically significant reductions in FEV_1 for these subjects at

baseline, making it unlikely that small or transient changes in responsiveness would be detected.

Frampton *et al*. (1995) exposed 30 healthy and 30 asthmatic subjects to 100 µg/m^3 H_2SO_4 (particle size 0.64 µm) or NaCl (particle size 0.45 µm) for 3 hours followed the next day by exposure to 0.08, 0.12, or 0.18 ppm O_3 for 3 hours. All exposures included intermittent exercise. Each subject received two of the three O_3 exposure levels. Exposure to H_2SO_4 or NaCl did not alter lung functions. Changes in spirometry following exposure to O_3 were small, consistent with the relatively low concentrations, short exposure duration, and moderate exercise levels. Changes in FVC 4 hours after O_3 exposure were similar to those found immediately after exposure. With H_2SO_4 pre-exposure, FVC decreased following O_3 in a concentration-response fashion. There was evidence of an interaction between aerosol and O_3 exposure for effects on FEV_1 and FVC among the asthmatic subjects but not the healthy subjects. The authors concluded that, for asthmatic subjects, H_2SO_4 alters the response to O_3 in comparison with NaCl pre-exposure.

In summary, the above results suggest that co-exposure of acidic aerosols and insoluble inert particles does not result in exacerbation of any effects. In contrast, studies in which exposures include both acidic aerosol and irritant/toxic gases which are also potential ambient air pollutants suggest that H_2SO_4 aerosol exposure may enhance airway responsiveness to the gaseous component.

7. THE EPIDEMIOLOGY OF ULTRAFINE PARTICLES

7.1. THE EPIDEMIOLOGICAL APPROACH

This section is concerned with patterns of ill-health observable in groups of people exposed to $PM_{2.5}$. In some of the reported studies, ill-health in different groups of people exposed to different levels of $PM_{2.5}$ has been compared. In other studies, ill-health in a single group of people has been compared at times of relatively high exposure and times of lower exposure. In either case, the aim was to establish the presence or absence of a statistical association between ill-health and $PM_{2.5}$, after allowing for other components of pollution, and other factors such as the weather and smoking habits.

Epidemiological studies can be sub-divided into two categories. Those in the first category are only considered to be capable of generating hypothesis for further research (Esteve *et al*., 1994). Studies in the second category are considered to be capable of testing hypotheses generated by epidemiological or toxicological evidence.

Previous sections of this report have indicated that some relationships between ill-health and $PM_{2.5}$ are more biologically plausible than others. In particular, children, the aged, and those with impaired respiratory health can be considered to be most at risk, while acidity and a synergistic relationship with ozone can be expected to be important aspects of exposure.

In the event, published epidemiological studies have, to date, failed to build upon biological plausibility by using the hypothesis testing techniques. As will be shown, all the studies tend only to be capable of generating hypotheses from broadly based objectives, rather than testing specific, targeted hypotheses. Indeed, several of the hypotheses - generating studies concerned with $PM_{2.5}$ are no more than re-analyses of studies previously used to generate hypotheses about PM_{10}. This failure to use hypothesis-testing approaches is the principal reason why the debate about the ill-health effects of exposure to $PM_{2.5}$, including ultrafine particles, is unresolved.

7.2. THE HEALTH EFFECTS OF HIGH CONCENTRATIONS OF POLLUTION

An assessment of the epidemiology of $PM_{2.5}$ must necessarily begin by reviewing the epidemiology of air pollution in general and ambient air pollution post Clean Air Legislation. The relevant questions are:

- Has air pollution caused ill-health?
- Is ambient air pollution associated with ill-health?
- Does ambient air pollution cause ill-health?

7.2.1. Has air pollution caused ill-health?

The answer to this question is undoubtedly – yes. In extreme conditions, such as the London smog of 1952, there is no argument that there is a causal relationship between air pollution and measures of ill-health, including mortality (Godlee *et al*., 1991).

7.2.2. Is ambient air pollution associated with ill-health?

The answer to this question is also – yes. At ambient levels of pollution there is a substantial body of epidemiological evidence of statistical association between air pollution and ill-health, as measured by a variety of parameters.

These measures of ill-health include:

- acute mortality (e.g. Schwartz *et al*., 1996)
- chronic mortality (e.g. Lloyd *et al*., 1985)
- hospitalisation (e.g. Thurston *et al*., 1994)
- symptoms (e.g. wheeze) (e.g. Dockery *et al*., 1996)
- physical measurements (e.g. lung function) (e.g. Peters *et al*., 1997)

and this evidence has accumulated from a variety of study designs, principally:

- ecological studies (e.g. Lloyd *et al*., 1985)
- time series analyses (e.g. Schwartz *et al*., 1996)
- hybrid studies (cohort studies with ecological variables) (e.g. Pope *et al*., 1995)

7.2.3. Does ambient air pollution cause ill-health?

7.2.3.1. Study design

A major difficulty in assessing the evidence is that the designs used do not fit easily into a classical, text-book categorisation of epidemiological study designs.

A simple dichotomy of study designs is that they are either analytical or descriptive.

In an analytical study, an estimate of exposure is available for each individual, either from direct measurement, or from some appropriate surrogate information, such as job in an occupational study. The relevant measure of ill-health is also recorded at the individual level, as may be measurements of other factors that need to be taken into account such as smoking history.

The gold standard for analytical studies is set by the prospective cohort study (Breslow and Day, 1987), closely followed by the retrospective cohort study and the case-control (or case-referent) study (Breslow and Day, 1980). These studies are considered to be 'hypothesis testing' studies, capable of tackling such questions as 'Is $PM_{2.5}$ associated with acute respiratory hospitalisation?' A weaker type of analytical study is the cross-sectional study with individual data. It is weaker because it has no longitudinal element, only studying a problem at one point in time. It can tackle weaker hypotheses such as 'Is $PM_{2.5}$ associated with today's prevalence of respiratory symptoms?'

In descriptive studies, estimates of exposure are only available at the group level. It is assumed that the exposure of each individual in the same group, neighbourhood, city or country has the same exposure. This group exposure data can be correlated with ill-health data collected at the group level and, perhaps the correlations can be modified by allowing for factors such as smoking, also collected at the group level.

The simplest of descriptive studies in which, for example, cancer rates in several countries are related to an index of industrialisation in those countries, are at the opposite end of the spectrum from the gold standard. Descriptive studies are considered to be 'hypothesis generating' studies, only capable of tackling such questions as 'Are there any statistical associations between measures of air pollution and acute hospitalisation rates that are worth further study?

In this dichotomy, ecological studies, time-series analyses, and hybrid studies are all descriptive studies.

In practice, however, the dichotomy is less rigid. An elaborate, well-conducted descriptive study can have more scientific value than a poorly conceived analytical study.

The study designs employed in environmental epidemiology make efforts of varying degrees of sophistication to bridge this gap between descriptive and analytical studies.

In an ecological study, (also referred to in the literature as a cross-sectional study (of groups), the exposure data, ill-health data and data for other factors are all available at the group level. The analysis uses regression techniques. The number of groups may be quite small. Graphical methods are often the most convincing presentations of the data.

In a time series study the measurements are again all at the group level. The difference from an ecological study is that the exposure and ill-health data are collected on a daily rather that an annual or seasonal basis. The quantity of data is therefore much greater and the analysis is within groups and much more complex.

In a hybrid study many of the advantages of the analytical approach are gained by studying a cohort of individuals for whom ill-health data and measurements of other factors are recorded, but exposure is still only available at the group level.

This discussion is illustrated in **Figure 7** in an attempt to demonstrate the difficulty of assessing the value of the reported environmental epidemiological studies.

7.2.3.2. Strength of association

In his classic discussion of association and causation Bradford Hill (1971) lists strength of association as his first criterion.

Most of the relative risks reported in ambient air pollution studies are < 1.50 and many are < 1.15 (Moolgavkar and Luebeck., 1996, McClellan and Miller., 1997). It is difficult to convince anyone accustomed to cohort and case- referent studies that they should regard these results as strong evidence because they would rarely be statistically significant or of any concern in occupational studies.

Nonetheless, they are relative risks of considerable importance to public health because they apply to large populations. Indeed they are alarming if they can be extrapolated to occupational groups, and they could hardly be much larger without causing a major breakdown in public health standards.

Weak evidence is not unconvincing simply because it is weak, it is unconvincing because:

- it may be due to confounding factors
- it may be an outcome of the chosen statistical analysis
- it may be extracted from highly correlated variables
- it may be due to study design

These factors affect almost all epidemiological studies, but they do not usually invalidate conclusions when the evidence is strong. Their impact when the evidence is weak is difficult to assess, but it is usually sufficient to justify a recommendation of caution when interpreting the results.

A **confounding factor** is a factor related both to exposure and ill-health. These relationships do not need to be logical, they only need to be statistical. For example, a study of lung cancer in two groups of workers will be confounded by smoking if one group just happens to contain a higher proportion of smokers. Confounding is a problem in environmental epidemiology because an ill-health effect attributed to air pollution may be due to a confounder. Many of these reports do not quote the crude relative risk for air pollution before correcting for confounders, but there must be a suspicion that if a relative risk of, for example, 2 or 3 is reduced to one of perhaps 1.15, then the residual relative risk could be the result of imperfect consideration of confounding factors or due to unforeseen factors.

For example, in a cohort mortality study of nickel/chromium platers, Sorahan *et al*., (1987) reported a statistically significant relative risk of 1.86 for stomach cancer in male workers. When they tested for an exposure gradient within the cohort, using the technique of regression models in life tables, they found no evidence of an association between exposure and stomach cancer and they attributed the observed excess risk to confounding factors of social class differences, quoting that there is a steep positive social class gradient for mortalities from all cancers, cancer of the stomach and cancers of the respiratory system.

Many confounding factors have been described in air pollution studies and either allowed for or ignored. They include smoking history, use of a gas fire, body mass index, peak daytime temperature, day of the week, and previous respiratory health history. There is a clear potential for the reported relative risks of 1.05-1.15 to be due to residual confounding. The most difficult confounder to allow for is the multi-dimensional variable known as social deprivation. This is known to be associated with both air pollution and ill-health, but there is no simple way of giving it a numerical value, and thereby analysing for it. The weather is another complex confounding factor and it does not appear to have a simple relationship with ill-health.

The possible impact of confounding on the validity of results varies from one study design to another. In ecological studies, the danger that an excess risk attributed to ambient pollution is really due to a confounder is most extreme. Often the only confounding factors included in the analysis are those associated with the weather, such as temperature. Group data for such factors as smoking and general health are sometimes included but their validity is difficult to justify.

In time series studies, the use of a group as its own control obviates the need to analyse for individual data, such as smoking and education level. The confounders

of importance in these studies are the weather and the season of the year. Whilst it is common practice to include these in the analyses, their complex effects on health patterns, and their high correlations with air pollution, mean that their effects on ill health may not be modelled sufficiently precisely to exclude a residual effect.

Hybrid studies present the best opportunity to overcome the threat of residual confounding. In these studies it is possible to allow for a host of factors at the individual level such as smoking, passive smoking, time spent indoors, occupational dust exposure, childhood illness, education level, alcohol consumption and weight. In these studies the criticism that the exposure data is group data rather than individual data is more justifiable than criticism on grounds of residual confounding.

The **statistical analyses** employed in these studies are not well established particularly in the case of time-series studies.

Compared to the calculation of a standardised mortality ratio in a cohort study, or an odds-ratio in a case-control or cross-sectional study, the techniques used in time-series studies are highly complex.

