

Report no. 650010 015

Toxicity of Ambient Air PM10

A critical review of potentially causative PM properties
and mechanisms associated with health effects

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May, 2000

This investigation has been performed by order and for the account of Ministry of Housing,
Spatial Planning and Environment, The Hague, the Netherlands.

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ABSTRACT

A critical review of potentially causative PM properties and mechanisms associated with health effects.

Here, studies focused on ambient particulate air pollution (PM) toxicity, particle hypotheses, and mechanisms were evaluated to investigate causality and plausibility of acute health effects associated with ambient exposure.

High-dose studies indicate that PM: 1) induces oxidative pulmonary inflammation and cardiorespiratory malfunctioning, which could contribute to a disease exacerbation mechanism. PM surface reactivity seems more important than PM mass, thereby prudently suggesting an important role for the anthropogenic (carbonaceous) fine fraction. The limited number of low-dose PM inhalation studies supports this suggestion. Coarse PM may still be important in health effects related to upper airways (like worsening of asthma); however, a role for secondary components (sulfates, nitrates) or ultrafine PM at levels occurring in ambient air have not yet been established. Evidence that (diesel) exhaust particles play a role in PM health effects is still marginal. Studies have indicated that mixtures of particles and gases like ozone may result in more toxicity than the components separately. Current dosimetry models predict that (older) people with cardiorespiratory diseases may receive increased PM doses upon exposure. Ambient PM toxicity studies have been intensified in recent years. The current limited data, however, have not yet resulted in sufficient evidence to be convincing in indicating: 1) a specifically important and causal role for one form of PM fraction or composition and 2) mechanisms explaining PM health effects in people considered to be at increased risk.

SAMENVATTING

Dit rapport bevat een evaluatie van studies gericht op buitenlucht fijn stof (PM) toxiciteit, deeltjeshypothesen en mechanismen, teneinde causaliteit en plausibiliteit van acute gezondheidseffecten beter te begrijpen.

Mechanistische studies met hoge doses wijzen uit dat PM 1) oxidatieve ontstekingsreacties induceert en 2) cardiorespiratoire functies vermindert, hetgeen biologisch plausibel lijkt met verergering van aandoeningen als mechanisme. PM oppervlakte-reactiviteit wordt belangrijker gevonden dan PM massa, hetgeen suggereert dat de antropogene (roethoudende) fijne PM fractie belangrijk is. De zeer beperkte gegevens uit inhalatie studies met lage PM doses ondersteunen dit vermoeden. De grovere ("coarse") PM fractie zou ook nog belangrijk kunnen zijn, met name daar waar het effecten in de hogere luchtwegen betreft, zoals verergering van astma. Een rol voor secundaire PM componenten (sulfaat, nitraat) of ultrafijn PM, op de niveaus zoals ze in de buitenlucht vóórkomen, is nog niet duidelijk aangetoond. Studies suggereren ook dat mengsels van PM en gassen zoals ozon tot meer toxiciteit leidt dan op grond van de afzonderlijke componenten verwacht kan worden. De huidige dosimetrie modellen voorspellen dat (oudere) mensen met cardiorespiratoire aandoeningen een grotere dosis kunnen binnenkrijgen.

De laatste jaren zijn studies naar de toxiciteit van PM toegenomen. De huidige gegevens hebben echter nog niet geresulteerd in voldoende bewijs om overtuigende aanwijzingen te kunnen geven over 1) een specifiek belangrijke en causale rol voor een bepaalde PM fractie of samenstelling en 2) mechanismen die PM gezondheidseffecten in personen die tot risicogroepen worden gerekend plausibel kunnen verklaren.

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1. Introduction

1.1 General introduction

Exposures to respirable ambient air particulates seem to pose a serious threat to human health in urban and rural populations all over the world. Even though the relative risk is small, there is a public health problem because of the large number of people exposed. The specific particle size fractions, the chemical or biological components and the most dominant sources that might be most responsible for these health effects are only marginally known. In addition, there are insufficient toxicity data that can explain the health effects as observed at relatively low particle levels and or provide a view on plausible biological mechanisms.

Recently, National Ambient Air Quality Standards (NAAQS, USA) and Air Quality Limit Values (EU) for particulate air pollution (*particulate matter*, PM₁₀) have been established and proposed, respectively. These values apply to the mass concentrations of particles with aerodynamic diameters lower than 2.5 µm (PM_{2.5}) and 10 µm (PM₁₀). These new primary standards and limit values intend to provide better protection against a wide range of PM-associated health effects. However, scientific sound evidence for a possible causal relationship is needed for a solid basis for these standards or limit values. Table 1 summarises the various new annual and daily mean air quality standards and limit values and the limitations for exceedances.

		Annual mean		Daily mean	
USA	PM ₁₀	50	3-y average of annual mean concentrations (2012*)	150	3-y average of the 99 th -percentile of 24-h mean concentrations (2012)
	PM _{2.5}	15	3-y average of annual mean concentrations (2012)	65	3-y average of the 98 th -percentile of 24-h mean concentrations (2012)
EU	PM ₁₀	40	(2005)	50	exceedance ≤ 35/year (2005)
		20	(2010)		exceedance ≤ 7/year (2010)

* Years in which standards and limit values have to be effective.

Mean values expressed as mass concentrations (µg/m³).

Promulgated/adopted in 1997 (USA) and 1998 (EU).

Next revision of standards and limit values in 2002 (USA) and 2003 (EU).

In 2003 EU will also consider the need for a limit value for PM_{2.5}.

A European multi centre study (APHEA I; Katsouyanni *et al.*, 1996; Katsouyanni and Touloumi 1998) on daily hospital admissions and mortality related to short-term exposure of (particulate)

air pollution has revealed a large number of data on regional differences in air pollution and health outcomes in adults and elderly. Total suspended particulates (TSP), black smoke (BS), and ozone (O₃) levels were positively but moderately associated with admissions for chronic obstructive pulmonary disease (COPD) in several western European cities with widely varying climates and air quality conditions (Anderson *et al.*, 1997; Spix *et al.*, 1998). O₃ levels were most strongly and consistently associated with daily admissions for respiratory causes, especially in elderly and during the summer season. Studies on total and cause-specific mortality in both Western and Central European cities (Katsouyanni *et al.*, 1997; Touloumi *et al.*, 1997; Zmirou *et al.*, 1998) revealed positive, but moderate associations between increased levels of PM, BS, and O₃ and excess daily number of deaths from cardiovascular or respiratory causes in Western European cities. In Central European cities similar associations were not observed. Collectively, these data point to regional differences in (particulate) air pollution-associated health effects in Europe and the data seem coherent with those from other studies, although the regression coefficients for PM are substantially smaller in these European studies compared to those from studies conducted in e.g. the USA.

The EU air quality objectives for PM₁₀ are stricter than in the USA air quality standards, resulting in the need in the EU for reductions in emissions from various sources to a much greater extent than formerly anticipated. For particulate matter it is estimated that emissions in cities will need to be reduced to ~ 50% of the present values. The new PM standards will be revised in 2002 (USA) and 2003 (EU) following a critical review of data from new studies on exposure, air quality, emission and source apportionment PM toxicity and health effects. In particular, in 2003 the EU will also consider whether the PM Daughter Directive should be adjusted or extended to control for e.g. the fine fraction of PM₁₀ (i.e. PM_{2.5}) or a source related PM fraction like (diesel) motor vehicle exhaust.

Toxicological studies on the identification of responsible particle size-fractions and components and on plausible views on biological mechanisms have to offer a rationale for the epidemiological findings and for effective control measures targeted on sources of the most important PM components. This plea for toxicological studies has also recently been published by the National Research Committee of the US National Academy of Sciences in order to guide the US Environmental Protection Agency towards a more targeted and intensified PM research (NAS, 1998). Similar needs will also be put forward in the Environment and Health Section of the 5th EU Framework Programme and has recently been identified in the European Science Foundation (ESF) position paper "Environment and Health Research for Europe" (ESF, 1998).

1.2 Epidemiological data on health effects

Epidemiological data suggest that current levels of particulate air pollution are statistically significantly and positively associated with adverse health effects. Lung function decline, increased respiratory symptoms, worsening of chronic obstructive pulmonary diseases (COPD), increased hospital admissions, as well as excess cardiovascular and respiratory morbidity and adult mortality appear to be associated with acute exposure to relatively low mass concentrations (below the current and proposed standards) of ambient particles (Tzonou *et al.*, 1992; Dockery *et al.*, 1993; Nitta *et al.*, 1993; Roemer *et al.*, 1993; Hoek and Brunekreef 1993, 1994; Dockery and Pope 1994; Van der Zee *et al.*, 1994; Dusseldorp *et al.*, 1995; Pope *et al.*, 1995; RIVM 1995; Moolgavkar *et al.*,

1995; Verhoeff *et al.*, 1996; Zmirou *et al.*, 1996; Dab *et al.*, 1996; Burnett *et al.*, 1997; Gielen *et al.*, 1997; Borja-aburto *et al.*, 1998). These studies indicate that associations are found at various geographical urban and rural areas with a rather large contrast in air quality conditions, like e.g. wood smoke, wind blown dust, industrial emissions, energy generation, (diesel) motor vehicle emissions, as well as acid aerosols. Many studies show associations with a number of indicators of ambient air and particle quality, like PM₁₀, black smoke, SO₄²⁻, and H⁺, but also with gaseous air pollutants like NO₂, O₃, CO, and SO₂. In a number of studies specific (aged) subpopulations with respiratory and cardiovascular diseases seem to be at increased risk to the adverse health effects associated with PM₁₀, possibly via worsening of their already compromised physiological conditions (Delfino *et al.*, 1997; Vedal *et al.*, 1998). An European multi centre study on short-term air Pollution related health Effects on Asthmatic Children (PEACE, Hoek *et al.*, 1997) did not show clear associations between PM₁₀, Black Smoke, SO₂ or NO₂ levels and peak expiratory flow, respiratory symptoms, and bronchodilator use (Roemer *et al.*, 1998). A recent study on short-term health effects of wintertime air pollution in asthmatic children in Paris showed positive associations between low levels of air pollution in general and SO₂ in particular, with increases of asthma and related (airway function) parameters (Segala *et al.*, 1998).

Associations between PM₁₀ and changes in cardiac rhythm are considered to be consistent with recent animal toxicity data on cardiovascular effects on concentrated PM_{2.5} and collected PM (Pope *et al.*, 1999). A recent study also presents data on the association between ambient PM₁₀ exposure and increased risk of postneonatal mortality in normal birth weight babies (Woodruff *et al.*, 1997). Epidemiological studies also showed that PM_{2.5} levels are positively associated with sensitive indicators of immunological alterations in children and adults, suggestive for a serious adverse effect of particulate air pollution on host defence regulations (Hahnagy *et al.*, 1998).

A number of epidemiological studies performed in the USA and Europe with adults and children also indicate statistical significant associations between long-term exposure to PM₁₀ or PM_{2.5} levels and increased mortality, lung function decline, and respiratory symptoms, in particular at urban areas with relatively high traffic density (Tzonou *et al.*, 1992; Dockery *et al.*, 1993; Nitta *et al.*, 1993; Wjst *et al.*, 1993; Edwards *et al.*, 1994; Pope *et al.*, 1995; Ackermann-Liebrich *et al.*, 1997; Braun-Fahrländer *et al.*, 1997; Abbey *et al.*, 1998). Analyses of causes of death revealed that lung cancer is also positively associated with PM₁₀ (Beeson *et al.*, 1998; Abbey *et al.*, 1999). A recent review (Katsouyanni and Pershagen, 1997) indicate that urban air pollution may be a risk factor for lung cancer with estimated relative risks up to about 1.5. Morphometric evaluation of main bronchus necropsy samples of individuals who died due to violent causes showed that lungs collected from a high PM pollution area demonstrated more histopathological damage in comparison with those from the clean area (Souza *et al.*, 1998) suggesting that PM may contribute to the pathogenesis of airway diseases.

The consistency of the epidemiological data base among different populations, air pollution situations, and weather patterns is even more remarkable because of the lack of knowledge on biological mechanisms for effects seen at the relatively low particle levels. The particles responsible for the observed adverse health effects are unknown but presumably present both indoors and outdoors and may consist of primary particles originating from stationary and mobile combustion sources as well as secondary particles from other sources (Schwartz, 1999).

Although not substantiated with data, it is believed that human health effects are associated more strongly with the fine fraction (0.1 – 2.5 μm) than with the coarse fraction (2.5 – 10 μm). It should be emphasised that it is not a general feeling. For example, this view is criticised because of serious measurement errors in the various PM size fractions (Lipfert and Wyzga, 1997). In addition, if corrected properly for the mass portion of PM_{2.5} in PM₁₀, associations with PM_{2.5} are about equally strong as with PM₁₀.

The question whether or not associations between health effects and ambient PM are causal or statistical is subject of intense dispute (Gamble and Lewis, *et al.*, 1996; McClellan and Miller, 1997; Lipfert and Wyzga, 1997). At present there are no indications for effective control measures targeted to specific particle components and sizes, emissions that contribute the most to the adverse health effects to reduce the human risk. A complicate issue is that different particle sizes and compositions may be related to different effects, depending on differences in deposition in airways and in chemical reactivity. Worsening of asthma may very well be related with larger particles, whereas edema formation, and subsequent cardiac function changes, may be the result of effects in the deep lung related to deposition of smaller particles there.

1.2.1 PM₁₀ size fractions and composition

Most of the epidemiological investigations on acute and chronic health effects have used the ambient PM₁₀ mass concentration as an exposure index. A limited number of studies have used PM_{2.5} mass levels as an index, however, the results show that on a microgram mass basis, compared to PM₁₀, the associations with PM_{2.5} are not stronger than can be expected on the PM_{2.5} mass content in PM₁₀. A very limited number of studies has used ultrafine particles (\sim PM_{0.1}) as an exposure index (Peeters *et al.*, 1997b; Pekkanen *et al.*, 1997; Penttinen *et al.*, 1998), but the results do not unequivocally suggest that the number concentration of ultrafine particles is associated with health effects in (asthmatic) children or adults and or that the positive associations are stronger compared to those with particle mass indices like PM₁₀, PM_{2.5}, TSP etc.

1.2.2 Traffic emissions

There is also evidence from epidemiological studies suggesting that long-term exposure to air pollution at urban areas with relatively high traffic intensity is associated with decreased lung function, increased prevalence of chronic respiratory symptoms like bronchitis and asthma, and lung cancer (Tzonou *et al.*, 1992; Dockery *et al.*, 1993; Nitta *et al.*, 1993; Wjst *et al.*, 1993; Pope *et al.*, 1995; Ackermann-Liebrich *et al.*, 1997; Braun-Fahrländer *et al.*, 1997; Abbey *et al.*, 1998, 1999; Beeson *et al.*, 1998). These relationships include associations with long-term levels of particulate pollution (TSP, PM₁₀, sometimes also including acid aerosols) and pollutants, which seem particularly, correlated with traffic emissions like e.g. NO₂.

A limited number of recently performed studies in the Netherlands showed that acute exposure to motor vehicle and (heavy) truck traffic emissions is positively associated with daily variation in chronic respiratory symptoms in children living near freeways (Van Vliet *et al.*, 1997; Brunekreef *et al.*, 1997). The data suggest that (heavy) motor vehicle emissions might be important in PM-associated health effects and that (ultra) fine particles could play a significant role.

1.3 PM₁₀ as a complex and heterogeneous mixture

Ambient particulate air pollution is a mixture of solid particles and liquid droplets that may vary in mass, size, and chemical composition, depending on the sources, and the meteorological conditions. PM levels relevant to human health effects are commonly expressed on the basis of the mass concentration of inhalable particles, defined to contain particles with an aerodynamic diameter equal to or less than 10 µm. This is because only these and smaller particles can penetrate into the airways and lungs. Air quality data have revealed that ambient aerosols have typical bimodal or trimodal mass or number distributions showing peaks occurring at coarse-mode, fine-mode, and ultrafine-mode particles (Whitby *et al.*, 1972; Annema *et al.*, 1994):

- a) The coarse fraction of relatively large inhalable particles, ranging from 1.0-2.5 to 10 µm, generally consists of particles which are mainly derived from soil (dust) and other crustal material and from mechanical wear processes. The generally chosen lower cut-off of 2.5 µm is arbitrary.
- b) Fine particulate fractions are defined to include particles with an aerodynamic diameter of about 0.1-2.5 µm. This fine-mode fraction contains a mixture of particles including carbonaceous material like soot (with possibly adsorbed reactive metals and organic compounds), and secondary aerosols like acid condensates, and sulphate and nitrate particles, and is derived direct or indirect primarily from combustion of fossil fuels used in power generation, and industry, and automobile engines. This fraction has the largest surface area and contributes also to the total particle mass concentration.
- c) The ultrafine of the particulates, with an average diameter of about 0.01-0.1 µm, is considered to be primarily derived from exhaust of automobile engines (diesel and otto types), however, its occurrence in ambient (urban) air and its mass contribution to the PM_{2.5} fraction is yet largely unknown. This fraction contains also the highest number of particles per volume.

The relative occurrence of the three particle modes in ambient air at various locations and during various air pollution circumstances is largely unknown. Whereas only slight increases in the levels of PM₁₀ are measured, there are indications that, during worsening of air pollution episodes, the mass and number concentrations of ultrafine particles may increase considerably (Hoekstra 1993; Oberdörster 1993; Bloemen *et al.*, 1995; Harrison *et al.*, 1996; Van der Wal and Janssen, 1996). Furthermore, it has been estimated that emissions from diesel- and gasoline-powered vehicles may contribute 20-40% of the ultrafine aerosol organic carbon emissions and this contribution is still increasing (Hildemann *et al.*, 1991; Curran *et al.*, 1993). This would suggest that (local) vehicle exhaust related emissions are a major source of ultrafine fractions, and may be also to PM-induced health effects. The precise contribution is obviously also dependent of the strength of this type of emission source and on meteorological conditions.

Epidemiology studies on ambient PM₁₀ have shown consistent health effects data from locations with a large contrast in emission sources, air quality conditions, and air quality indices. Across Europe large regional differences in the levels of particulate air pollution may exist, whereby Western PM levels are highly correlated with Central European levels and these levels are not correlated with Southern European and Scandinavian sites (Hoek *et al.*, 1997). PM₁₀/BS ratios between all these sites show large variations, with a tendency of lower ratios in urban areas with high (diesel) motor vehicle emissions, suggesting that BS might be considered as a better indicator for the carbonaceous emissions from traffic. The health impact of these regional differences in

particulate pollution and the specific role of motor vehicle exhaust (ultra)fine particles remains unclear and is not endorsed by epidemiological research (Hoek *et al.*, 1997).

Intra-site analyses in urban areas show that $PM_{2.5}$ is highly correlated with PM_{10} , but not with $PM_{10-2.5}$ and site-to-site correlations are high for $PM_{2.5}$ but not for $PM_{10-2.5}$ (Wilson and Suh *et al.*, 1997). These observations are in good agreement with the different emissions from the source-types from which they originate. These authors conclude that epidemiological studies using PM_{10} may provide useful information on health effects of fine rather than coarse particles.

It must be stated clearly that PM_{10} and $PM_{2.5}$ are not single components, but complex and heterogeneous mixtures, varying in particle size and composition, dependent on location, weather conditions, season, and sources and emissions. Many components may be adsorbed to the carbon core like e.g. acids, partly neutralised salts, aliphatic and (polycyclic) aromatic organic compounds, sometimes in oxidised forms, metals (heavy metals, transition metals), and biomaterial like allergens, pollen fragments, and endotoxins. The relative importance of all these components for health effects is largely unknown. A simplified impression of the complex chemical and structural organisation of PM is shown in Fig. 1. Toxicity testing of the complex ambient PM mixture requires prudent research, that can apply sophisticated statistical designs and evaluation of results (Cassee *et al.*, 1998).