They can involve:

- auto-correlated series (in which one observation of pollution or ill-health is related to the previous ones and the following ones)
- long-term trends (in pollution and/or ill-health)
- short-term peaks (due to pollution incidents or ill-health epidemics)
- non-linear dose-response curves (only to be analysed by non-parametric methods such as splines)
- use of indicator variables (e.g. for day of week, smoking status)
- regression with highly correlated variables (e.g. TSP and PM_{10})

Suitable techniques are available for all these problems, but their use collectively, or in sequence, to estimate effect parameters is open to argument.

Doubts about the validity of the analysis of time series studies is based on unease rather than any specific areas of criticism. The methodology for retrospective cohort studies was first published by Case *et al*., (1954). The analyses for case-referent studies was published by Mantel and Haenszel., (1959). Thus there have been four decades in which to refine the methods and investigate what can go wrong. Time series studies have only reached the literature in the 1990's following publication of suitable methodology in 1986.

The estimation of coefficients of effect for **highly correlated variables** is a well-known problem in all fields of statistical endeavour. Given that many of the components of air pollution are highly correlated with one another, and with many social deprivation variables, how can the most likely 'real' effects be determined. Should one choose:

- those with the most statistical significance
- those with the biggest effects on ill-health
- those with the least measurement error
- those with the largest elasticity (as claimed in one publication)

or even

- those with the greatest biological plausibility.

Indeed, these choices are not independent of one another. It is quite possible that the most statistically significant component of pollution will also be the one with the least measurement error, simply because it is the one with the least measurement error.

In an ideal world (e.g. a laboratory) one would study populations in which the correlations were much lower or absent.

The concern about **study design** is that the designs employed are all descriptive. In the absence of individual exposure data, there is no evidence that individuals with the higher exposures are the individuals suffering the reported ill health.

In theory it is quite possible for a study of ambient pollution and ill health based on group data to reach a fallacious outcome. If the individuals experiencing ill health in the area of high pollution, and those experiencing ill health in the area of low pollution, are actually identified, it may be found that ambient pollution can be demonstrated not to be the causative factor.

7.2.3.3. Consistency

The second of Bradford Hill's criteria for causality is consistency. He proposes that an effect is more likely to be causal if it is observed repeatedly by different investigators, in different places, in different circumstances and times.

It can be argued that the evidence for the ill health effects of ambient pollution scores very highly on grounds of consistency. Several groups of investigators, in several countries using at least three different study designs have arrived at positive conclusions. These conclusions relate to a wide range of health measures ranging from exacerbation of respiratory symptoms in susceptible subjects to premature death from respiratory or cardiovascular disease in the population at large.

Not only are the results consistent in outcome, they are also consistent in their estimates of excess risk. Relative risks for mortality generally show that about 5% of deaths in highly polluted areas are attributable to the pollution, while relative risks for non-lethal outcomes are generally rather larger.

It can be argued that these consistent results must be demonstrating a real, causative, effect because it is not conceivable that all the studies are subject to the same flaw of design, bias or confounding.

On the other hand it can be argued that the results are in fact too consistent and that the results are very inconsistent in terms of specific risk factors. Consistency in this area of research is not a novelty. Mapping exercises in various parts of the world have consistently demonstrated large associations between industrialisation / population density / poverty / social class mix etc. and death from all causes / cancer / cardiovascular disease. As ambient pollution is related to industrialisation etc., it is no surprise that studies show a relation between pollution and ill health however hard the investigators try to exclude other aspects of industrialisation etc. It also appears that aspects of design and analysis could lead to the variety of specific pollutants found to be harmful, rather a complex mix of causative relationships which depend upon the circumstances of the study.

To summarise at this stage:

- Extreme levels of air pollution have caused ill-health, including death.
- Ambient air pollution is statistically associated with ill-health
- There may be a weak causal relationship between ambient air pollution and ill-health, but it is far from being proven.

7.3. CONSTITUENTS OF AIR POLLUTION

To take the debate about air pollution in general forward to a debate about components of air pollution it can be argued that the specific components of air pollution that are associated with ill-health have not been identified.

This is mainly because:

- studies have not included all components
- analyses have not discriminated among components
- confounders have not been adequately allowed for

The first objection is transparently obvious. If several components of air pollution are statistically associated with ill-health, but only one is included in a study, then that one will be reported as associated with ill-health, while the remainder will be in the category of unmeasured confounders. The other two objections have been discussed above.

Therefore further studies are needed in which

- all suspect components are considered
- the influence of confounders is minimised

and, if possible

- more valid designs are used
- more discriminatory analyses are used

Any assessment of $PM_{2.5}$ epidemiology must build upon the arguments in the ensuing **Section 7.4** and assess the extent to which the requirements in this section have been met in recent publications.

It is patently obvious that if the role of ambient air pollution in relation to ill-health is unclear, then the role of coarse particles must be more unclear and the role of small particles even less clear unless recent studies have incorporated major advances in study design, measurement of confounders and statistical analyses.

7.4. RECENT PUBLICATIONS

Recent publications relevant to the small particulate issue have been critically assessed. The summaries below concentrate on the few, vital, elements of each study listed in **Section 7.5**. They do not consider other aspects that may, from a different perspective, be considered to invalidate the study. For example, the summaries do not consider:

- whether the environmental measurements are appropriate
- whether the environmental measurements are accurate
- whether the populations are representative
- whether the ill-health criteria are precisely defined
- whether the data collection processes are unbiased

Each study can be criticised on these grounds and many more, but these criticisms are deemed to be of secondary importance to the issue at stake.

The key questions asked of each report are simply:

(i) Is the study design suitable for reaching detailed conclusions?

(ii) Have all suspect components of pollution been considered?

(iii) Have all relevant confounders been allowed for?

(iv) Have effect estimates allowed for correlations?

and assuming all other aspects of the study are at least adequate,

(v) What contribution does the study make to the debate about $PM_{2.5}$?

7.5. RELEVANT REPORTS

7.5.1. Mortality

A Schwartz *et al*. (1996)

- The study is a time series analysis of daily mortality in six eastern U.S cities (Boston, MA; Knoxville, TN; St Louis, MO; Steubenville, OH; Portage, WI and Topeka, KS) between 1976 and 1987. (It is a re-working of the Harvard 6 cities study).
- The combined base population for all six cities is 5.9 million.
- Some but not all suspect components are included, namely, $PM_{2.5}$, TSP, SO_4 and H^+ but not O_3.
- The confounding effect of the weather is allowed for, social confounders are ignored because of the time series design.
- Components were considered singly in 6 cities separately and 6 cities combined. Comparison was principally by size effect measured from 5^{th} to 95^{th} percentile of exposure. $PM_{2.5}$ found to be the biggest contributor to daily mortality.

❖ A descriptive study showing a consistent, weak, effect of $PM_{2.5}$ across 6 cities, with some indication that $PM_{2.5}$ is the most likely harmful component.

B Dockery *et al*. (1993)

- The study is a hybrid cohort study with ecological variables of mortality in 8111 white subjects aged 25-74 in six eastern U.S cities (Boston, MA; Knoxville, TN; St Louis, MO; Steubenville, OH; Portage, WI and Topeka, KS)

between 1975 and 1991. (It is another re-working of the Harvard 6 cities study).

- All relevant pollutants considered, namely, $PM_{2.5}$, TSP, SO_4, H^+, SO_2, O_3, NO_2.
- Many social confounders were allowed for including smoking, educational level, body mass index, measured in the cohort study.
- City specific mortality rates were calculated allowing for cohort factors. These rates were related to pollution levels for the 6 cities. Pollutants were considered separately and compared for effect across the range of pollution TSP, $PM_{2.5}$ and SO_4 had similar effects.

❖ A descriptive study showing a $PM_{2.5}$ effect but not able to discriminate between three competing components. The ecological analysis between cities is not a convincing form of analysis.

C Pope *et al*., (1995)

- A hybrid cohort study with ecological variables of mortality in 552,138 persons aged at least 30, in 50 American states, the district of Columbia and Puerto Rico, between September 1982 and December 1989.
- Only SO_4 and $PM_{2.5}$ were measured.
- Many confounders were allowed for including race, smoking, body mass index, alcohol, education, occupation and ambient temperature.
- SO_4 and $PM_{2.5}$ were analysed separately, SO_4 related to cardiopulmonary and lung cancer mortality, $PM_{2.5}$ related to cardiopulmonary mortality. An ecological analysis gave similar results.

❖ A hybrid cohort study showing an association between $PM_{2.5}$ and cardiopulmonary mortality but not discriminating between $PM_{2.5}$ and SO_4, and not considering other pollutants.

D Dockery *et al*., (1992)

This report was superseded by A above. In this smaller version of A, however, TSP was found to be of greater importance than $PM_{2.5}$. Acidity was found to be of marginal importance.

7.5.1.1. Summary of mortality studies

These three studies of mortality provide limited evidence of association between $PM_{2.5}$ levels and mortality. The evidence from the ecological cohort studies is not convincing because these studies do little more than recapitulate the well-known fact that mortality in industrialised regions is higher than that in non-industrialised ones. The reason is known to be multifactorial and it is also well-known that separating the effects of the various factors is an unsolved problem. The time series study uses such a complex analytical process that the validity of its findings is open to argument.

Studies that can evaluate which individuals are affected by exposure, and what their individual exposure is likely to have been, are needed if these associations between $PM_{2.5}$ and mortality are to be given credence.

7.5.2. Hospitalisation

E Thurston *et al*., (1994)

- The study is a time-series analysis of respiratory admissions to 22 acute care hospitals in Toronto, Ontario, Canada (base population 2.4 million), for six week periods during July and August of 1986, 1987 and 1988.
- All relevant components of pollution were measured, namely, $PM_{2.5}$, TSP, PM_{10}, SO_4, H^+, SO_2, NO_2 and O_3.
- The confounding effect of ambient temperature was allowed for, the time-series approach allowed for social confounders.
- A sequential approach was adopted to variable selection. Many components were statistically significant on their own, but O_3 and H^+ were selected in competing analyses.

❖ A time-series study of hospital admissions showing a statistically significant effect of $PM_{2.5}$ that disappeared when considered in conjunction with O_3 and H^+.

F Burnett *et al*., (1997)

- Time-series analysis of cardio-respiratory hospitalisation in Toronto, Ontario, Canada (base population 2.4 million) during the summers (May-September) of 1992, 1993 and 1994.
- All relevant components of pollution were measured, namely, $PM_{2.5}$, TSP, PM_{10}, SO_4, H^+, SO_2, NO_2, CO, O_3 and COH.
- Allowance made for the confounding effect of weather.
- Considered singly COH and O_3 were most important judged by statistical significance. $PM_{2.5}$ statistically significant on its own but its effect explained by gaseous components.

❖ A time-series analysis in which $PM_{2.5}$ had little effect on cardio-respiratory hospitalisation after allowing for gaseous components.

G Delfino *et al*., (1997)

- Times-series analysis of respiratory admissions to 25 hospitals in Montreal, Canada (base population 3 million) between June-September 1992 and 1993. A control group consisted of psychiatric and gastrointestinal illnesses.
- Most pollutants measured including $PM_{2.5}$, PM_{10}, O_3, SO_4 and H^+.
- Allowance made for the confounding effect of weather.
- Summer 1992 and Summer 1993 analysed separately. Effects only found in young and old. Comparisons between pollutants inconclusive based on separate analyses .

❖ A time-series analysis showing a statistically significant effect of $PM_{2.5}$ on respiratory hospitalisation, but only in the elderly and not distinguishable from other particulate effects.

7.5.2.1. Summary of hospitalisation studies

These time series studies of hospitalisation use complex analytical techniques that are difficult to evaluate. In any case any associations between air pollution and hospitalisation have not been demonstrated to be due to $PM_{2.5}$.