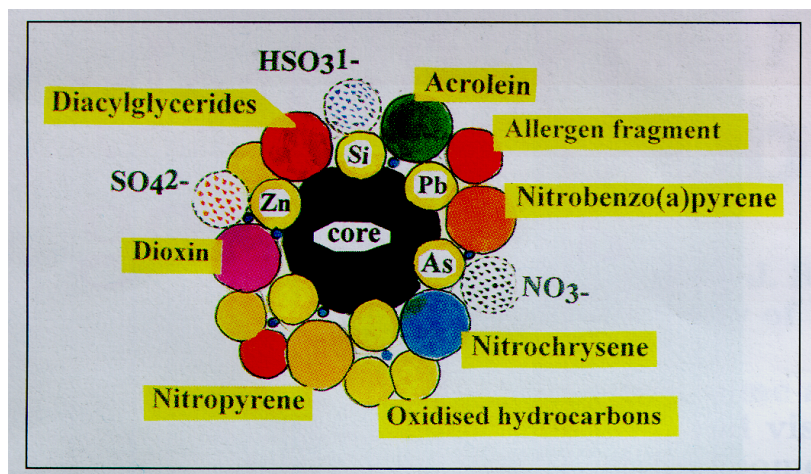


Figure 1 A simplified artistic illustration of the possibly complex chemical heterogeneity of ambient air PM and its suggested (bio) organic and inorganic components (Richards, 1997).

1.4 Important questions related to plausible mechanisms and critical causative components

Several questions, which have been considered as highly important (NAS 1998; ESF, 1998), have to be answered in the years to come by targeted toxicology studies on PM_{10} and fractions of PM_{10} . In addition to studies focused on acute effects, also mechanisms underlying chronic effects should be investigated. The most important questions can be formulated as below:

1. Can the epidemiological data on acute health (cardiorespiratory) effects of PM₁₀, PM_{2.5}, and ultrafine particles be confirmed in toxicological studies?
2. Do toxic effects depend on specific ambient air pollution situations and on dominant source categories as e.g. traffic emissions? In other words, which chemical-biological properties and compositions of PM do play a role?
3. To what extent do primary carbonaceous, secondary inorganic and non-antropogenic aerosols contribute to human health effects?
4. What is the specific role of gasoline or diesel motor exhaust particles?
5. Is the air pollution mixture of particles and gases relevant to the health effects?
6. What are the biological mechanisms underlying adverse health effects from ambient PM?
7. Are the results of toxicological studies sufficiently representative and do they provide sufficient plausibility for human health effects at ambient levels with respect to
 - a) exposure-deposition-dose-effect relationships,
 - b) possibly threshold levels,
 - c) biological mechanisms and human subpopulations at increased risk?
8. Can toxicology studies improve our understanding of the health effects associated with long-term exposure to ambient particulate matter and do acute these studies provide early markers for these effects?
9. To what extent do indoor PM play a role?

This report evaluates and reviews the toxicological literature on particulate air pollution, the current hypotheses in ambient PM toxicity, the various proposals for possibly important PM fractions, the current concepts of proposed biological mechanisms of action, and the deposition estimations on particle of various sizes. An effort is made to present a current view on causality and plausibility explaining the human health effects associated with PM exposure.

2. Potential PM properties and mechanisms underlying PM₁₀-associated health effects

2.1 Toxicological data on various PM₁₀ fractions, components and specific sources

Most of what is known on the toxicity of (ultra)fine PM is derived from chronic exposure studies and the use of high (overload) concentrations of e.g. TiO₂, Al₂O₃, carbon black and silicates (Nolte *et al.*, 1991/1993; Oberdörster *et al.*, 1994; Mauderly *et al.*, 1994; Nikula *et al.*, 1995; Driscoll *et al.*, 1996). Studies with H₂SO₄ and polyfluortetrafluoroethylene pyrolysate have indicated pulmonary toxicity of (ultra)fine particles (Chen *et al.*, 1995; Oberdörster *et al.*, 1996). Some of these studies also suggest that the number concentration and large surface area of the (ultra) fine particles, rather than the mass, correlate with pulmonary toxicity like irritant potency, inflammation, edema, fibrosis, and carcinogenesis (Ferin *et al.*, 1992; Oberdörster *et al.*, 1992; Oberdörster 1993; Oberdörster *et al.*, 1995). The relatively high surface reactivity of these (ultra) fine particles is suggested to be linked with its acidic nature or oxidative ability, the latter possibly through adsorbed reactive organic compounds and metal ions (Oberdörster 1993; Utell and Samet 1993). Among the proposed mechanisms underlying these responses, a significant role for inflammation and cell proliferation has been postulated involving free (oxygen) radical formulation. Attention has also been given to the strong oxidative potential of (ultra) fine diesel exhaust particles, an important part of the carbonaceous ultrafine fraction of particulate matter. These particles show to be tumorigenic in the respiratory tract of animals and are considered to be causally linked with development of asthma and chronic obstructive pulmonary disease, as well as with edema formation (Sagai *et al.*, 1993; Heinrich *et al.*, 1995; Ichinose *et al.*, 1995). Most of the above mentioned experimental animal studies have been performed with healthy.

Recent experimental animal studies using intratracheal instillation (2.5-5 mg/rat) as well as *in vitro* incubation of fine or coarse PM fractions from various sites (~ 0.4-10 µm; mineral dusts, PM₁₀ collected on filters from ambient air, fly ash samples) cause pulmonary cytotoxicity, inflammation, and production of reactive oxygen species (Hatch *et al.*, 1985; Ghio *et al.*, 1992; Ghio and Hatch 1993; Gilmour *et al.*, 1996b; Pritchard *et al.*, 1996; Li *et al.*, 1996, 1997; Donaldson *et al.*, 1997). A limited number of these studies give indications that toxicity of ambient PM may correlate with particle size, particle (oxy) reactivity and transition metal composition (Pritchard *et al.*, 1996; Dreher *et al.*, 1996; Nadeau *et al.*, 1996; Donaldson *et al.*, 1997; Vincent *et al.*, 1997; Goegan *et al.*, 1998). In general, however, there is at present no evaluated inhalation toxicity data base that could:

- a) explain the exposure-response relationships at relatively low PM levels as observed in epidemiological studies,
- b) give a basis on the causal PM fractions and components (particle mass, numbers, sizes and chemical-biological composition related to sources, and
- c) provide plausible biological mechanisms understanding of adverse health effects in human risk populations.

2.2 PM₁₀ size fractions and composition

A number of PM components have been considered as possibly responsible for the adverse health effects of PM, but none of them seems complete causality and plausibility.

Possibly important PM₁₀ size fractions and components

- Fine secondary acid particles or acid-coated particles (“acids”)
- Fine carbonaceous particles with soluble (oxy) reactive components (“metals”, “organics”)
- Insoluble ultrafine particles (“ultrafines”)
- Particles contaminated with biological components (“bio-organics”)
- Particles and gases combinations (“mixtures”)

The evidence for these most likely particle hypotheses are described below. These data mainly originate from controlled animal and human inhalation studies with specific PM components or, to a limited extent, PM sampled by filters from ambient air.

2.2.1 Acid (coated) particles

Epidemiological findings have demonstrated associations of health effects and short-term exposure to ambient air pollution indicated by levels of sulphate and hydrogen ions. This observation supports a hypothesis that sulphuric acids and their (partly) neutralised salts, mostly present in the range of fine particles, might be causally related to the health effects. Data on controlled human-clinical and laboratory animal exposures are often advocated as supportive for this hypothesis (Utell and Samet 1993; Schlesinger and Chen, 1994; Schlesinger 1995).

Human clinical studies remain almost completely limited to the study of acid aerosols, primarily of H₂SO₄, with the majority of these focussing on respiratory symptoms and pulmonary function. A number of studies have confirmed previous findings that healthy subjects do not experience decrements in lung function following single exposures to H₂SO₄ of various particle sizes at levels up to 2,000 µg/m³ for 1 h, even with exercise and prevention of neutralization by ammonia in the mouth. Mild lower respiratory symptoms occur at exposure concentrations in the mg/m³ range, particularly with larger particle sizes. Acid (H₂SO₄) aerosol exposure can decrease bronchial mucociliary clearance and pulmonary immune defence in healthy subjects at levels as low as 100 µg/m³ (Leikauf *et al.*, 1984; Zelikoff *et al.*, 1994, 1997).

Asthmatic subjects appear to be more sensitive than healthy individuals to the effects of acid aerosols on lung function. Adolescent asthmatics have small decrements (6-8%) in lung function in response to H₂SO₄ at exposure levels above peak ambient levels (70-110 µg/m³ for 30-40 min plus 10 min exercise; MMAD = 0.6) (Koenig *et al.*, 1983, 1989). Even in studies reporting an overall absence of effects on lung function following acid exposure, occasionally asthmatics appear to show clinically important effects.

Although airway inflammation in asthmatic subjects is considered to play a role in the exacerbation of asthma, no studies have examined the effects of acid aerosol exposure on this type of effects. There is little, if any, effect of low-concentration acid aerosol exposure (regardless of particle size) on airway responsiveness in healthy or asthmatic subjects. In the few studies documented, elderly people and individuals with COPD (chronic obstructive pulmonary disease) do not appear to be particularly susceptible to the effects of submicron acid aerosols on lung function.

Very high concentrations ($> 4000 \mu\text{g}/\text{m}^3$) of acid sulphates are required to cause mortality in otherwise healthy animals, depending on acid particle mass and size and the animal species tested. In general, exposure to H_2SO_4 at levels $<1000 \mu\text{g}/\text{m}^3$ (MMAD $>0.4 \mu\text{m}$) does not produce physiologically significant changes in standard tests of pulmonary mechanics. However, concentrations as low as $100 \mu\text{g}/\text{m}^3$ of H_2SO_4 (MMAD = $0.08 - 0.3 \mu\text{m}$) altered the respiratory tract clearance after 1 h (Chen *et al.*, 1991; Zelikoff *et al.*, 1997) in guinea pigs. Some small effects on morphology were observed after exposing rabbits 1-2 hr/day for 1 year (Schlesinger *et al.*, 1992). The relative potency of (partly) neutralised acid to induce similar effects is always smaller than H_2SO_4 . Emphysema was observed after exposing rats during 6 h/day for 15 weeks to $(\text{NH}_4)_2\text{SO}_4$ (Godleski *et al.*, 1984). There are no data available of laboratory animals with compromised airways exposed to acids.

Recent animal studies show that, besides mass concentration, also the number concentration of sulphuric acid particles is important for effects (Chen *et al.*, 1993, 1995a). Chen *et al.*, (1992, 1995b) also demonstrated that short-term inhalation of H_2SO_4 -coated (ultra)fine particles like carbon or ZnO enhances the toxic potency of the acid with respect to decreased alveolar macrophage function, compared with the same concentration of H_2SO_4 delivered for inhalation as single particle or droplet. These acid-coated particles caused effects at concentrations of 20-30 $\mu\text{g H}_2\text{SO}_4/\text{m}^3$. In addition, these data showed again that acid aerosols cause only minor pulmonary inflammatory responses and changes in lung tissue morphology. Studies were conducted to investigate possible differences between ammonium sulphate and ammonium nitrate aerosols. These data showed that several weeks inhalation exposure resulted in lung function decrements and cellular and immunologic changes with the general response order of sulphate $>$ nitrate, except for lung permeability and oedema formation, which responded most to the nitrate (Loscutoff *et al.*, 1985; Kleinman *et al.*, 1995). Long-term exposure studies in healthy dogs with particle-associated neutral sulfur (IV) and acidic sulfate and hydrogen ions caused only marginal respiratory and pulmonary effects but no pathological changes of severe nature, supporting a relatively less potent and maybe even antagonistic role of these components in inducing chronic effects following long-term exposure (Heyder *et al.*, 1999, and references cited therein).

2.2.2 Particles coated with soluble (reactive) organic or metallic components

Recent experimental animal studies show that in healthy animals and animals with cardiopulmonary diseases using intratracheal instillation (high dosage of 1-5 mg/rat of fine and coarse particles ($\sim 0.4-10 \mu\text{m}$; mineral dusts, PM collected from ambient air or from anthropogenic, combustion sources (fly ash)) cause cytotoxicity, inflammation, and production of oxygen radicals in lungs as well as myocardial toxicity and arrhythmic changes (Hatch *et al.*, 1985; Ghio *et al.*, 1992; Ghio and Hatch 1993; Li *et al.*, 1996, 1997; Watkinson *et al.*, 1998; Killingworth *et al.*, 1997; Kodavanti *et al.*, 1997; Murphy *et al.*, 1998). The oxidative potency of these types of particles to induce lung toxicity *in vivo* such as iron ions adsorbed to titaniumoxide, ambient PM

from various locations, or residual oil fly ash, seems to be proportional with the content of some of the 'first-row' transition metals associated with the particles (Keeling *et al.*, 1994; Becker *et al.*, 1996; Gilmour *et al.*, 1996; Li *et al.*, 1996; Pritchard *et al.*, 1996; Costa and Dreher, 1997; Kodavanti *et al.*, 1997; Dreher *et al.*, 1997; Adamson *et al.*, 1999). These findings support the view that ambient PM₁₀ has free (oxygen) radical activity causing lung inflammation and tissue injury. Also humic-like (organic) substances of a (semi)quinone-type molecular structure have been suggested to be the similar causal factor for diesel and other carbonaceous particles, as well as for ambient PM (Sagai *et al.*, 1994; Ichinose *et al.*, 1995; Kumagai *et al.*, 1995; Ghio *et al.*, 1996). All these organic and metallic components are able to participate in electron transfer and redox cycling reactions resulting in free radical generation (O₂⁻, OH, lipid peroxides). Ambient particle chemistry via solubility or ionizability of these components seems to be a prerequisite for (oxidative) reactivity, as demonstrated with extracted or chelated PM samples which lose their biological activity as well as with positive correlations of the effects with the content of soluble components. It has been demonstrated from *in vitro* experiments reported by Ghio *et al.* (1999) that the secondary PM aerosol component sulfate may play a certain connecting role in the generation of oxygen-based free radicals by PM by functioning as a ligand for the particle-associated iron. This might suggest a possible role of secondary aerosol components like sulfate in PM health effects, not by acting as a direct toxicant but by facilitating toxicity of reactive metal constituents. This option warrants further studies.

A number of *in vitro* studies using PM₁₀ fractions sampled from ambient air and incubations with cultures of human airway and lung cells has also been performed. These data show that residual oil fly ash (ROFA), TSP, PM₁₀ and PM_{2.5} have soluble transition metal- or endotoxin-mediated free radical activity, causing oxidative stress, inflammation, and immunotoxic responses (Donaldson *et al.*, 1996; Becker *et al.*, 1996; Gilmour *et al.*, 1996; Donaldson *et al.*, 1997; Samet *et al.*, 1997; Vincent *et al.*, 1997; Becker and Soukup, 1998; Bonner *et al.*, 1998; Frampton *et al.*, 1999). Metal solubility may play a dominant role and PM fractions < 2.5 µm show a larger solubility and metal release than PM fractions > 2.5 µm (Smith *et al.*, 1998). Recently however, Becker and Mohn (1998) presented *in vitro* data suggestive of coarse ambient PM being more potent than fine PM to provoke inflammatory responses, in which endotoxin may play an important role. Also *in vitro* data from Hornberg *et al.*, (1998a,b) on genotoxicity of ambient fine and coarse PM collected from an urban area characterized by a high traffic density, suggests that coarse PM may have comparable or even higher activity than fine PM. The data indicate that biological effects of coarse fraction PM cannot be excluded yet.

2.2.3 Ultrafine insoluble particles

This hypothesis considers the possibility that ultrafine (< 0.1 µm) insoluble particles, freshly generated in ambient air by combustion processes or by gas-particle conversion reactions, are responsible for adverse health effects. Evidence supporting this hypothesis is originating from animal inhalation studies with highly toxic singlet particles in an aerosol mixture from pyrolysis of polyfluortetrafluoroethylene (~ 20-30 nm, ~ 9 µg/m³) (Oberdörster *et al.*, 1996). Earlier studies with ultrafine TiO₂ also showed marked pulmonary inflammation, possibly related to their large surface area and increased interstitial access (Oberdörster *et al.*, 1992). Oxidative cardiopulmonary damage and changes in blood coagulability are proposed as the pathogenic mechanism for these types of highly toxic particles (Oberdörster *et al.*, 1995; Seaton *et al.*, 1995; Adamson and Prieditis, 1995).

A recent inhalation study with short-term exposures to near ambient mass and number concentrations of ultrafine metal oxide and carbon particles failed to show any pulmonary biological response in healthy rats. This suggests that the generic, physical nature of ultrafine particles are not important for pulmonary effects (Roth *et al.*, 1998; Ziesenis *et al.*, 1998). Whether similar effects will also be seen in animal models for cardiopulmonary diseases, remains to be determined (Roth *et al.* 1998; Ziesenis *et al.*, 1998).

It is unclear whether earlier studies with non-environmentally relevant ultrafine particles such as titaniumoxide, aluminiumtrioxide, quartz, and carbon black can contribute to this 'ultrafine hypothesis', because most of these studies focused on tumor formation and lung fibrosis. In addition, these chronic exposures resulted in so-called "overload" conditions triggering an inflammatory and/or genotoxic response.

There is no evidence so far for a role of environmentally relevant levels and types of ultrafine particles in acute morbidity and mortality associated with exposure to ambient PM, except for diesel exhaust particles and also carbon black (Li *et al.*, 1999). It should be noted that also in those (instillation) studies extremely high doses were used. Sceptics argue against such a role because of the very tiny masses of these particles in ambient air. However, if numbers of particles are more important than mass or surface area, which is still unknown, ambient ultrafine particles might have a role in inducing adverse health effects. Preliminary data from epidemiological studies carried out in Germany (Peeters *et al.*, 1997) suggest that health effects show stronger associations with the number concentrations of ambient PM than with mass concentration of PM₁₀. However, a similar study performed in Finland (Pekkanen *et al.*, 1997) failed to show a relationship between health effects and PM number concentrations. On the other hand, the same group (Penttinen *et al.*, 1998) found a statistical association between numbers of particles in ambient air and asthma responses.

2.2.4 Biological components of PM

Another aspect of adverse health effects by PM, which has not received much attention yet, is that of motor vehicle exhaust-related PM could have the potential to act like an adjuvans in the immune response and would enhance allergic sensitisation. There are a limited number of data from mechanistic animal toxicity studies with diesel exhaust particles, which show that this could play a role in e.g. exacerbation of asthma-type response, either by influencing the cytokine production (Diaz Sanchez *et al.* 1996; Diaz Sanchez *et al.* 1997; Diaz Sanchez, 1997), by enhancing the IgE response (Diaz Sanchez, 1997) or by acting as vectors for submicron fragments of pollen grains which would otherwise too small to deposit in human airways (Knox *et al.*, 1997; Behrendt *et al.*, 1992; Behrendt *et al.*, 1997).

2.2.5 Combination of particles and gases

A number of experimental studies has suggested that combined exposure to fine (acidic) particles and (in)organic gases or vapors, like e.g. O₃, NO₂, SO₂, HNO₃, aldehydes, caused acute effects in lower airways (Last *et al.*, 1986; Warren *et al.*, 1986; Last and Warren, 1987; Jakab, 1992; Jakab *et al.*, 1993; Jakab and Hemingway, 1994; Creutzenberg *et al.*, 1995; Hemingway *et al.*, 1996; Vincent *et al.*, 1997; Bolarin *et al.*, 1997). The results of these studies suggest that 1) particles can act as a carrier delivering toxic amounts to the target sites i.e. deep lung, or 2) that the life of free

radicals arising from the interaction of oxidants (O₃, NO₂) with biomolecules in the lung is increased during acidic conditions.

All these studies have been conducted at relatively high particle mass concentrations. There is at present no clear view on whether this type of gas-particle interactions also occur at environmentally relevant concentrations. Recently, a 4-week inhalation study of 0.5 µm particles combined with exposures to carbon black (50-100 µg/m³), ammonium bisulphate (70 µg/m³), and ozone (300 µg/m³) was conducted. The results show more deleterious effects than exposure to the components alone (Kleinman *et al.*, 1996; Bolarin *et al.*, 1997). These effects included decreased alveolar macrophage function, increased lung collagen concentration and lung cell turnover rates, although without indications for increased lung permeability and inflammation. An *in vitro* study focused on pre-exposure to ozone and subsequent exposure to mineral particles, has revealed that ozone is directly stimulating the uptake of particles in airway cells. This might play a role in impaired particle clearance from the lungs and might further contribute to increased morbidity following exposure to both O₃ and ambient particles (Churg *et al.*, 1996).