7.5.3. Lung function measurements

H Neas *et al*., (1995)

- A longitudinal cohort study of peak expiratory flow rate in 85 children in Uniontown, Pennsylvania during the summer of 1990.

❖ This study is not large enough and does not have a sufficient participation rate to merit critical appraisal.

I Peters *et al*., (1997)

- Time-series study of peak expiratory flow in 89 asthmatic children in Sokolov, Czech Republic during the winter of 1991-1992.
- Most relevant pollutants measured.
- Confounding effect of weather allowed for.
- Pollutants only considered singly. SO_4 was the strongest predictor as measured by statistical significance.

❖ A small respiratory function study of a susceptible population. No effect of $PM_{2.5}$ reported.

J Raizenne *et al*., (1996) (companion paper to L)

- A cross sectional study of pulmonary function in 10,251 children living in 24 communities in the United States and Canada between 1988-1991.
- Most relevant pollutants considered. The study targeted at FPSA.
- Social factors related to parents considered individually. No attempt made to test $PM_{2.5}$ with other pollutants.

❖ A cross-sectional study analysed by ecological methods. No reason to regard $PM_{2.5}$ as an independent adverse component of pollution.

7.5.3.1. Summary of lung function studies

These studies do not show a relationship between lung function and $PM_{2.5}$.

7.5.4. Respiratory symptoms

K Schwartz *et al*., (1994)

- The study is a hybrid cohort and time-series analysis of respiratory symptoms in 1,844 children in six eastern U.S cities (Boston, MA; Knoxville, TN; St Louis, MO; Steubenville, OH; Portage, WI and Topeka, KS). (It is another re-

working of the Harvard 6 cities study). Symptoms were recorded for 1 year for each city between 1984-1988.

- All relevant components of pollution were considered, namely, $PM_{2.5}$, TSP, SO_4, H^+, SO_2, NO_2 and O_3.
- Many confounders were allowed for. The weather was part of the time-series analysis and indoor air pollution, gas stoves and parental smoking were included in the cohort data. The analysis had to be confined to non-winter months because of infectious diseases in winter.
- Pollutants considered singly and those showing statistically significant results were then considered together. TSP and O_3 were related to cough, TSP to Lower Respiratory Symptoms, TSP somewhat related to Upper Respiratory Symptoms.

❖ A descriptive study showing no statistically significant effect of $PM_{2.5}$ on respiratory symptoms in children.

L Dockery *et al*., (1996)

- A cross-sectional, ecological study of respiratory symptoms in 13,369 white children aged 8-12 years from 24 communities in the United States and Canada between 1988-1991.
- Most relevant components of pollution were included. The study was targeted at FPSA.
- Social factors such as parental smoking, education, parental history of asthma were allowed for.
- Pollutants were considered singly. H^+ was related to bronchitis as was SO_4.

❖ A cross-sectional study analysed by ecological methods. No effect of $PM_{2.5}$ was found.

M Abbey *et* al., (1995a)

- A cross-sectional, ecological study of chronic respiratory symptoms in 1,868 non-smoking Seventh-Day Adventists living in the vicinity of nine California airports between 1977-1987. Each participant had resided since 1966 and was over 25 years of age in 1974.
- $PM_{2.5}$ estimated from airport visibility data.
- Airway obstructive disease (AOD), bronchitis and asthma analysed along with covariates of age, education, gender and previous symptoms.
- $PM_{2.5}$ considered singly and then checked for surrogate relationships with TSP, PM_{10}, O_3, SO_4 and average visibility. $PM_{2.5}$ showed relationship to development of new cases of chronic bronchitis but not AOD or asthma. Changes in severity of these latter two parameters were related more to other pollutants than $PM_{2.5}$.

❖ A cross-sectional study analysed by ecological methods. A relationship between $PM_{2.5}$ and development of chronic bronchitis was proposed.

7.5.4.1. Summary of respiratory symptoms studies

These studies do not demonstrate any convincing relationship between respiratory symptoms and $PM_{2.5}$, although they do suggest that acidity is an important aspect of air pollution as far as respiratory symptoms are concerned.

7.5.5. Summary of relevant reports

None of the studies meets all the requirements for a study aimed at demonstrating causation.

(i) The designs are not suited to testing hypotheses;

(ii) Not all aspects of air pollution have been included;

(iii) Not all possible confounders have been allowed for;

(iv) Correlations have not been adequately allowed for;

(v) The studies have not targeted $PM_{2.5}$.

The recent reports relevant to fine particulate air pollution A-M, show:

- No effect of fine particles on respiratory symptoms.
- No effect on pulmonary function or hospitalisation, or no effect that cannot be attributed to other components of pollution.
- A consistent effect of fine particles on daily mortality, but an effect for which there are competing explanations.

The reported effect that fine particulate pollution has on daily mortality is not convincing because:

- It is based on descriptive studies. There is no indication of new thinking having been brought to bear on the issue, many reports are re-analysis of old studies.
- There is not yet a consistent approach to the collection of environmental data,
- A wide range of confounders have been shown to be important. The residual relative risk could be due to residual confounding.
- ❖ There is no effective way of selecting the harmful component (if any) from a set of highly correlated alternatives.

7.6. SUMMARY

- There is limited evidence of a weak association between exposure to ambient fine particulate air pollution and acute and chronic mortality.
- All arguments that there is no causal link between coarse particulates and ill-health apply with greater weight to $PM_{2.5}$.

The following papers are concerned with the epidemiology of air pollution but they do not contribute to a discussion of $PM_{2.5}$.

Choudhury *et al.* (1997)

Hefflin *et al.* (1994)

Gordian *et al.* (1996)

Moolgavkar *et al.* (1997)

Lipsett *et al.* (1997)

Koenig *et al.* (1989)

Ware *et al.* (1986)

Gielen *et al.* (1997)

Nicolai (1997)

Timonen and Pekkanen (1997)

Braun-Fahrlander *et al.* (1997)

Styer *et al.* (1995)

Abbey *et al.* (1995b)

Abbey *et al.* (1995c)

8. DISCUSSION AND CONCLUSIONS

8.1. AIM OF THIS REPORT

The aim of this report has been to review the available data on the health effects of $PM_{2.5}$ including ultrafine particles. This fraction of environmental particles is considered highly respirable to man and when inhaled will readily penetrate to and deposit within the alveolar regions of the lung. The current perception is that such particles in the environment, especially during episodes of high urban pollutant levels, are responsible for a number of health problems in a proportion of the general population.

A criticism that is expressed by many people is the apparent lack of correlation between the levels supposedly associated with such effects and the perceived no effect levels of many particulates in workplace exposures or those tested in clinical studies or experimental animal studies. One important consideration when attempting to rationalise this dilemma is that in general the proportion of the population affected by environmental pollutants appears to be composed of those that suffer already from some health impairment and are therefore pre-disposed to the development of further symptoms etc. In contrast, the majority of workers exposed occupationally and subjects used in experimental trials are usually in good health. Furthermore, whenever an effect is being considered it will invariably be related to the dose received. As described in **Section 4**, dose by inhalation is not quantified easily. Using well-defined deposition models and comparisons of particle deposition patterns between healthy subjects and those suffering some forms of pulmonary disease it is apparent that the changes observed in the latter, such as asymmetric deposition, may lead to localised regions receiving high doses of deposited particles. Doses received by such regions with exposure to near ambient concentrations might well then be approaching effect levels that are only achieved uniformly in the normal lung at considerably higher atmospheric concentrations.

8.2. EXPERIMENTAL ANIMAL STUDIES

Experimental animal studies have been used to investigate the consequences of exposure to particles which can be considered as possible surrogates for $PM_{2.5}$. Caution may be required in extrapolation of the effects seen in the animals to potential effects in man. For example, the majority of studies with insoluble particles have been conducted at atmospheric concentrations that induce an overload of the lung clearance mechanism and a range of subsequent pathological responses. While overloading can occur in all experimental species, the use of the rat as the generally preferred species has led to the discovery that the persistent presence of particles in the lung can lead to substantial inflammatory responses in this species, responses not seen in other species including primates, despite the fact that the latter may clear any such deposited particles more slowly than rodents. Man can be considered to be similar to experimental primates, information from past occupational exposure to high dust concentrations, such as in coal miners, showing that while high dust burdens can be found in miners lungs comparable to those of overload in the rat, they do not appear to give rise to the same type of overt inflammatory response seen in the rat. It now appears that the rat represents a specific species problem with respect to the pulmonary response to particulates and until these species differences are fully resolved caution must be exercised in extrapolating the results of the effects of particles in the rat lung to potential effects in man.

8.3. OVERLOAD

The high particulate lung burdens that can occur in man are usually associated with past occupational exposure and now current hygiene standards are designed to prevent this occurring. Such standards are set considerably higher than ambient particulate levels and hence the latter are considered unlikely to induce any form of particle overloading in the normal populace. It is conceivable that localised overloading might occur in health impaired individuals for reasons discussed above regarding deposition patterns and this, in conjunction with pre-existing lung conditions, might promulgate further respiratory problems. If localised overloading does contribute to further impairment of health it is likely that this will be over extended time periods and not associated with short term excursions of airborne particles and acute respiratory responses.

8.4. THE AGED POPULATION

While pre-existing disease appears to pre-dispose individuals to the effects of ambient pollutants, there is also increasing evidence that the aged in general may be more susceptible. This is unlikely to be due to overall deposition patterns since these differ little with age in healthy humans. However, a number of features of cardiorespiratory functions, such as cardiac output reserve, lung volume, FEV_1 and maximum oxygen uptake, decline with age. Other morphological changes such as loss of lung elasticity, enlargement of alveolar ducts and loss of alveolar septae also occur with age. All of these changes, while having little effect on gross deposition, may alter localised deposition patterns and clearance, thus leading to increased particle burdens and possibly therefore, initiation or promulgation of pollutant-linked respiratory disease. Another aspect to the aged is that over their lifespan they will have had more exposure to particulates that may have provided the opportunity to accumulate particles or induce lung damage. Taking these points together with the general fact that many people over 75 have some form of heart disease, hypertension or obstructive lung disease, it is not surprising that if ambient particulates are responsible for adverse health effects, the aged population are a more to likely target than the younger population.

8.5. THE YOUNG POPULATION

In contrast to the aged, it has been said that the young may also be somewhat more susceptible to environmental particles. This could be attributed to increased total and alveolar deposition of the fine (0.2 µm) to ultrafine fractions of $PM_{2.5}$ in young children, possibly in combination with the fact that during early childhood many of the in-built mechanisms to resist disease are still developing and therefore potentially more susceptible to inhaled insults than in individuals where these are fully developed.

It should also be noted with respect to both the aged and young, that they are also considered more susceptible to the effects of the gaseous components of ambient pollution. The current experimental evidence for additive or even synergistic effects of the gaseous and particulate components of ambient pollutants raises concerns for these members of the general populace.

8.6. TOXICOLOGICAL MECHANISMS

It is apparent from studies that have investigated the mechanisms whereby apparently inert particles may induce lung responses that trace components, including metals, may influence this substantially. Surface adsorbed components, especially acidic materials can also enhance particle effects but it is likely in this instance that this would be in the shorter term since unless there is continual deposition the adsorbed material would be expected to rapidly react with or disperse into the surrounding tissue. Although most studies of these types have been conducted in animals, the mechanisms and possible potentiation of effects by adsorbed materials may well be applicable to man and effects in some animal studies have been reported at near ambient concentrations.