Collectively, although the evidence is rather limited, these data suggest that the mixture of air pollution (PM plus oxidant gases) might be more important than PM alone. Moreover, it might be suggested that PM modulates the adverse health effects induced by (oxidant) gases.

2.3 Traffic emissions

Of particular relevance for the issue of health effects of ambient PM is the exposure to particulate emissions from motor vehicle sources, e.g. diesel exhaust. Most of what is known about long-term carcinogenic effects of exposure to diesel exhaust comes from epidemiological occupational studies (for a review see HEI 1994; Heinrich *et al.*, 1995). It has been recently recognised from experimental animal studies that e.g. tumorigenic effects of relatively high exposure concentrations of diesel exhaust are not related to the exposure of the PAHs adsorbed onto the particle, but the effects are more likely the result of the surface reactivity of the particles (Nikula *et al.*, 1995). In addition, longer-term exposure of laboratory animals to diesel engine exhaust can also result in non-carcinogenic, structural airway tissue damage and allergic airway inflammation and hyperresponsiveness, for which the particle fraction seems important (Nagai *et al.*, 1996; Sagai *et al.*, 1998; Lim *et al.*, 1998).

Short-term effects of exposure to diesel exhaust particles have also drawn attention. Experimental animal data show lung function impairment, increased airway responsiveness, pulmonary edema, impairment of alveolar macrophage function, and enhancement of IgE antibody production following instillation (0.4 – 0.8 mg/mouse) or inhalation (3.0 – 6.0 mg/m³) of diesel exhaust particles (Sagai *et al.*, 1994; Ichinose *et al.*, 1995; Fujimaki *et al.*, 1997; Ohta *et al.*, 1999). In addition, there is also recent human evidence that controlled short-term inhalation of diesel exhaust, below occupational exposure limits, causes inflammatory responses in the respiratory tract and lung function decrements (Rudell *et al.*, 1990, 1996; Blomberg *et al.*, 1998; Salvi *et al.*, 1999).

A number of *in vitro* studies with incubation of airway cells and diesel exhaust particles shows that these particles are capable of inducing inflammatory-like responses and may also act as adjuvans enhancing the allergic responses (Tkamo *et al.*, 1997; Steerenberg *et al.*, 1997; Bayram *et al.*, 1998; Fujieda *et al.*, 1998).

These data on diesel exhaust PM demonstrate respiratory toxicity at relatively low mass levels, proposing a possible role in ambient PM induced adverse health effects.

2.4 Real-world ambient PM

Data from field studies with contrast in traffic density and PM levels in Sao Paulo and Florence (experimental animals) and Mexico City (humans) have shown that serious histopathological changes in upper and lower airways can occur. These effects may be linked with effects on the immune system and respiratory morbidity and mortality as found at those locations in epidemiological studies (Böhm *et al.*, 1989; Calderon-Garcidueñas *et al.*, 1992, 1993; Lemos *et al.*, 1994; Saldiva *et al.*, 1992, 1994; Gulisano *et al.*, 1997). The relative contribution of traffic-related PM exposure compared to gaseous pollutants is not clear but is expected to be substantial.

The recent development of ambient PM concentrators seems to provide promising opportunities for toxicity studies with 'real-world' ambient particulates under relatively controlled conditions (Sioutas *et al.*, 1995, 1997). This concentrator is able to increase the concentration of ambient PM in the particle size range of 0.15-2.5 μm to levels about 25 times their ambient values and to supply it to an exposure chamber for controlled animal and human studies. Data for New York and Boston have been reported in proceedings of symposia and some recent peer-reviewed journal papers (Godleski *et al.*, 1996; 1997; Gordon *et al.*, 1998a,b; Zelikoff *et al.*, 1998; Clarke *et al.*, 1999; Goldsmith *et al.*, 1999). Using rat models of pulmonary hypertension or chronic bronchitis, short-term exposure to concentrated PM_{0.1-2.5} (2-6 hr/day; 3 days; mass levels ranging between ~50-700 $\mu\text{g}/\text{m}^3$) reveals sometimes positive sometimes negative results on (extra)-pulmonary effects for which no explanation has been found yet. Many current ambient air PM toxicity studies are therefore focussed to find a better effect-relevant exposure indicator, which might be particle chemistry and composition. In general, the experimental ambient PM exposure studies frequently show additional decrements in lung function, airway hyperresponsiveness, increases in airway inflammation and mucus secretion, edema, specific electrocardiogram changes related to cardiac dysfunction, as well as mortality (all compared to similar exposures of healthy animals). Similar effects were seen with concentrated PM exposures of healthy dogs (personal communications Sioutas and Godleski). If these types of effects would have occurred in humans, it would have formed a plausible indication for the increased morbidity and mortality as observed in epidemiological studies and a first sign of a causal link of ambient PM_{2.5} exposure and adverse human cardiac-respiratory effects.

2.5 Host responsiveness

The data base on health effects of ambient particulate matter points to specific host responsiveness. Increased mortality, morbidity, and respiratory symptoms and complaints (cough, irritation) are particularly found in specific subgroups of the general population. These risk groups may include 1) elderly people, presumably with a weak physical condition, 2) children, and 3) people with pre-existing cardio-respiratory diseases like congestive heart disease, pulmonary hypertension, asthma, chronic bronchitis, emphysema, as well as airway infections. Exposure to PM is suggested to result in an exacerbation of these diseases, possibly resulting in excess morbidity and mortality. The mechanism underlying the finding that pre-existing cardio-respiratory disease is a major risk factor for health effects of ambient PM may be partly based on dosimetry and biological sensitivity. Increased PM deposition in compromised airways, larger impairment of lung particle clearance, as well as a higher biological sensitivity of compromised lungs compared to healthy lungs, have been suggested as contributing mechanisms (Bohning *et*

al., 1982; Miller *et al.*, 1995; Freijer *et al.*, 1997; Kim *et al.*, 1997). It is likely that the responses of the diseased or compromised cardio-respiratory system to inhaled PM are greater than those in healthy lungs. This may lead to a considerable shift in the dose-response curve towards lower PM doses, resulting in an increased risk of morbidity and death in the most susceptible groups.

2.6 Deposition and clearance of PM in airways and lung

The process from exposure concentration to respiratory tract (RT) dose is called deposition. Particle deposition in the RT may depend on radial diffusion, gravitational sedimentation, and impaction and interception, but also on properties of particles like chemical reactivity and hygroscopicity. This implicates that the size and nature of a particle determines the fate in the airways. Fig. 2 shows that the total deposition fraction of coarse mode particles (2.5-10 μm) and ultrafine mode particles (<0.1 μm) are significantly higher compared to the fine mode (0.1 - 2.5 μm) for both human and rat. In general, alveolar deposition increases with decreasing particle size (Fig. 2). Recent calculations for a typical urban PM₁₀ aerosol of the fine and coarse particle mass and number doses in different airway and lung regions show striking differences between the two particle fractions (Venkataraman and Kao, 1999). Fine particles prefer to deposit in the lower airways and lungs, whereas coarse particles deposit more in tracheobronchial regions and upper airways.

Table 2. Averaged deposition in human and rat lung (tracheo-bronchial plus alveolar regions) for fine mode PM (0.1 – 2.5 μm)¹.

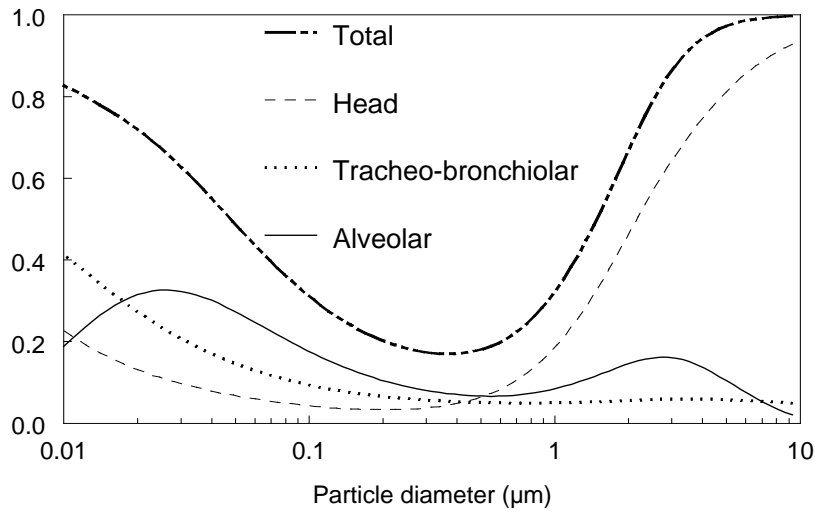
	HUMAN	RAT		Ratio
<u>Input values</u>				
Tidal volume (TV)	500	1.5	ml	
Breathing frequency (Bf) ²	20	130	min ⁻¹	
Exposure concentration (C)	0.1	1	mg/m ³	
Exposure duration (t)	24	4	hr	
Deposition fraction (f _d)	25	20	%	
Body weight ²	75	0.25	kg	
Lung surface ³	640581	3461	cm ²	
<u>Calculations</u>				
Deposition (TV x Bf x C x t x f _d)	1.44	0.047	mg	
Correction for deposition efficiency	0.36	0.009	mg	40
Correction for body weight	4.8	37.4	mg/kg	0.13
Correction for lung surface	0.56	2.70	ng/cm ²	0.21

¹ Calculations are based upon data from Anjilvel and Ashgarian (1995), using the Multiple-Path model for Particle Deposition (version 1.1), developed by RIVM and CIIT (Cassee *et al.*, 1999).

² Standardised value for a male.

³ Values from Jarabek *et al.*, (1989).