Soluble particles, especially acidic particles such as sulphuric acid, would be expected to have mainly short term acute effects if exposure is episodic. Studies in both experimental animals and man have shown discrepancy between effect concentrations in experimental studies and pollutant episodes with regard to a range of normal pulmonary physiological functions. At high concentrations, often at least an order of magnitude higher than environmental levels, effects are observed, suggesting that in the normal individual it is likely that there will be little, if any, health effects resulting from air pollution episodes. A limited number of studies with subjects with mild health impairment, such as asthmatics, suggest that some may be more susceptible to effects at lower concentrations, sometimes approaching the upper levels recorded in pollutant episodes. While this is quite conceivable, since airway hyperresponsiveness is commonly associated with a number of lung conditions and will be expected to make an individual markedly more susceptible to exposure to an irritant material, the evidence for this is not always consistent.

Some evidence from animal and human studies exists to suggest that single or repeated exposure to acidic aerosols may affect mucociliary clearance. The changes observed are probably not of concern to the normal healthy person but, as before, may exacerbate some respiratory problems present already in some heath impaired individuals.

The findings discussed above indicate that while particles representative of $PM_{2.5}$ can have effects in healthy humans and animals at high concentrations, it is less likely that this will occur at concentrations relevant to episodic increases in environmental particles. Some evidence from both human and animal studies or extrapolation from dosimetric considerations indicate effects at such concentrations might occur in some individuals who already suffer some forms of health impairment. This possibility is enhanced further when the data on combined exposure to particles and gaseous pollutants is considered. In animals there is evidence to suggest that acidic particles may enhance in a synergistic manner the effects of gaseous components of air pollution such as O_3. Studies with humans have also suggested acid aerosols may enhance airway responsiveness to the same gaseous components. These studies point to the view held by many that health effects associated with episodic increases in urban airborne pollutants arise from an additive or synergistic combination of exposure to both the particulate phase and the gaseous phase.

Considering the extensive amount of data generated in both humans and experimental animals on particle dosimetry, mechanisms, acidity, health impairment contributions etc., there is still no proven underlying biological mechanism for the apparent contribution of $PM_{2.5}$ to pollutant induced cardiorespiratory diseases. The generally inert nature (biologically) of low concentrations of inhaled particles is at

variance with the substantial adverse health effects supposedly induced by exposure to such low concentrations of particles. It is more than likely that other components of ambient pollutants such as O_3, NO_2 etc., all of which can exert marked biological responses at low concentrations and which are invariably present when episodic increases in particulates are measures, play a substantial role in induction of the impaired health effects. The particulate phase may then exacerbate effects in the already compromised tissue.

8.7. ULTRAFINE PARTICLES

$PM_{2.5}$, being a generic term for the fraction of airborne particles having sizes of < 2.5 µm, encompasses those particles of < 0.1 µm and termed UF in this review. Recent studies with animals and the known effects of such particles in man suggest that the potential for UF particles to induce health effects may be greater than that perceived from the effects seen with larger particles of similar materials that still fall within the $PM_{2.5}$ fraction. A clear understanding of how these relate to and initiate enhanced lung responses is still to be resolved but high particle numbers and the increased deposition efficiency of UF particles in the lung are probably two important factors. Evidence to date suggests the state of aggregation (individual singlets posing the greatest risk) together with a rapid uptake into or reaction with the lung epithelium also contribute to the apparent higher biological reaction potential of such particles. Additionally, the ease with which such aggregates undergo disaggregation within the tissue is likely to influence the severity and duration of tissue responses. Aggregation is certainly an important moderating factor in UF particle toxicity, as seen for the studies on PTFE fumes. In the context of ambient UF particles, while the primary particles of diesel exhaust are of UF size and could be expected to have greater potential toxicity than other ambient particles of larger size, extensive clinical and epidemiological studies on the health effects of diesel exhaust do not raise untoward concerns other than those expressed already regarding environmental particulate pollutants. This can be attributed to the fact that UF particles will ultimately and often rapidly aggregate, the rate being dependent upon concentration and physicochemical characteristics such as charge. Information on the degree of aggregation of ambient UF particles is sparse but recent data on freshly generated diesel particulate collected from a tractor exhaust showed that already 40% of the particles analysed and counted by electron microscopy were aggregates (Bérubé et al., 1999). Since individual aggregates were composed of large numbers of primary particles, it is clear that the great majority of individual primary UF particles aggregate extremely rapidly once generated.

The emphasis of UF particle effects has been placed predominantly on insoluble or poorly soluble particles but it is apparent from a number of studies that have used UF acidic soluble particles that these also appear to have greater potency to induce effects compared with those representing the higher size ranges within $PM_{2.5}$, when expressed in gravimetric terms. Once again, this can probably be attributed to some of the factors of particle numbers etc. discussed above.

8.8. EPIDEMIOLOGY STUDIES

The perception of a relationship between adverse health effects and exposure to particulate air pollution has come from epidemiology studies. Study design, epidemiological techniques etc. can have a considerable influence on the correlations and conclusions drawn when considering ambient pollutants and for

such reasons a critique of the application of epidemiology in this context is included in the review.

The vast majority of epidemiology studies on ambient particulate pollutants consider either TSP or PM_{10}. This is understandable since up to the present these have been the fractions collected traditionally to measure levels of airborne particles. Very few studies to date therefore address $PM_{2.5}$ specifically and those available have been reviewed. These provide limited evidence of an association between $PM_{2.5}$ levels and acute and chronic mortality but it is considered that any associations between air pollution and hospitalisation have not been demonstrated to be due to $PM_{2.5}$. A number of studies do not demonstrate a relationship between lung function or respiratory symptoms and $PM_{2.5}$ but there is a suggestion that acidity is an important aspect of air pollution with respect to respiratory symptoms.

The apparent relationships that have been shown are relatively weak and not convincing. Some of the reasons for this lie in the fact that they are descriptive studies, not designed to test hypotheses for relationships and some are re-analyses of older studies which were considered to also be flawed. Many of the weaknesses of the studies lie with the environmental data used to derive relationships. The mortality studies and many others to date have generally used environmental data generated for reasons other than looking at health effect associations. The data were collected by area monitors, many of which were sufficiently distant from the population being compared to raise questions on what was the real exposure of groups or individuals? Methodology may also reduce the tenability of using such data to link to health effects, for example using $PM_{2.5}$ concentrations derived from visibility assessments will invariably give rise to marked variation from what may have been the real exposure concentrations experienced by the people being assessed in the study, in addition to the geographical differences between population and sampling / measurement points.

The emphasis of many studies on the particulate component of the atmosphere does not take into account other components of the air pollution, such as gases, as already discussed and which may potentially play a more important role. These may not have been included in evaluations but inevitably there is no effective way of identifying or selecting out an individual harmful component from a mixture of potentially harmful components. Additionally, TSP, PM_{10} and $PM_{2.5}$ are generic terms for fractions of the particulate phase, the chemical and physical characteristics of which may vary widely by region but which have not been fully characterised in the past. Experimental studies suggest that variations in these could also influence health effects.

Another important weakness in most of these studies is that the large populations used were taken from almost a decade ago or longer when it is generally accepted that pollution was higher. What is required and what may provide more substantial evidence for any relationship between ambient pollution and health effects is more recent surveys for which robust and relevant data (geographically) is compared with groups or individuals. These data are being generated and further definitive evaluation of these important potential relationships must wait until the outcome of such studies are reported.

8.9. CONCLUSIONS

- Dosimetric consideration of inhaled $PM_{2.5}$ suggest that asymmetric deposition patterns in some individuals with obstructive lung diseases might result in localised doses from near ambient concentrations that might enhance the already existing conditions.
- Particles of low solubility pose a limited risk to health but animal experiments imply that trace metals and adsorbed components associated with some particle types may enhance pulmonary responses.
- Many of the experimental studies have been conducted at high concentrations and used the rat as experimental species. It is now evident that the rat lung may over-respond to the presence of particles in the lung, especially at high doses, and thus results in this species and their extrapolation to man may need to be interpreted with caution.
- Ambient acidic particles probably pose the greatest risk to health and there is a suggestion from epidemiological studies that acidity is an important aspect of air pollution with respect to respiratory symptoms.
- There is no effect of concern on pulmonary function in normal healthy individuals at concentrations of acidic aerosols as high as 1000 $\mu g/m^3$. Effects that may have biological significance may occur at concentrations below 100 $\mu g/m^3$ in the most sensitive asthmatic individuals.
- There is evidence to suggest that acidic particles may enhance in a synergistic manner the effects of gaseous components of air pollution such as O_3, adding support to the view that health effects associated with episodic increases in urban airborne pollutants arise from an additive or synergistic combination of exposure to both the particulate phase and the gaseous phase.
- UF particles (particles < 100 nm diameter) may pose a greater health risk due to higher particle numbers and deposition efficiencies in the lung and greater biological reaction potential, but further studies or evidence will be required for a full evaluation to be made.
- There is a limited number of epidemiology studies that have specifically addressed $PM_{2.5}$. These appear to provide limited evidence of an association between $PM_{2.5}$ levels and acute and chronic mortality available at present. However, this is not convincing for several reasons including study design, lack of robust correlation between environmental data and reported exposed population and inability of identifying or selecting out one individual harmful component ($PM_{2.5}$) from an ambient mixture of a number of potentially harmful components.
- The overall pattern that emerges is that $PM_{2.5}$, at normal ambient levels or those seen during episodic pollutant increases, poses limited risk, if any, to normal healthy subjects. Individuals suffering already from cardiorespiratory disease or pre-disposed to other respiratory diseases such as asthma may be at risk of developing adverse responses to exposure to increased ambient levels of $PM_{2.5}$ but more robust evidence is required to substantiate this.

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GLOSSARY OF ABBREVIATIONS

µm	Microns
BAL	bronchoalveolar lavage
Ca	Calcium
COH	Coefficient of haze
COPD	Chronic obstructive pulmonary disease
Cr	Chromium
Fe	Iron
FEV_1	Forced expiratory volume in 1 second
FP	Fine particles (usually $PM_{2.5}$ or $PM_{2.1}$)
FVC	Forced vital capacity
GSD	Geometric Standard Deviation
H^+	Hydrogen ion – a measure of aerosol acidity
H_2SO_4	Sulphuric acid
HNO_3	Nitric acid
Il-6	Interleukin-6 cytokine
Il-8	Interleukin-8 cytokine
Mg	Magnesium
MMAD	Mass median aerodynamic diameter
mRNA	Messenger RNA
NaCl	Sodium chloride
NH_3	Ammonia
Ni	Nickel
nm	Nanometers
NO_2	Nitrogen Dioxide
O_3	Ozone
PG-TiO_2	Pigment grade titanium dioxide
PTFE	Polytetrafluoroethylene
ROFA	Residual oil fly ash
SG_{aw}	Specific airway conductance
SO_2	Sulphur Dioxide
SO_4	Sulphate ion
SR_{aw}	Specific airway resistance
TiO_2	Titanium dioxide
TNF	Tumour necrosis factor (cytokine)
TSP	Total suspended particulate
UF	Particles of diameter < 100 nanometers
UF-TiO_2	Ultrafine titanium dioxide
V	Vanadium
V_R	Ventilation rate
ZnO	Zinc oxide