A. Human



B. Rat

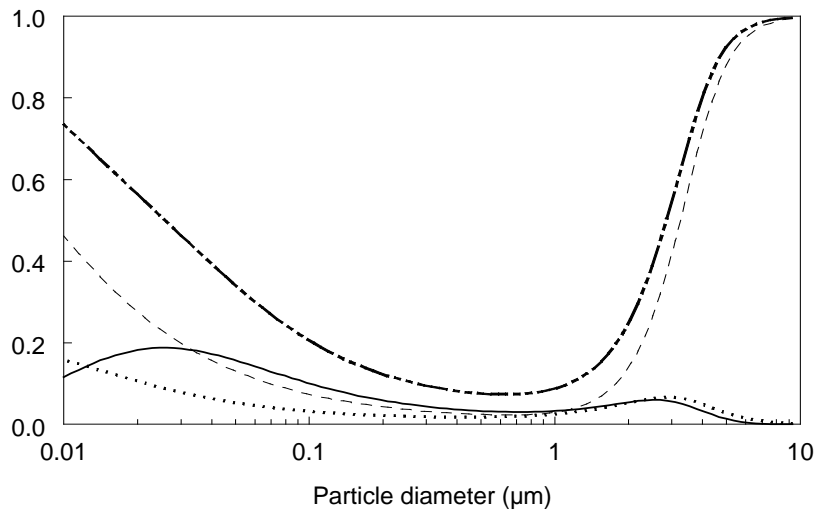


Figure. 2 The deposition fraction in alveolar, tracheo-bronchiolar, and head regions and the total deposited fractions in human (A) and rat (B) for an adult male inhaling monodisperse aerosols consisting of particles of different sizes. Results are based on calculations with the MPPD computer programme developed by the Chemical Industry Institute of Toxicology and RIVM (Anjilvel and Asgharian, 1995). Default values are listed in Table 1, except for the particle density for which 1 g/cm^3 has been used.

On interpreting data of animal inhalation toxicity studies and compare it with data from human studies, it is more important to consider the inhaled dose rather than the exposure concentration. Pulmonary deposition fractions e.g. are roughly twice as high in humans compared to rats. Furthermore, it can be estimated that rat inhalation toxicity studies with exposures up to several mg per cubic meter of (resuspended) TSP, PM₁₀ or PM_{2.5} will result in comparable inhaled dose estimates in airways as can be expected from a plausible human exposure scenario (Anjivel and Ashgarian 1995, Vincent *et al.*, 1997).

The ventilation rate of a person varies largely over the day, depending on the level of activity and the oxygen requirement related to this activity. Differences exist between subjects, depending on body mass, the presence of pulmonary disease and smoking habit. In general, subjects with compromised airways or smokers breathe at a higher rate than healthy people and non-smokers (Tobin *et al.*, 1983). Children have a much lower ventilation rate than adults. It has been estimated by particle deposition modelling that in compromised airways the deposition pattern of particles is changed compared with healthy airways (Miller *et al.*, 1995; Kim *et al.*, 1997). Due to increased ventilation and altered airway geometry, the total deposition may be enhanced and shifted toward the alveolar region. The finding that a pre-existing cardiopulmonary disease and worsening of the pathological condition is a major risk factor for health effects of PM seems therefore partly explained by deposition calculations. This suggests that particle deposition may be markedly increased in people with COPD or asthma compared to healthy lungs (Miller *et al.*, 1995; US-EPA 1995; Freijer *et al.*, 1997). Using the ICRP model, it can be estimated that the overall deposition of various PM size fractions in compromised airways is suggested to be ~ 3-5 times compared to healthy airways (Miller *et al.*, 1995; see Table 3 below (Freijer *et al.*, 1997)). It might be assumed that locally deposition is much higher in compromised versus healthy lungs.

*Table 3. Calculations of total deposited dose of ultrafine, fine and coarse PM fractions in human airways and lungs, using the ICRP model and expressed relative to the dose in a healthy adult male at light exercise and standardised to respiratory tract tissue mass (Freijer *et al.*, 1997)*

Subjects*	Ambient PM fractions		
	Ultrafine < 0.1 µm	Fine 0.1-2.5 µm	Coarse 2.5-10 µm
▪ Adult male	1.0	1.0	1.0
▪ Children, < 10 year old	1.2 - 1.7	1.1 - 2.5	1.4 - 1.7
▪ Adult male			
at rest or sleep	0.3 - 0.4	0.2 - 0.4	0.3 - 0.4
at heavy exercise	1.9 - 2.0	1.4 - 1.7	2.0
▪ Adult male with COPD	4.0 - 4.4	3.7 - 5.0	4.2 - 4.3

* at light exercise, unless stated otherwise

Once deposited, in particular insoluble particles have to be cleared and clearance rates will affect the retention time and consequently the pulmonary toxicity. In general, deposited particles are cleared at varying rates depending on the airway compartment, favouring a lower clearance in lower airway regions. Physical exercise will enhance deposition in the respiratory tract. Remarkably,

humans have considerably longer retention halftimes than rats and most other rodent species. There is evidence suggesting that inhaled particle clearance is impaired in disease-compromised lungs (Bohning *et al.*, 1982; Chen *et al.*, 1991; Zelikoff *et al.*, 1997)

2.7 Possible biological mechanisms of action of PM

Little is known about the biological mechanisms underlying the health effects of ambient PM and the increased host responsiveness in specific subgroups of the population. A number of PM toxicity investigations earlier reviewed in this chapter were initiated with respect to study biological mechanisms underlying ambient air PM-associated health effects. From these studies and the limited number of epidemiological studies focusing on specific end points, a number of hypotheses on mechanisms can be put forward according to whom these effects might occur:

Possible biological mechanisms of health effects of PM₁₀

- Oxidative stress
- Airway inflammation
- Oedema formation
- Impaired gas diffusion resulting in hypoxic stress
- Cardiac (right ventricle) dysfunction and impaired pulmonary circulation
- Increased plasma viscosity and blood coagulation
- Immunotoxicity
- Neurological dysfunction

The specific role of airway epithelial cells and alveolar macrophages in these processes is getting particular attention because the activation of these cells upon contact with ambient air particles might result in a subsequent release of pro-inflammatory and pro-coagulant mediators and of reactive oxidant species which may interact with other cells, and damage the epithelium, resulting in systemic effects (Moody, 1993; Oberdörster, 1993, Driscoll *et al.*, 1997). Alveolar macrophages are considered to play a crucial role in the pathogenesis of structural changes in airways. Inhaled particles are phagocytosed and transported by alveolar macrophages for effective removal and by phagocytosing macrophages become activated and induce a cascade of beneficial or pathogenic events. Macrophages, at their protective role against foreign bodies, also via the immune system, thereby function normally producing various (oxygen) radicals, proteins and lysosomal enzymes, all orchestrating effectively the inflammatory and immune responses and tissue repair processes. It has recently been suggested that perhaps the very first airway effects of oxidants and particles might be caused by the airway epithelium. Pulmonary epithelium has been shown to be able to release chemotactic and other inflammatory mediators upon a toxic insult, which can trigger a further response resulting in an involvement of inflammatory cells and a cascade of pathogenic events leading to tissue injury. At normal functioning, it is proposed that macrophages and epithelium usually deactivate themselves by release of other, down regulating mediators. It is hypothesised that

unregulated or persistent release of these and other factors by macrophages and compromised epithelium may cause structural airway tissue injury, fibrosis, and possible neoplasms. A schematic view on these proposed mechanisms of oxidative stress, inflammation, and pro-coagulant status is shown in Fig. 3.

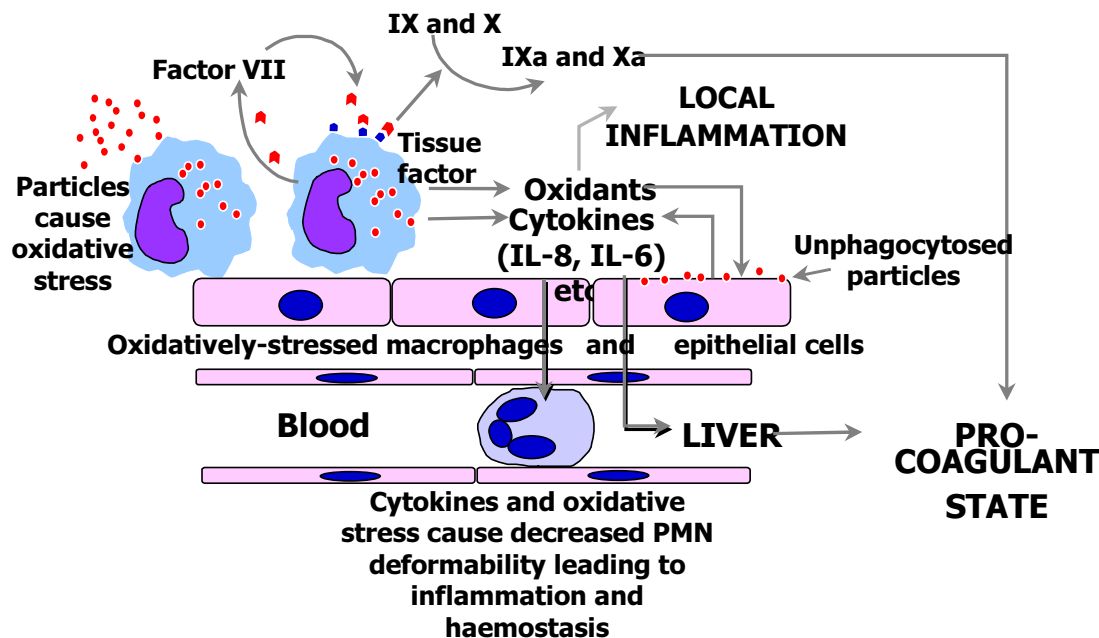


Figure 3. Possible mechanisms for the production of local inflammation and a systemic pro-coagulant state after PM10/2.5 exposure. Scheme was kindly supplied by Dr. Kenneth Donaldson, Napier University, Edinburgh.

The evidence for this oxidative stress, inflammation, and pro-coagulant mechanism from ambient PM fractions is limited and is suggested by one specific epidemiological study (Peeters *et al.*, 1997a), and a few *in vivo* experimental animals studies (Li *et al.*, 1997; Kodavanti *et al.*, 1997; Killingsworth *et al.*, 1997; Watkinson *et al.*, 1997), and a limited number *in vitro* studies with human and animal cells (Donaldson *et al.* 1996; Becker *et al.* 1996; Gilmour *et al.*, 1996; Donaldson *et al.*, 1997; Goldsmith *et al.*, 1997; Samet *et al.*, 1997; Vincent *et al.*, 1997; Becker and Soukup 1998; Bonner *et al.* 1998,). The oxidative stress exerted on cells by particles results in production and release of e.g. pro-inflammatory and immune-regulating cytokines. It is suggested that this process is at least partly mediated through activation of the transcription of nuclear factor kappa B (NFκB), one key regulator of intracellular signal transduction and gene activation (Driscoll *et al.*, 1997).

Recently, Kadiiska *et al.* (1997) showed *in vivo* evidence of free radical formation in rat lungs following intratracheal instillation of oil fly ash. This free radical production appears to be associated with soluble metals in the fly ash.

Another suggested mechanism (Godleski, 1998), proposed for cardiotoxic effects and related mortality following acute exposure to ambient PM_{2.5} and PM₁₀, includes effects like autonomic neurological responses, myocardial hypoxia, systemically circulating inflammatory cytokines and toxins (Fig. 4). These responses to PM may lead to direct effects on heart cells, with subsequent changes in the cardiac electrical systems, including (brady)arrhythmia, low frequency/high frequency ratio increases, heart rate variability increases, impaired atrioventricular conduction, fatal ventricular fibrillation (with T-wave flipping as a possible indicator), and ultimately death. The systemic effects may be caused by receptor stimulation, CNS responses, apnea, bronchoconstriction, as well as by formation of reactive oxygen species, release of pro-inflammatory mediators, and pulmonary inflammation. The evidence for this cardiotoxic mechanism is extremely limited and is only suggested by a few epidemiological studies (Pope *et al.*, 1999) and *in vivo* inhalation or instillation studies on experimental animals (Godleski *et al.*, 1996, 1997; Gordon *et al.*, 1998; Watkinson *et al.*, 1998). The data in these experimental studies show that effects are sometimes larger in animal models for cardiopulmonary diseases compared to healthy animals. Killingsworth *et al.* (1997) studied the effects following inhalation of fly ash in rats and found that chemokines (MIP-2) in heart tissue were stimulated and that macrophages in the heart contain in fact true fly ash particles.

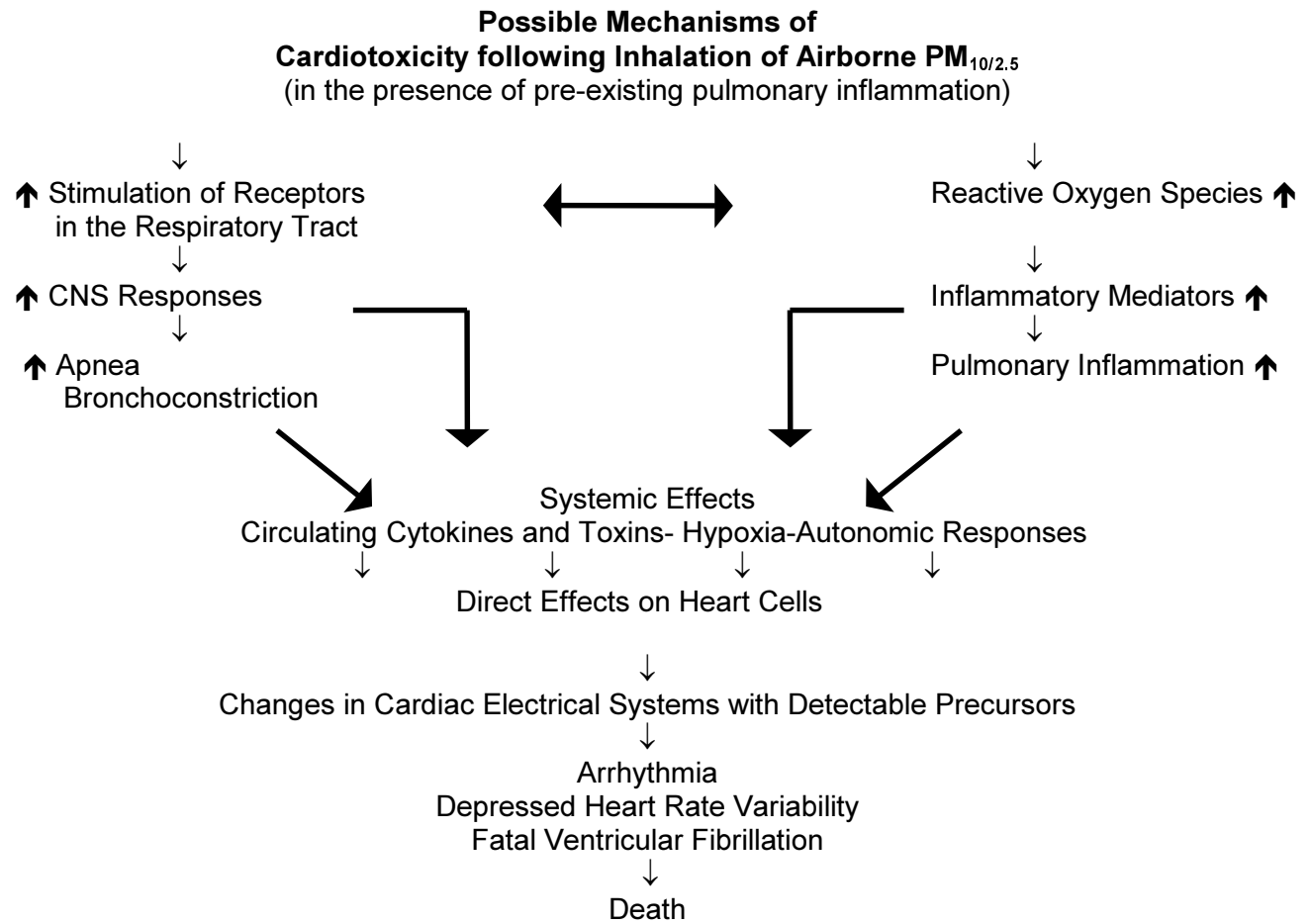


Figure 4. A schematic impression of possible cardiotoxic mechanisms underlying PM-induced effects related to morbidity and mortality (slightly modified after Godleski et al. (1996).

3. Toxicity data and health relevance of PM₁₀ properties

3.1 Importance of the total air pollution mixture

Numerous epidemiological studies indicate that current PM levels are associated with excess cardiopulmonary (premature) mortality, morbidity, including hospital admissions, asthma exacerbation and medication use, as well as respiratory symptoms and lung function decrements. Because of the consistency of these data it is advocated that the mass concentration of PM₁₀ or PM_{2.5} are causal risk factors and indeed no likely alternative causal factor has been identified yet, although the co-correlation of PM with other air pollutants prevents such a conclusive analysis.

Remarkably, associations between health effects and ambient PM are found at various geographical urban and rural areas in many countries. The data show a range of relative low risks depending on the effect parameters and specific study and locations. It is unknown whether these differences reflect the exposure to the rather large contrast in air quality conditions, like e.g. wood smoke, wind blown dust, industrial emissions, energy generation, motor vehicle emissions, as well as acid aerosols. Contrast analyses to shed some light on this have not yet been performed. It may be assumed that the ambient PM mixtures will vary between those locations with respect to particle size distribution and chemical composition and source contributions. In addition, many studies show indistinguishable auto-correlation of PM and TSP, PM₁₀, PM_{2.5}, BS, SO₄²⁻, and H⁺, and also with pollutants like O₃, NO₂, NO, CO, and SO₂. Collectively, these data indicate that causality and plausibility of PM induced adverse health effects is far from clear.

3.1.1 Toxicological evidence for the relevance of ambient PM fractions

For the analysis of the relationship between PM source and human risk RIVM uses a simplified strategy called 'the Pentagon model'. This discriminates between major particle size fraction and chemical compositions: coarse (2.5 – 10 µm), fine (0.1 – 2.5 µm), ultrafine (< 0.1 µm), primary (carbonaceous) and secondary (acid condensates, and sulphate and nitrate particles).

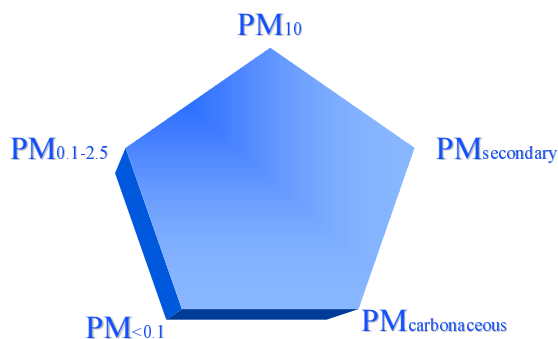


Figure 5. Pentagon Model for critical PM fractions associated with health effects

3.1.2 PM₁₀ size fractions and composition

Toxicology data on ambient PM components in general are difficult to extrapolate to environmentally relevant concentrations because most of the studies were initiated with a mechanistic perspective using relatively high levels of chemically and biologically uniform particles. Relatively high levels of acid aerosols do elicit lung function effects in healthy people and asthmatics following short-term exposures, although, for obvious ethical reasons, only mild asthmatics have been investigated. In addition, no airway inflammatory responses have been observed in these studies. Bronchial mucociliary clearance seems to be decreased following exposure to acid aerosols. Recent laboratory animal data, however, show that sulphuric acid can evoke some toxicity if bound at low levels to ultrafine solid particle cores.

A limited number of field exposure studies and studies using inhaled concentrated fine particles in experimental animal models have shown that urban air and particulate matter fractions of it can cause serious effects which might possibly be related to the effects on morbidity and mortality as observed in epidemiological studies (Saldiva *et al.*, 1992; Lemos *et al.*, 1994; Saldiva *et al.*, 1994; Godleski *et al.*, 1996, 1997, Gulisano *et al.*, 1997; Gordon *et al.*, 1997; Zelikoff *et al.*, 1998; Clarke *et al.* 1999).

A number of experimental animal *in vivo* exposure studies have reported on toxicity of PM₁₀ or TSP, sampled from ambient air or diesel exhaust by filters and administered to animals by inhalation and instillation, giving the first evidence that these PM fractions can exert airway and lung (cell) injury (Sagai *et al.*, 1994; Gilmour *et al.*, 1996; Pritchard *et al.*, 1996; Nadeau *et al.*, 1996; Costa and Dreher, 1997; Donaldson *et al.*, 1997; Li *et al.*, 1997; Vincent *et al.*, 1997; Goegan *et al.*, 1998, Adamson *et al.*, 1999; Frampton *et al.*, 1999). The toxicity measured by inflammation, oxygen radical production, and lung tissue damage, appears to correlate with the content of soluble reactive (first row transition) metals and organics and reactivity is reduced upon chelation or extraction. A PM instillation study done by Dreher *et al.* (1996) gave a first indication that “fine” mode particles might be more toxic *in vivo* than a “coarse” mode fraction, correlating very well with the solubility and reactivity mechanism and their combustion nature and origin. This mechanism is assumed to be much less valid in coarse particles due to their non-combustion nature and origin leading to relatively non-soluble metals in its fraction¹. On the other hand, *in vitro* studies with human monocytes (Becker and Mohn, 1998; Hornbey *et al.*, 1998) show that cellular-toxicity and inflammation may also be associated with the coarse fraction (2.5-10 µm) and its biological components.