Figure 1 Composition of a Typical UK Urban $PM_{2.5}$ Fraction

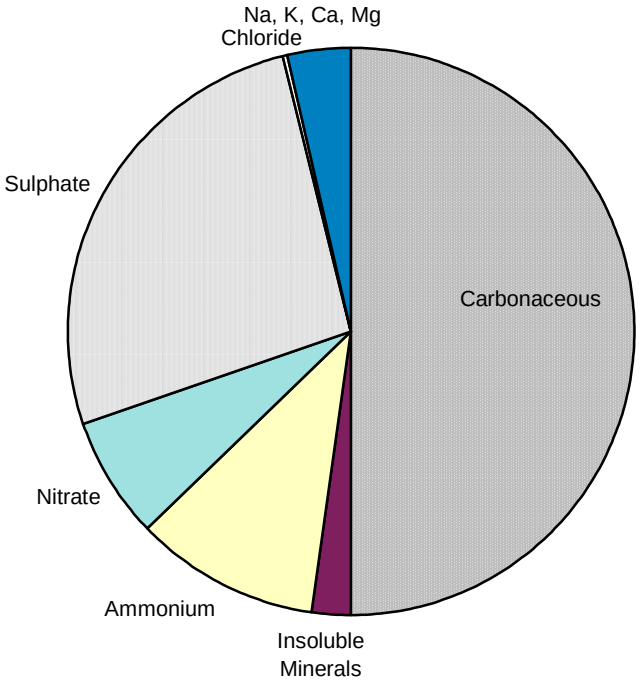


Figure 2 Size Distribution Profile of Typical Urban Ambient Particulates

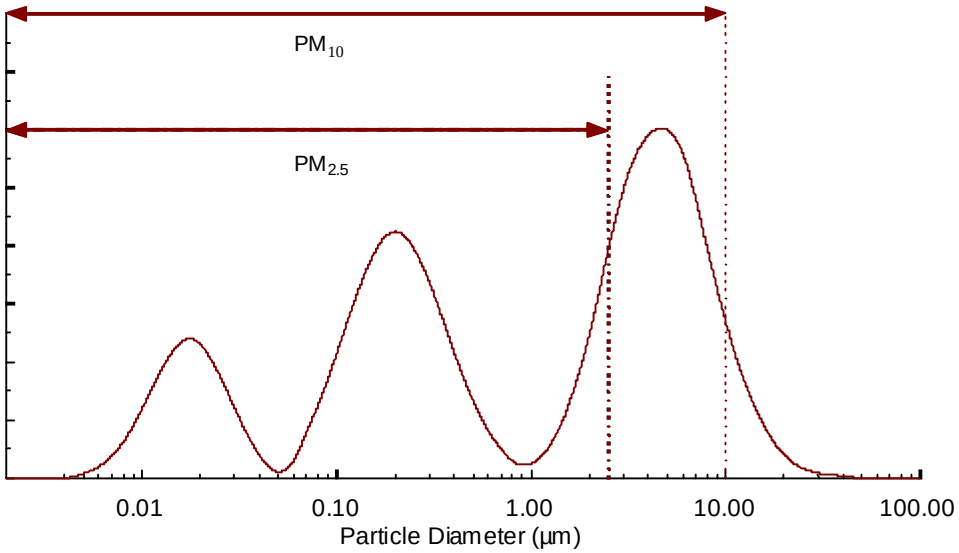


Figure 3 Regional Deposition Efficiencies in the Adult Human Engaged in Light Work

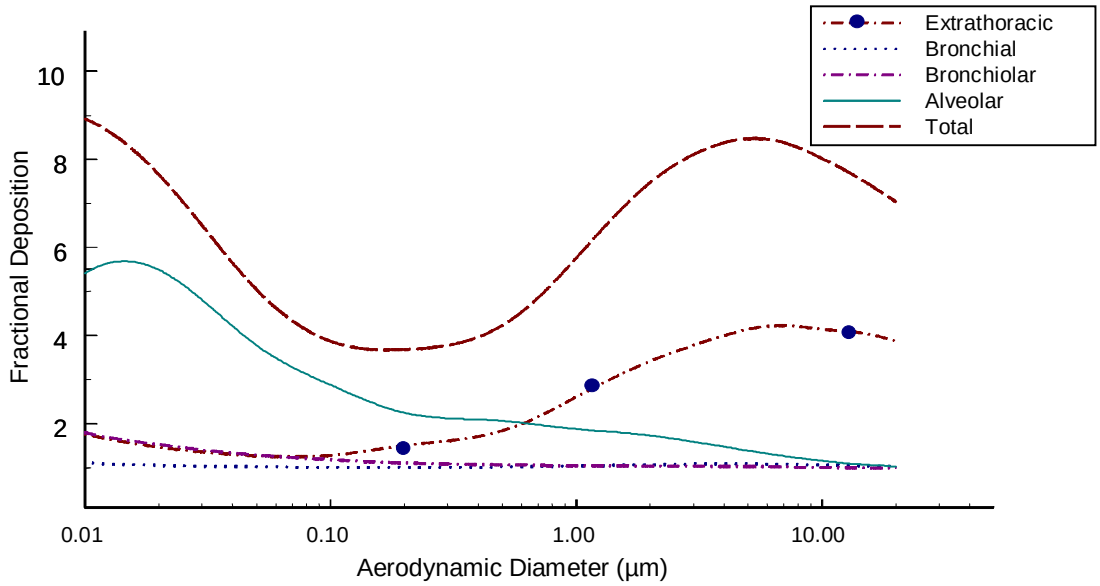


Figure 4 Comparison of Alveolar Deposition Between Nose and Mouth Breathing

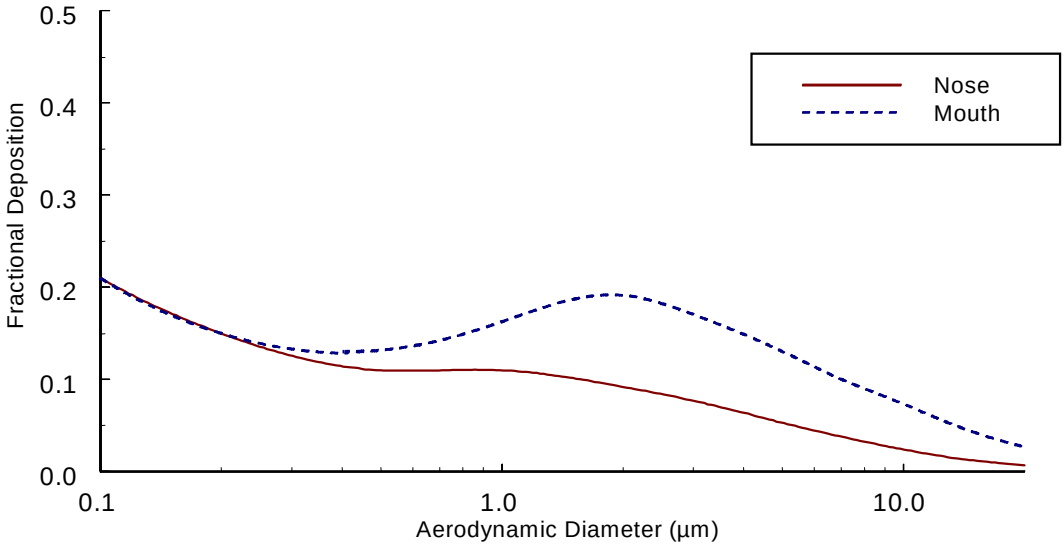


Figure 5 Alveolar Deposition Pattern for Non-hygroscopic and Hygroscopic Aerosols

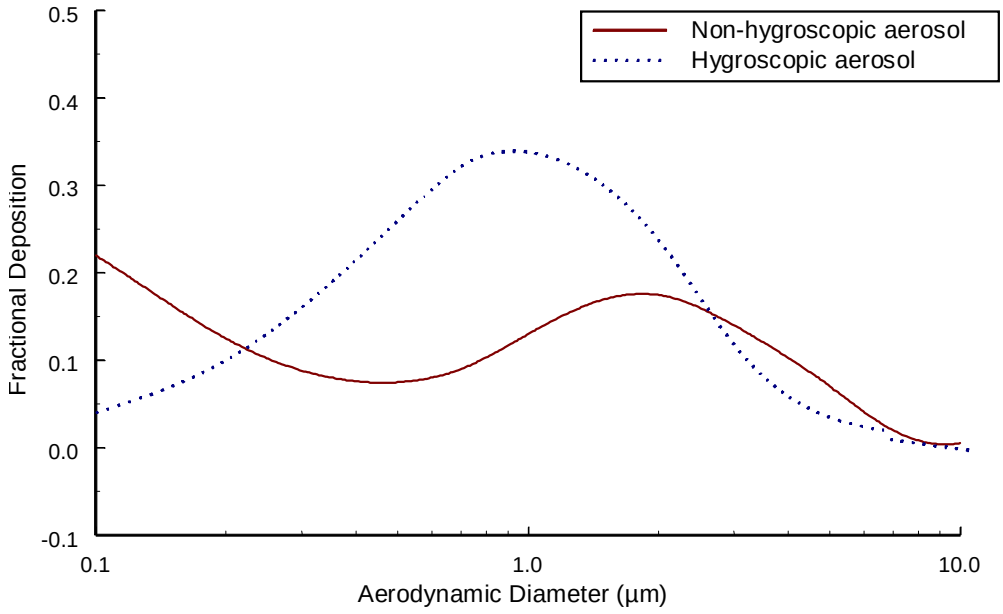


Figure 6 Change in Pulmonary Resistance in the Guinea Pig in Relation to Particle Size