Toxicological evidence for a possible role of ultrafine PM in ambient conditions is not available, and existing data relate only to environmentally unrealistic, non-soluble particles like Polyfluortetrafluoroethylene pyrolysate, carbon black, and certain metal oxides. Yet, recent data (Roth *et al.*, 1998; Ziesanis *et al.*, 1998) with exposures to metal oxides and carbon black particles suggest that the generic nature of such ultrafine particles, as just physical entities with a clean surface and a narrow size distribution, does not seem to play a role in short-term toxicity. It may very well be that when such ultrafine particles have a high surface reactivity because of adsorbed reactive components, they may become toxic. There is only very limited evidence that

¹ Due to the high dosages in a range of 1-5 mg/rat, which can be orders of magnitude beyond a dose expected to be inhaled under ambient-relevant conditions it is difficult to extrapolate to possible acute toxicity in humans at ambient levels (Henderson *et al.*, 1995). However, these types of studies are useful to evaluate the relative toxicity of PM samples and the possible underlying mechanisms of adverse health effects.

such a mechanism might play a role for coating of acids on ultrafine inert particles and for PAH-type of organics in diesel exhaust (Chen *et al.*, 1992, 1995; Sagai *et al.*, 1994).

A possible role of the primary, carbonaceous (combustion-derived) fraction of PM₁₀ deserves specific attention. Inflammation is considered an important process in many diseases related to PM-associated morbidity and mortality. Secondary aerosols in exposures at (near-ambient levels) have not or hardly been shown to be able to induce effects in general and inflammation in particular. The carbonaceous fraction of PM may play a significant role because animal toxicity data suggest that levels of soluble components, all adsorbed on to the carbonaceous core of PM, correlate with inflammatory reactions and these responses can be blocked by chelation, extraction, and radical scavengers. Such data need confirmation in realistic animal inhalation studies using exposures resulting in comparable internal doses as can be expected from a plausible human exposure scenario. Deposition models estimate that the inhaled dose of experimental animals for several hours to particle mass concentrations of 1-5 mg/m³ meet that criterion (Vincent *et al.*, 1997; Anjivel and Ashgarian 1995).

The finding that pre-existing cardiopulmonary disease and worsening of the pathological condition is a major risk factor for health effects of PM₁₀ seems to be supported by data from particle deposition modeling (Miller *et al.* 1995, Freijer *et al.*, 1997; Kim and Kang, 1998; see also Table 2), showing that particle deposition may be markedly increased in people with COPD or asthma when compared to deposition in healthy lungs. PM deposition models also estimate that the total deposition fraction of the coarse mode particles (2.5 - 10 µm) and ultrafine mode particles (<0.1 µm) are significantly higher compared to the fine mode (0.1 - 2.5 µm) for both human and rat (Anjivel and Ashgarian 1995). In general, pulmonary deposition increases with decreasing particle size (Venkataraman and Kao, 1999).

3.1.3 Traffic emissions

Toxicity studies on repeated exposures of experimental animals and humans to direct real-world ambient air in urban areas of São Paulo, Mexico City and Florence suggest that more adverse health effects occur at locations with higher traffic density, possibly due to the higher level of (fine) particulate air pollution (Böhm *et al.*, 1989; Calderon-Garcidueñas *et al.*, 1992; Saldiva *et al.*, 1992; Calderon-Garcidueñas and Roy-Ocotla 1993; Pereira *et al.*, 1993; Lemos *et al.*, 1994; Gulisano *et al.*, 1997). These studies demonstrate serious adverse health effects in airways, including lesions in upper and lower airway tissue, increased airway reactivity, and immunotoxic effects.

Many studies on diesel exhaust in the past have focussed on chronic exposure and tumorigenic and non-tumorigenic structural airway effects (Heinrich *et al.*, 1995; Nagai *et al.*, 1996; Kim *et al.*, 1998). Recently performed controlled human studies on acute diesel exhaust exposures showed substantial respiratory and systemic effects on inflammation and lung function (Salvi *et al.*, 1999; Rudell *et al.*, 1990, 1996; Blomberg *et al.*, 1998). It is also assumed from these studies using ceramic particle traps that these effects are not exclusively evoked by the particles (Blomberg *et al.*, 1998). Experimental animal inhalation studies with short-term exposures revealed that diesel exhaust particles (Sagai *et al.*, 1994).

4. Summary and conclusions

Epidemiological studies have observed statistical associations between short-term, and to a limited extent also long-term, exposure to increased ambient particulate air pollution (*particulate matter*, PM) and increased morbidity and mortality. Even though the relative risks are small, there is a public health concern because of the large number of people exposed and the existence of high-risk groups. Epidemiological data show positive associations of health effects with mass levels of secondary aerosols or representative air quality indicators (SO_4^{2-} , NO_3^- , H^+). Epidemiological studies, however, have not yet shown evidence to conclude that health effects are preferentially associated with a particular PM size-fraction (coarse, fine, or ultrafine. The associations with $\text{PM}_{2.5}$ compared with PM_{10} are similar if the mass portion of $\text{PM}_{2.5}$ in PM_{10} is taken into account. A number of epidemiological studies shows that, besides PM, significant and many times even stronger associations of health effects also exists with O_3 , NO_2 , CO, and SO_2 . Data from epidemiological studies do not yet allow adequate conclusions on the relevance of traffic- and, in particular, diesel exhaust-related particles in acute PM_{10} -associated health effects.

The possible responsible PM size fractions (PM_{10} , $\text{PM}_{2.5}$, ultrafine-mode particles) and chemical or biological components as well as their respective emission sources, are still unclear and subject of dispute. The biological hypotheses for mechanisms underlying these adverse health effects are just beginning to develop. Collectively, these uncertainties complicate the PM health risk assessment and standard setting as well as application of the most (cost-) effective emission and risk control, because such strategies should be targeted on responsible components and linked to their most important sources. Despite these uncertainties, regulatory decisions resulted recently in new and more tight air quality standards (USA) and limit values (EU) for ambient air PM, forcing larger PM emission reductions than formerly anticipated. For a targeted and justified standard setting (next revision in 2003) control policy focused on critical sources and effective cost effective risk reduction, however, new data should be extracted from studies on (population and personal) exposure, air quality, emissions and sources, and PM toxicity.

In general, the current limited number of conducted inhalation toxicity studies on respiratory and cardiac effects following exposure to sampled or concentrated ambient PM fractions (coarse, fine, or ultrafine) in both healthy volunteers or laboratory animals (both healthy and diseased) has not yet resulted in sufficient evidence to conclude that ambient PM levels may play a causal biologically plausible role in PM-associated adverse cardiorespiratory effects. Specific conclusions on the toxicological evidence for various PM fractions and components, and on plausible mechanisms are as follows:

- Current inhalation toxicity data do not strongly favour a particular PM size fraction or chemical composition explaining PM-associated health effects.
- Particles of different sizes might be preferentially involved in specific health effects because of their size-dependent airway deposition pattern, favouring smaller particles ($<0.2 \mu\text{m}$) to deposit more deeply in the lower airways. People with compromised airways (asthma, COPD) seem to receive a higher dose of PM.
- A limited number of mechanistic studies (*in vivo* and *in vitro*) using high PM doses show that TSP, PM_{10} , $\text{PM}_{2.5-10}$ and $\text{PM}_{2.5}$ are able to induce acute cardiopulmonary injury and

inflammation, whereby toxicity appears to correlate with the PM content of soluble, transition metals and maybe also with organic constituents. This might suggest an important role for the anthropogenic, carbonaceous mode of ambient PM. Surface area chemistry and (oxy) radical reactivity of fine mode particles might therefore be more relevant for explaining biological effects than the generic, physical nature of ultrafine particles or the non-soluble components of coarse particles. However, none of these studies used inhalation exposures.

- A few animal toxicity studies have shown significant positive interactions between e.g. PM and O₃ in inducing pulmonary toxicity, suggesting that the mixture of air pollution (oxidants) might be more important than PM alone.
- Based on their cardiopulmonary toxic potency and the stimulation of inflammatory processes, the data from toxicological studies suggest that the primary carbonaceous, anthropogenic fractions of PM₁₀ and PM_{2.5} may be more relevant compared to the secondary fractions for adverse human health effects in airways, lungs, and the heart. The secondary PM components like acids, sulphates and nitrates might still serve as a proxy for the carbonaceous fraction of PM in epidemiological studies.
- Toxicological evidence for adverse health effects caused by traffic-derived PM from acute studies is still only marginal.
- Data from limited PM mechanism studies have suggested various pathophysiological processes in airways, lungs, and heart, which may play a role in adverse health effects in specific human subpopulations believed to be at increased risk. Airway deposition models predict that the PM₁₀-associated increased host responsiveness in (older) people with cardiorespiratory diseases may be partly related to a markedly increased internal PM dose in the airways. Most of the mechanism data indicate that PM fractions are able to
 - 1) induce inflammation and immunotoxicity in airways and lungs related to oxidative stress, and
 - 2) impair respiratory and cardiac neurological functions.

Since all of these processes play an important role in various diseases related to PM₁₀-associated acute excess morbidity and mortality, an exacerbation mechanism of ambient PM for cardiorespiratory effects in susceptible human populations might be biologically plausible.

Because these toxic effects appear also to correlate with the content of soluble reactive PM components absorbed onto the surface (transition metals, reactive PAHs; responses can be blocked by chelation, extraction, and radical scavengers) a specific role for the anthropogenic, carbonaceous, fine ($\leq 2.5 \mu\text{m}$) fraction is prudently suggested, however, other fractions like coarse mode PM (2.5-10 μm) might still contribute to a certain extent to the observed health effects, maybe in particular for upper airway and tracheobronchial effects.

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