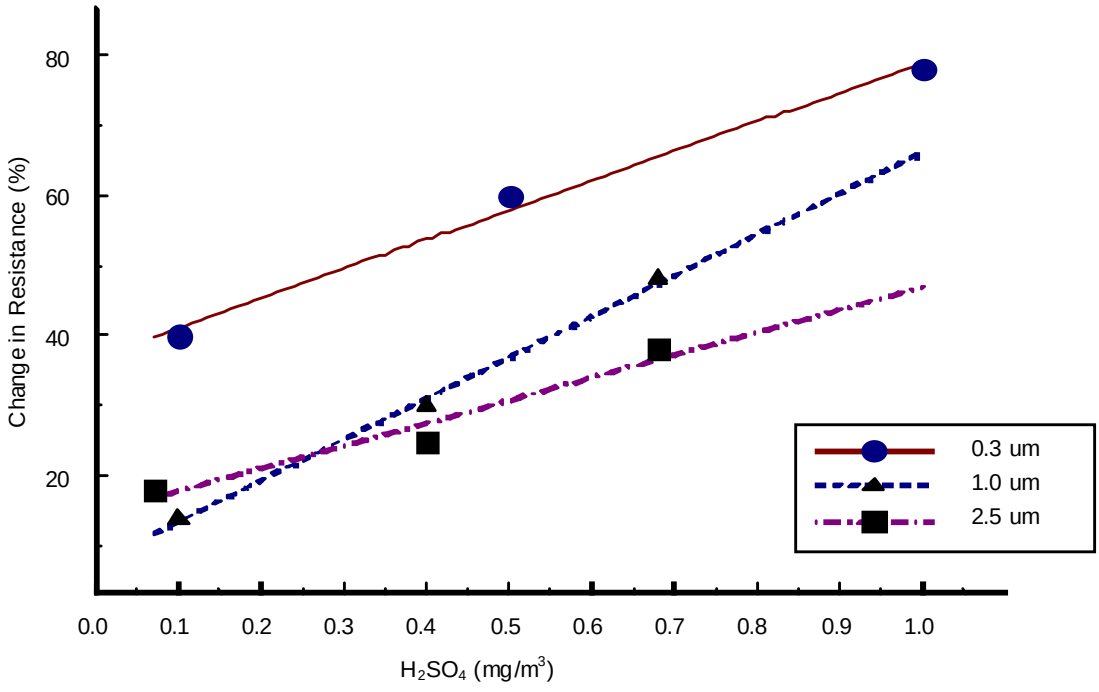


Figure 7 The Scientific Value of Epidemiological Designs

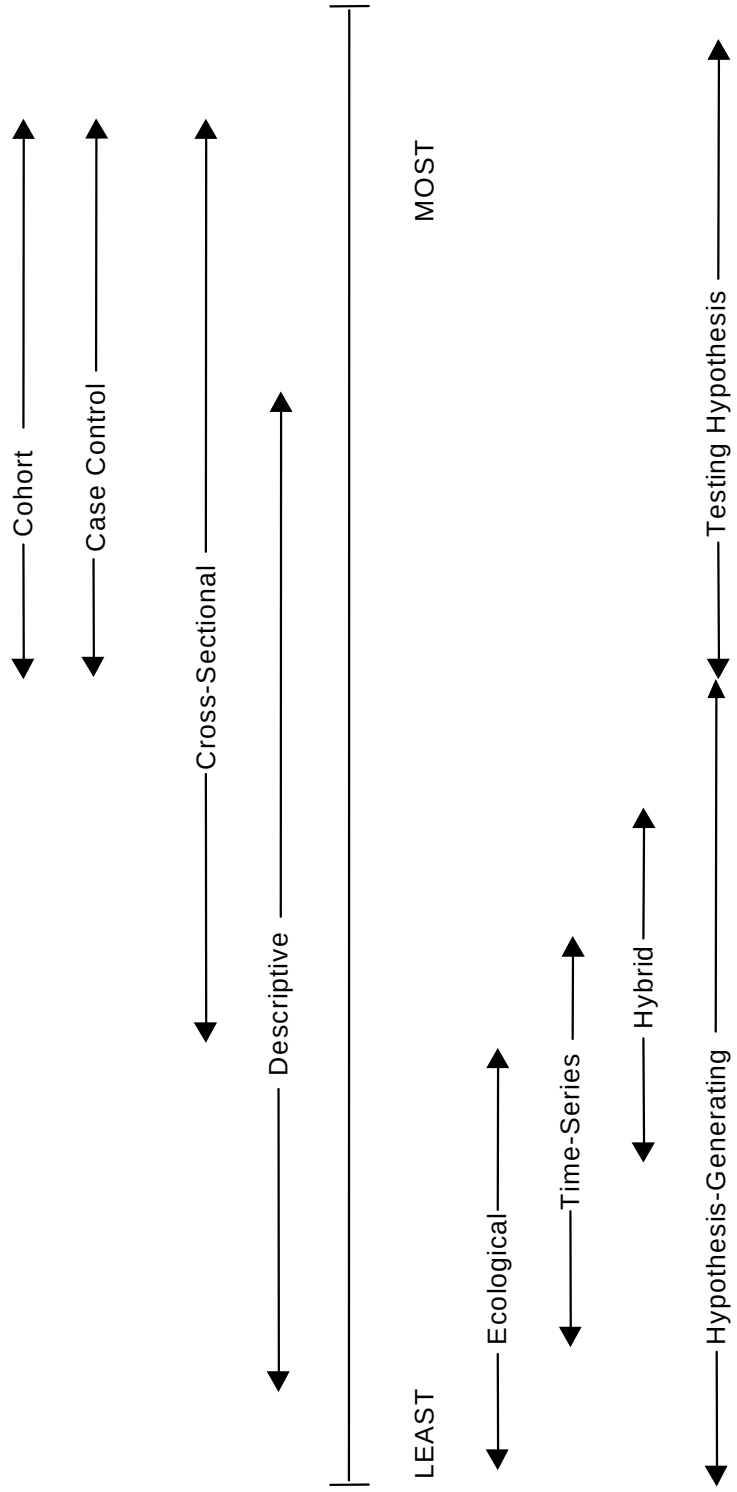


Table 1 Limit Values, Action Levels and Implementation Dates from EU Directive on Particulate Matter

Limit Values for Particulates

	Averaging period	Limit value	Margin of tolerance	Date by which limit value is to be met
Stage 1				
1. 24 hour limit value for the protection of human health	24 hours	50 µg/m^3 PM_{10} not to be exceeded more than 25 times per year	50% on entry into force of this Directive, reducing on 1 January 2001 and every 12 months thereafter by equal annual percentages to reach 0% by 1 January 2005	1 January 2005
2. annual limit value for the protection of human health	calendar year	40 µg/m^3 PM_{10}	20% on entry into force of this directive, reducing on 1 January 2001 and every 12 months thereafter by equal annual percentages to reach 0% by 1 January 2005	1 January 2005
Stage 2				
1. 24 hour limit value for the protection of human health	24 hours	50 µg/m^3 PM_{10} not to be exceeded more than 7 times per year	(to be derived from data and to be equivalent to the Stage 1 limit value)	1 January 2010
2. annual limit value for the protection of human health	calendar year	20 µg/m^3 PM_{10}	50% on 1 January 2005 reducing every 12 months thereafter by equal annual percentages to reach 0% by 2010	1 January 2010

Note: The Directive states that indicative limit values under stage 2 are to be reviewed in the light of further information on health and environmental effects, feasibility and experience in the application of stage 1 limit values in the Member States

Table 2 Relationship of Particle Numbers, Surface Area and Settling Velocity to Particle Size at Unit Density

Particle Diameter (µm)	Number of Particles in 1µg	Surface Area (m^2/g)	Settling Velocity (cm/sec)
0.01	2×10^{12}	6×10^{6}	5×10^{-6}
0.1	2×10^{9}	6×10^{5}	8×10^{-5}
1.0	2×10^{6}	6×10^{4}	3.5×10^{-3}
2.5	1.2×10^{5}	2.4×10^{4}	2×10^{-2}
10	2×10^{3}	6×10^{3}	0.3

Table 3 Species Sensitivity to Particle-Induced Lung Inflammation, Epithelial Hyperplasia and Fibrosis

Particulate Material	Exposure Duration	Relative Species Sensitivity	Reference
Diesel exhaust	30 months	rat > mouse	Henderson *et al*, (1988)
Diesel exhaust	30 months	rat>mouse>hamster	Heinrich *et al*, (1986)
Carbon black	13.5 months	rat > mouse	Heinrich *et al*, (1995)
Titanium dioxide	13 weeks	rat > mouse	Oberdörster, (1994)
Talc	24 months	rat > mouse	NTP, (1993)
Petroleum coke	24 months	rat > monkey	Klonne *et al*, (1987)
Lignite/activated carbon	12 months	rat>monkey, guinea pig > mouse	Gross and Nau, (1967)
Shale	24 months	rat > monkey	MacFarland *et al*, (1982)

Table 4 Relative Irritant Potency of Sulphates

Sulphuric acid*	100
Zinc ammonium sulphate	33
Ferric sulphate	26
Zinc sulphate	19
Ammonium sulphate	10
Ammonium bisulphate	3
Cupric sulphate	2
Ferrous sulphate	0.7
Sodium sulphate	0.7
Manganous sulphate	-0.9

From Amdur et al. (1978b)

* Sulphuric acid is given an irritancy of 100%

Table 5 Effects of acidic particles on pulmonary mechnical function

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Mode	Concentration ($\mu g/m^3$)	Particle Size (µm MMAD)	Exposure Duration	Observed Effect	Reference
H_2SO_4	Rat	Whole body	2370	0.5	14 weeks	No significant change in tidal volume, respiratory frequency, pulmonary resistance, dynamic compliance, arterial pH and partial pressure of CO_2 in arterial blood	Lewkowski *et al.* (1979)
H_2SO_4	Rat	Whole body	6350	0.44	6 weeks	Significant decrease in partial pressure of CO_2 in arterial blood	Lewkowski *et al.* (1979)
H_2SO_4	Rat	Whole body	6590	0.31	13 weeks	Significant increase in arterial pH	Lewkowski *et al.* (1979)
H_2SO_4	Guinea pig, M Hartley	Whole body	1000, 3200	0.54	24 hr/day, 3-30 days	Hypo- to hyperresponsive airways	Kobayashi and Shinozaki (1993)
H_2SO_4	Rabbit, M NZW	Nose-only	250	0.3	1 hr/day, 5 days/week, up to 12 months	No significant change in pulmonary resistance. Hyperresponsive by 4 months	Gearhart and Schlesinger (1986)
H_2SO_4	Guinea pig, M Hartley 260-325 g	Nose-only	300	0.08	1 hr	No significant change in vital capacity, inspiratory capacity, alveolar volume, total lung capacity. A significant decrease in diffusing capacity, CO (3 hr post exposure)	Chen *et al.* (1991)
H_2SO_4	Guinea pig, M Hartley 290-410 g	Head-only	200	0.06	1 hr	No significant change in pulmonary resistance	Chen *et al.* (1992b)
$(NH_4)_2SO_4$	Guinea pig, M Hartley, 10 wk	Whole body	1000	0.4	6 hr/day, 5 days/week, 1 or 4 weeks	No significant change in residual volume. Significant increase in functional residual capacity, vital capacity, total lung capacity, CO diffusing capacity, dynamic compliance and change in distribution of ventilation as measured by nitrogen washout technique	Loscutoff *et al.* (1985)
$(NH_4)_2SO_4$	Rat, M SD, 14 wk	Whole body	1000	0.4	6 hr/day, 5 days/week, 1 or 4 weeks	Significant increases in residual volume and functional residual capacity and change in distribution of ventilation as measured by nitrogen washout technique	Loscutoff *et al.* (1985)

Note: The use of the term significant in the above table implies statistical significance unless stated otherwise

Table 6 Effect of particles and acid sulphates on microbial infectivity

Particle	Species, Gender, Strain, Age, or Body Weight	Exposure Mode	Concentration ($\mu g/m^3$)	Particle Size (µm MMAD)	Exposure Duration	Observed Effect	Reference
$(NH_4)_2\ SO_4$	Mouse, F CD-1, 30 days	Whole body	1,000	Sub-micrometer	3 hour/day, 20 days	No Change	Aranyi *et al.* (1983)
TiO_2	Guinea pig, F, Dunkin-Hartley 300-350g	Whole body	23,000	95%< 1.98 µm	20 hour/day, 14 days	No change in susceptibility to *Legionella pneumophila* administered 1-6 days post exposure but AM with heavy particle burden did not ingest bacteria	Baskerville *et al.* (1988)
H_2SO_4	Mouse, F CD-1, 30 days	Head-only	543	0.08	2 hour	No Change	Grose *et al.* (1982)
H_2SO_4	Mouse, F CD-1, 30 days	Head-only	365	0.06	2 hour/day, 5 days	No Change	Grose *et al.*(1982)
Carbon black	Mouse, Fswiss, 20-23g	Nose - only	10,000	2.4	4 hour/day, 4 days	No change in no. of *S. aureus* or *P. mirabilis* recovered in lung after bacterial challenge or on intrapulmonary killing of bacteria administered 1 day post exposure; no effect on proliferation of *L monocytogenes*; no effect on proliferation or elimination of influenza A virus; no change in albumin level in lavage 4 hours after bacterial challenge; no change in neutrophils in lavage 4 hours after challenge	Jakab (1993)
Volcanic ash	Mouse, F, CD-1, 4-8 weeks	Whole body	9,400	0.65	2 hour	No change in susceptibility to bacteria (*Streptococcus*) or virus administered 0 or 24 hours post exposure; no change in lymphocyte response to mitogens	Grose *et al.* (1985)
TiO_2	Mouse, Harlan-Olac 8 weeks	Whole body	2,000 , 20,000	95%< 1.98 µm	20 hour/day, 2 to 4 weeks	Reduced clearance of *P. haemolytica* administered after exposure in proportion to exposure duration at 20,000 $\mu g/m^3$ only	Gilmour *et al.* (1989)
TiO_2	Mouse, Harlan-Olac, 8 weeks	Whole body	20,000	95% <1.98 µm	20 hour/day, 10 days	Reduced clearance of *P. haemolytica*, persistent up to 10 days post exposure	Gilmour *et al.* (1989)
Coal dust	Mouse, F, Swiss CD-1, 20-24g	Whole body	2,000	80%< 10 µm; 50%< 5 µm	7hr/day, 5 days/week, 6 months	No change in susceptibility to influenza virus administered after 1, 3 and 6 months exposure; decrease in interferon level in lung at 3 months; no change in inflammatory response to virus	Hahon *et al.* (1985)
Carbon black	Mouse, F Swiss, 20-23g	Nose - only	4,700 - 6,100	2.45	4 hour/day, 4 days	No effect on susceptibility to infection from *S. aureus* administered 1 day post exposure; no effect on intrapulmonary killing of bacteria by alveolar macrophages.	Jakab (1992)

Table 7 Functional response patterns of the canine respiratory system exposed to neutral and acid aerosol

Respiratory and Non-Respiratory Lung Function	Biological Marker	Response to Neutral Aerosol	Response to Acid Aerosol
Lung mechanics			
lung volumes		–	–
lung elasticity	static lung compliance	↓*	–
	dynamic lung compliance	–	–
Alveolar-capillary barrier			
gas transfer	diffusing capacity	↓	–
permeability	albumin concentration	↑*	–
Macrophage-mediated particles clearance	detection of intrapulmonary activity after inhalation of radiolabelled insoluble particles	↑*	↓*
Macrophage-associated defence capacity			
oxidative defence	release of superoxide	↓*	–
phagocytic capacity	phagocytosis index	↓*	–
	fraction of phagocytosing cells	–	↓*
lysosomal activity	β-N-acetylglucosaminidase concentration	↑*	↑*
intracellular particle dissolution	extracellular activity of radiolabel from phagocytosed radiolabelled particles of moderate solubility	↑*	↓*
Type II cell function	alkaline phosphatase concentration	–	↑
Oxidant status of extracellular proteins	methionine sulphoxide concentration	↓*	–
	carbonyl concentration	↓*	–
Cell injury	lactate dehydrogenase concentration	–	–

From Heyder et al. (1997) Key: –- no change detected; * statistical significant changes detected; ↑ increased; ↓ reduced.

Table 8 Human Studies on Exposure to Diesel Exhaust

Study	Findings	Reference
13 Cases of acute exposure, Utah and Colorado coal miners	Acute reversible sensory irritation, headache; nervous system effects, bronchoconstriction were reported at unknown exposures	Kahn *et al.* (1988)
161 Workers, two diesel bus garages	Eye irritation, headache, dizziness , throat irritation and cough and phlegm were reported at various incidences	El Batawi and Noweir (1966)
13 Volunteers exposed to three dilutions of diesel exhaust for 15 min to 1 hr	No significant effects on pulmonary resistance were observed as measured by plethysmography	Battigelli (1965)
12 healthy non-smoking volunteers exposed to diluted diesel exhaust for 1 hr with light work, with and without particle filtration of exhaust	Most prominent symptoms were irritation to eyes and nose. Airway resistance and specific airway resistance increased during exposure. Effects were not significantly reduced with filtered exhaust, indicating the effects were induced by the gaseous phase	Rudell *et al.* (1996)
232 Workers in four diesel bus garages were administered acute respiratory questionnaires and before and after workshift spirometry. Compared to lead acid battery workers previously found to be unaffected by their exposures	Prevalence of burning eyes, headache, difficult or laboured breathing, nausea, and wheeze were higher in diesel bus workers than in comparison population	Gamble *et al.* (1987a)
Pulmonary function of 60 diesel-exposed compared with 90 non-diesel exposed coal miners over workshift	No significant differences in ventilatory function changes between miners exposed to diesel exhaust and those not exposed	Ames *et al.* (1982)
240 Iron ore miners matched for diesel exposure, smoking and age were given bronchitis questionnaires and spirometry pre- and postwork shift	Among underground miners (surrogate for diesel exposure), smokers and older age groups, frequency of bronchitis were higher. Pulmonary function was similar between groups and subgroups except for differences accountable to age	Jörgensen and Svensson (1970)
210 Locomotive repairmen exposed to diesel exhaust for an average of 9.6 years in railroad engine houses were compared with 154 railroad yard workers of comparable job status but no exposure to diesel exhaust	No significant differences in VC, FEV_1, peak flow, nitrogen washout, or diffusion capacity nor in the prevalence of dyspnea, cough, or sputum were found between the diesel exhaust-exposed and non-exposed groups	Battigelli *et al.* (1964)
283 Male diesel bus garage workers from four garages in two cities were examined for impaired pulmonary function (FVC, FEV_1, and flow rates). Study population was compared to a non-exposed 'blue collar' population	Analyses within the study populations population showed no association of respiratory symptoms with length of exposure. Reduced FEV_1 and FEF_{50} (but not FEF_{75}) were associated with increasing exposure time. The study population had a higher incidence of cough, phlegm, and wheezing unrelated to exposure time. Pulmonary function was not affected in the total cohort of diesel-exposed but was reduced with 10 or more years of exposure	Gamble *et al.* (1987b)

Table 8 Human Studies on Exposure to Diesel Exhaust (contd.)

Study	Findings	Reference
Differences in respiratory symptoms and pulmonary function were assessed in 823 coal miners from six diesel equipped mines compared to 823 matched coal miners not exposed to diesel exhaust	Underground miners in diesel-use mines reported more symptoms of cough and phlegm and bad lower pulmonary function. Similar trends were noted for surface workers at diesel-use mines. Pattern was consistent with small airway disease but factors other than exposure to diesel exhaust thought to be responsible	Reger *et al.*(1982)
Changes in respiratorysymptoms and function were measured during a 5-year period in 280 diesel-exposed and 838 non-exposed U.S. underground coal miners	No decrements in pulmonary function or increased prevalence of respiratory symptoms were found attributable to diesel exhaust. 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust than in miners exposed to diesel exhaust	Ames *et al.* (1984**)**
Respiratory symptoms and function were assessed in 630 potash miners from six potash mines using a questionnaire, chest radiographs and spirometry.	No obvious association indicative of diesel exposure was found between health indices, dust exposure, and pollutants. A higher prevalence of cough and phlegm, but no differences in FVC and FEV_1, were found in these diesel-exposed potash workers when compared to predicted values from a logistic model based on blue- collar staff working in non-dusty jobs	Attfield *et al.* (1982)
Workshifts changes in pulmonary function were evaluated in crews of roll-on-roll-off ships and car ferries and bus garage staff. Pulmonary function was evaluated in 6 volunteers exposed to diesel exhaust, 2.1 ppm NO_2 and 600 $\mu g/m^3$ particulate matter	Pulmonary function was affected during a workshift exposure to diesel exhaust, which normalised after a few days with no exposure. Decrements were greater with increasing intervals between exposures. No effect on pulmonary function was observed in the experimental exposure study	Ulfvarson *et al.* (1987)
In a repeat of the above study the exhausts of trucks on the roll-on-roll-off ships were fitted with microfilters. Pulmonary function was evaluated in subjects from 3 groups; those exposed to filtered diesel exhaust (n=24), non-filtered (n=18) and both (n=6). All were compared to an unexposed control group (n=17)	Those exposed to non-filtered diesel exhaust showed a 5% shift-related decrement in FVC was seen, but no changes in FEV_1. Exposure to filtered then non-filtered resulted in a decrement in FVC from 10% to 5%. Filtered alone had a decrement of 2% FVC. No subsequent shift-related changes in FEV_1 were noted, with or without filters	Ulfvarson and Alexandersson. (1990)
Respiratory symptoms and pulmonary function were evaluated in 17 stevedores exposed to both diesel and gasoline exhausts in car ferry operations; control group consisted of 11 on-site workers	Stevedores had lower baseline lung function consistent with an obstructive ventilatory defect compared to controls . No significant shift-related changes were seen between the two groups for FVC or FEV_1	Purdham *et al.* (1987)

Table 8 Human Studies on Exposure to Diesel Exhaust (contd.)

Study	Findings	Reference
Mortality assessment of 8490 London transport maintenance workers employed for at least 1 year between 1967 and 1975	No increased incidence of non-malignant or malignant disease associated with exposure to diesel exhaust	Rushton *et al.* (1983)
Mortality assessment of potash miners and millers employed for at least 1 year between 1940 and 1967 (2743 underground workers and 1143 surface workers)	No excess mortality associated with exposure to diesel exhaust	Waxweiler *et al.* (1973)
Cohort of 34156 members of a heavy construction equipment operators union with a potential exposure to diesel exhaust	Lower than expected mortalities. No association between diesel exhaust exposure and lung cancer	Wong (1985)
Case-control study of 502 male lung cancer cases and 502 controls without tobacco-related diseases	No association between diesel exhaust exposure and risk of lung cancer	Hall & Wynder (1984)
Respiratory morbidity was assessed in 259 miners in 5 salt mines by respiratory symptoms, radiographic findings and spirometry. Two mines used diesels extensively, 2 had limited use, one used no diesels in 1956, 1957, 1963, or 1963 through 1967. Several working populations were compared to the salt mine cohort	After adjustment for age and smoking, salt miners showed no symptoms, such as increased prevalence of cough, phlegm, dyspnea or air obstruction (FEV_1/FVC) compared to aboveground coal miners, potash workers or blue collar workers. FEV_1 FVC, FEF_{50}, and FEF_{75}, were uniformly lower for salt miners in comparison to all the comparison populations. No changes in pulmonary function were associated with years of exposure or cumulative exposure to inhalable particles or NO_2	Gamble *et al.* (1983)
As above. Salt miners were grouped into low, intermediate and high exposure categories based on tenure in jobs with diesel exposure	A statistically significant dose-related association of phlegm and diesel exposure was noted. Changes in pulmonary function showed no association with diesel tenure. Age- and smoking-adjusted rates of cough, phlegm, and dyspnea were 145, 169, and 93% of an external comparison population. Predicted pulmonary function indices showed small but significant reductions; there was no dose-response relationship	Gamble *et al* (1983)
Pilot study of 129 bus company employees classified into three diesel exhaust exposure categories - clerks, bus drivers, and bus garage workers	The most heavily exposed group (bus garage workers) had a fourfold increase in risk of dying from cardiovascular disease, even after correction for smoking and allowing for 10 years of exposure and 15 years or more of induction latency time	Edling and Axelson (1984)

Table 8 Human Studies on Exposure to Diesel Exhaust (contd.)

Study	Findings	Reference
Cohort of 694 male bus garage employees followed from 1951 through 1983 were evaluated for mortality from cardiovascular disease. Sub-cohorts categorised by levels of exposure were: clerks, bus drivers and bus garage employees	No increased mortality from cardiovascular disease was found among the members of these five bus companies when compared with the general population or grouped as sub-cohorts with different levels of exposure	Edling *et al.* (1987)
1256 cases of lung cancer in U.S. railroad workers matched to 2385 controls who had worked for the railroad for at least 10 years or more. Deaths recorded between 1981 and 1982	After adjustment for smoking and previous exposure to asbestos a small, but significant, elevated lung cancer risk was related to diesel exposure	Garshick *et al.* (1987)
Retrospective cohort study of 55407 American railroad workers aged 40-64 in 1959 who had worked for 10-20 years. Mortality determined retrospectively through 1980.	Of 55407 deaths 1694 were attributable to lung cancer. Relative risk = 1.45 in workers aged 40-44 in 1959; Relative risk = 1.33 in workers aged 45-49. The highest risk for cancer was for those workers who had the highest potential cumulative exposure. No adjustment for smoking.	Garshick *et al.* (1988)

Note: The use of the term significant in the above table implies statistical significance unless stated otherwise

Table 9 Clinical studies in humans exposed to acid aerosols

Exposures	Subjects	Particle Size (µm MMAD)	Duration	Exercise	Temp (°C)	RH (%)	Symptoms	Lung Function	Other Effects	Comments	Ref.
(1): air (2): H_2SO_4 100 µg/m^3 (3): carbon black 200 µg/m^3 (4): acid-coated carbon with 100 µg/m^3 H_2SO_4	15 Healthy 15 Asthmatic 18 to 45 yrs	1.0	1hr	V ≈ 50 L/min	22	50	Healthy subjects more symptomatic in air	Largest decrements in FVC with air exposure	No change in airway responsiveness	Smoking status of subjects not stated	Anderson *et al.* (1992)
Mouthpiece study: H_2SO_4 vs NaCl 3000 µg/m^3	18 asthmatics 23 to 37 yrs	0.4	16 min	With and without exercise	24	<10 vs 100	No effects	Increases in SR_{aw} with low RH conditions; no exposure-related effects		Postulated that effects seen in other studies due to secretions or effects on larynx	Aris *et al.* (1991)
Air H_2SO_4 : Healthy: 363, 1128, 1578 µg/m^3 Asthmatic: 396, 999, 1,460 µg/m^3	21 healthy 21 asthmatic 18 to 45 yrs	0.85 to 0.91	1hr	10 min X 3 47 to 49 L/min	21	50	Healthy: slight increase in cough with highest concentrations Asthma: dose related increase in lower respiratory symptoms	Healthy: No effects on lung function or airway reactivity Asthma: ↓FEV_1 0.26 L with H_2SO_4 1,460 µg/m^3			Avol *et al.* (1988)
Air H_2SO_4 46, 127 and 134 µg/m^3	32 asthmatics 8 to 16 yrs	0.5	40 min	30 min rest, 10 min exercise 20 L/min/m^2	21	48	No exposure related effect	No exposure related effects. One subject increased SR_{aw} 14.2% with acid exposure		Did not produce findings of Koenig *et al.*, 1983	Avol *et al.* (1990)

Table 9 Clinical studies in humans exposed to acid aerosols (contd.)

Exposures	Subjects	Particle Size (µm MMAD)	Duration	Exercise	Temp (°c)	RH (%)	Symptoms	Lung Function	Other Effects	Comments	Ref.
NaCl 1000 µg/m^3 H_2SO_4 1,000 µg/m^3	16 healthy 20 to 39 yrs	0.9	2 hr	10 min x 4 = 40 L/min	22	40			Mucins from bronchoscopy: no effects on mucin recovery or changes in glycoproteins		Culp *et al.* (1995)
NaCl 1,000 µg/m^3 H_2SO_4 1,000 µg/m^3	12 healthy 20 to 39 yrs	0.9	2 hr	10 min x 4 ≈ 40 L/min	22	40	4/12 subjects: throat irritation with acid exposure	No exposure related effects	BAL findings: No effects on cell recovery, lymphocyte subsets, AM function, fluid proteins		Frampton *et al.* (1992)
NaCl or H_2SO_4 100 µg/m^3 followed by O_3 0.08, 0.12, or 0.18 ppm	30 healthy 30 asthmatic 20 to 42 yrs	0.45 0.64	3 hr 3 hr	10min x 6 Healthy: 33 to 40 L/min; asthmatics : 31 to 36 L/min	21	40	No pollutant effects	Healthy subjects: no significant effects Asthmatics: ozone dose-response following H_2SO_4 pre-exposure, but not NaCl			Frampton *et al.* (1995)
Mouthpiece: (1): Air; H_2SO_4 70, 130 µg/m^3 (2): Air; H_2SO_4 70 µg/m^3 with and without lemonade	22 asthmatics 12 to 19 yrs	0.72	40 min 45 min	10 min 30 min @ 30L/min	22	65	No effects	Significant decreases in FEV (37 ml / µmol H^+) and FVC at 2 to 3 min but not 20 min after exposure	Significant correlation between baseline airways responsiveness and $\Delta FEV_1/H^+$ (R^2 =0.3)	Large variability in oral NH_3 levels	Hanley *et al.* (1992)

Table 9 Clinical studies in humans exposed to acid aerosols (contd.)

Exposures	Subjects	Particle Size (µm MMAD)	Duration	Exercise	Temp (°c)	RH (%)	Symptoms	Lung Function	Other Effects	Comments	Ref.
Mouthpiece: Air; H_2SO_4 68µg/m^3; SO_2 0.1 ppm; H_2SO_4 +SO_2; HNO_3 0.05 ppm	9 asthmatics with exercise - induced bronchospasm 12 to 18 yrs	0.6	40 min	10 min	25	65	No effects	↓FEV 6% after H_2SO_4 compared with 2% after air			Koenig *et al.* (1989)
Mouthpiece: Air; H_2SO_4 35 or 70 µg/m^3	14 asthmatics with exercise-induced bronchospasm 13 to 18 yrs	0.6	45 or 90 min	≈ 23 L/min	22	65		↓FEV_1 6% after H_2SO_4 35 µg/m^3 for 45 min, 3% after 70 µg/m^3. Smaller changes after 90 min exposures			Koenig *et al.* (1992)
Mouthpiece: Air; $(NH_4)_2$ SO_4 ≈ 70 µg/m^3 H_2SO_4 ≈ 74 to 82 µg/m^3 with and without lemonade	8 healthy 9 asthmatics 60 to 76 yrs	0.6	40 min	10 min 17.5 L/min for asthmatics 19.7 L/min for healthy subjects	22	65		No significant effects. Correlation between increase in resistance and oral ammonia levels in asthmatics			Koenig *et al.* (1993)
Mouthpiece: Air; O_3 0.12 ppm + 0.3 ppm NO_2; 0.12 ppm O_3 + ppm NO_2 + 68 µg/m^3 H_2SO_4 ; 0.12 ppm O_3 + 0.3 ppm NO_2 + 0.05 ppm HNO_3	28 asthmatics 12 to 19 yrs	0.6	90 min x 2 days	3 x resting expired volume	22	65	No exposure related effects	No exposure related effects	No effects on airway responsiveness	6 subjects with moderate or severe asthma did not complete protocol	Koenig *et al.* (1994)

Table 9 Clinical studies in humans exposed to acid aerosols (contd.)

Exposures	Subjects	Particle Size (µm MMAD)	Duration	Exercise	Temp (°C)	RH (%)	Symptoms	Lung Function	Other Effects	Comments	Ref.
H_2O $H_2SO_4 \approx 2{,}000$ µg/m^3	22 healthy 19 asthmatic 18 to 48 yrs	20 10 1	1 hr	40 to 45 L/min	≈10	74 to 100	Increased total score with larger acid particles	No exposure related effects	No effects on airway reactivity	4 asthmatic subjects unable to complete exposures because of symptoms	Linn *et al.* (1989)
Air; ozone 0.12 ppm; H_2SO_4 100 µg/m^3 ozone + H_2SO_4	15 healthy 30 asthmatic 18 to 50 yrs	≈ 0.5	6.5 hr/day x 2 days	50 min x 6 @ 29 L/min	21	50	Symptoms unrelated to exposure	↓FEV_1 & FVC in O_3 , similar for healthy & asthmatic subjects. Greater fall in FEV_1 for acid+O_3 than O_3 alone, marginally significant interaction	Increased airway responsiveness with O_3, marginal further increase with O_3 + acid	Average subject lost 100 ml FEV_1 with O_3, 189 ml with O_3 +acid Original findings replicated in 13 subjects	Linn *et al.* (1994)
NaCl ≈ 100 µg/m^3 $H_2SO_4 \approx 90$ µg/m^3	17 asthmatic 20 to 57 yrs 17 COPD 52 to 70 yrs		2 hr	Asthmatics : 10min @ 4x minute vol; COPD: 7 min @ 4x minute vol;	21	30	No pollutant effects	Asthmatics: ↓FEV_1 slightly greater after acid than after NaCl. COPD: No effects.			Morrow et al (1994)
Mouthpiece: NaCl 350 µg/m^3; H_2SO_4 350 µg/m^3; high NH_3; H_2SO_4 , low NH_3	15 asthmatic 19 to 50 yrs	0.80	30 min	10 min 3x resting volume		20 to 25		Greater fall in FEV_1 with low NH_3 (19%) than with high NH_3 (8%)			Utell *et al.* (1989)

Note: The use of the term significant in the above table implies statistical significance unless stated otherwise
RH = Relative humidity
BAL = Bronchoalveolar lavage; AM = Alveolar macrophage

APPENDIX 1 DEPOSITION MECHANISMS

Deposition of aerosols within the respiratory tract is governed by five principal mechanisms, namely inertial impaction, gravitational sedimentation, Brownian diffusion, interception and electrostatic precipitation. These are described below. Of these, impaction, sedimentation and diffusion can be considered to be of the major importance in the context of normal, non-fibrous aerosols.

Impaction

Impaction occurs where the airstream undergoes a directional change Figure (a). The momentum of the particle is such that it is unable to change course and deposits on the wall of the airway. Particles of aerodynamic size of greater than 0.5 µm may deposit by this mechanism. This mechanism can operate only where there is a combination of both velocity and directional change and is confined predominantly to the upper respiratory tract and higher branching points in the tracheobronchial system of man but can operate down to the alveolar duct region of smaller experimental animals. Factors influencing deposition by this mechanism include the physical size and density of a particle and breathing pattern.

Gravitational Sedimentation

All particles are subjected to gravity and when this force exceeds other forces to which the particle is subjected, such as velocity and buoyancy, the particle will deposit on the wall of the respiratory tract Figure (b). This mechanism predominates in the lower regions of the respiratory tract where velocities are low. Factors influencing deposition by this mechanism are those mentioned above for impaction with the addition of residence time within the respiratory tract.

Brownian Diffusion

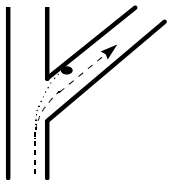
Very fine particles, i.e. those less than approximately 0.5 µm are subject to bombardment by gas molecules and thus acquire random movement in air, termed Brownian movement. Within the respiratory tract particles moving in such a manner may contact the wall of the airway and deposit Figure (c). Deposition by this mechanism is favoured by air velocities being low or absent and therefore predominates in the bronchiolar and alveolar regions.

Interception

Where there is a change in direction of the airflow, irregularly shaped particles such as fibres or fume aggregates may make partial contact with the wall of the airway and become deposited Figure (d).

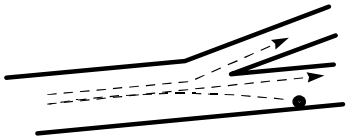
Electrostatic Charge

Aerosols generated for inhalation experiments may sometimes carry substantial electrostatic charge as a result of the methods of generation employed. Such charges can enhance the fraction and site of deposition of the inhaled aerosol by both particle-particle charge interaction and particle-respiratory tract charge interaction.

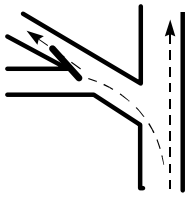


a) IMPACTION

c) BROWNIAN DIFFUSION



b) GRAVITATIONAL SETTLING



d) INTERCEPTION