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*Hum Exp Toxicol* 2006; 25; 559

DOI: 10.1177/096032706072520

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# Particulate matter properties and health effects: consistency of epidemiological and toxicological studies

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Identifying the ambient particulate matter (PM) fractions or constituents, critically involved in eliciting adverse health effects, is crucial to the implementation of more cost-efficient abatement strategies to improve air quality. This review focuses on the importance of different particle properties for PM-induced effects, and whether there is consistency in the results from epidemiological and experimental studies. An evident problem for such comparisons is that epidemiological and experimental data on the effects of specific components of ambient PM are limited. Despite this, some conclusions can be drawn. With respect to the importance of the PM size-fractions, experimental and epidemiological studies are somewhat conflicting, but there seems to be a certain consistency in that the coarse fraction (PM<sub>10-2.5</sub>) has an effect that should not be neglected. Better exposure characterization may improve the consistency between the results from experimental and epidemiological studies, in particular for ultrafine particles. Experimental data indicate that surface area is an important metric, but composition may play an even greater role in eliciting effects. The consistency between epidemiological and experimental find-

ings for specific PM-components appears most convincing for metals, which seem to be important for the development of both pulmonary and cardiovascular disease. Metals may also be involved in PM-induced allergic sensitization, but the epidemiological evidence for this is scarce. Soluble organic compounds appear to be implicated in PM-induced allergy and cancer, but the data from epidemiological studies are insufficient for any conclusions. The present review suggests that there may be a need for improvements in research designs. In particular, there is a need for better exposure assessments in epidemiological investigations, whereas experimental data would benefit from an improved comparability of studies. Combined experimental and epidemiological investigations may also help answer some of the unresolved issues. *Human & Experimental Toxicology* (2006) 25, 559–579

**Key words:** ambient particulate matter; epidemiology; health effects; particle composition; toxicology

## Introduction

For many years, ambient particulate matter (PM) has been regarded as a serious health problem. PM comprises a mixture of several compounds, including carbon-centered combustion particles, secondary inorganics, and crustal-derived particles. These compounds may contribute, with different potential, to the PM-induced health effects. Nevertheless, current air-quality standards are based on the total mass of suspended particles and do not discriminate between the different constituents of outdoor PM. The assumption that all PM are equally toxic is supported, to some extent, by epidemiological studies. Time series studies from many cities through-

out the world have shown limited variations in risk estimates despite exposures that varied widely with respect to sources.<sup>1–3</sup> However, the idea that the particle characteristics are without significance to PM-effects seems inconsistent with basic toxicological principles, and has thus been questioned by others.<sup>4</sup> Experimental studies have indicated wide variations in inflammatory and other effects of different particle types. This would suggest a comparable particle type-specific effect variation in epidemiological studies.

Revealing the particle characteristics or components responsible for PM-induced health effects is pivotal, and may, in the long-term, allow for more effective regulatory initiatives to improve outdoor air quality. This review attempts to summarize the present knowledge on particle properties and their effectiveness in eliciting respiratory and cardiovascular responses, and whether experimental and

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Received 27 September 2005; revised 28 February 2006; accepted 16 March 2006

epidemiological studies are consistent with each other, with respect to the importance of different particle properties.

### Varying composition of ambient air particles

PM is divided into different size fractions, PM<sub>10</sub> (PM  $\leq 10 \mu\text{m}$ ), PM<sub>2.5</sub> (PM  $\leq 2.5 \mu\text{m}$ ) and PM<sub>0.1</sub> (PM  $\leq 0.1 \mu\text{m}$ ), based on the aerodynamic diameter (50% cut off) of the particles, and measured as mass. Alternative metrics are particle number concentrations, surface area or organic and elemental carbon (OC/EC). However, PM consists of very heterogeneous groups of components, comprising particles from several different sources, including traffic, small- and large-scale biomass combustion, waste incineration, industrial processes, long-range transported air pollution, road abrasion and resuspension, car brake debris, and fugitive dusts from soil erosion. Whereas the coarse fraction (PM<sub>10-2.5</sub>) is often dominated by crustal material, the fine (PM<sub>2.5</sub>) and ultrafine fractions (PM<sub>0.1</sub>) mostly consist of carbonaceous combustion particles. Sulphates and nitrates contribute to particle mass and are considered as markers of long-range transported PM.<sup>5</sup> Metals may originate from combustion sources, but also from resuspension of car brake debris or crustal material.<sup>6-9</sup> Polycyclic aromatic hydrocarbons (PAH) and similar compounds are emitted from traffic, but are also abundant in PM from sources such as small-scale biomass combustion.<sup>10</sup> In addition, various volatile and semi-volatile compounds have been found in PM.<sup>11</sup> An important part of PM consists of biological material, including endotoxins and bacteria, beta-glucans and fungal spores, allergens and pollen.<sup>12-15</sup> Endotoxins seem to occur predominantly in the coarse fraction and, to a lesser extent, in the fine fraction.<sup>16-18</sup> Mineral particles may dominate in areas where sand is used to increase friction on icy roads or studded tires have led to increased road pavement abrasion, or in areas exposed to wind-blown sand or soil.<sup>19,20</sup> The contribution from the different sources varies with time, season, location and climate, resulting in spatial and season-dependent variations in concentrations and properties.<sup>21-23</sup>

### Epidemiological studies, uniformity of the size of risk estimates?

Global epidemiological studies have revealed associations between ambient air particle concentrations and mortality in the population. Although studies of the effects of short-term changes in exposure have been performed in areas varying in air pollution

composition, they show a similar range of risk estimates for morbidity or mortality.<sup>1-3,24-26</sup> However, the multi-centre time series studies, Air Pollution and Health – A European Approach (APHEA) and National Mortality Morbidity Air Pollution Study (NMMAPS), indicate the occurrence of some heterogeneity with respect to risk estimates between locations. This observation is further corroborated by seasonal analyses, which showed stronger effects on mortality in the summer in the Northeast of the US, whereas there were smaller or no differences between seasons in other areas.<sup>22</sup> These variations in risk estimates between different cities could be due to differences in particle composition, although other factors may also be involved.

For morbidity, the evidence for uniformity or heterogeneity of risk estimates is less abundant. Generally, morbidity registries have been less reliable than mortality registries – one of the reasons for fewer studies on morbidity than on mortality. A meta-analysis of US time series studies revealed an increase in hospital admissions by the elderly (>65 years) for respiratory diseases. Other studies showed increases in morbidity of similar magnitude for other age groups, including children.<sup>27</sup> The NMMAPS data on hospital admissions for pneumonia, chronic obstructive pulmonary disease (COPD), and cardiovascular disease, seem to indicate some heterogeneity in risk estimates between different locations.<sup>25</sup> Studies on the prevalence of allergy appear to indicate regional heterogeneity that may be associated with air pollution, but the overall data on particle-induced allergy are not conclusive.<sup>28-30</sup>

Exposure to increased levels of air pollution over longer time periods has been associated with an increased risk of mortality and morbidity due to cardio-respiratory diseases.<sup>31-34</sup> These cohort studies indicated associations that were considerably stronger for longer exposure times, compared to short-term increases in air pollution and showed increased risks of dying from elevated exposure to PM<sub>2.5</sub> and sulphates. For the locations included in the Six Cities Study, the risk estimates are not uniform, again indicating some heterogeneity also for the long-term effects of particle exposure.<sup>31,35</sup>

### PM-induced health effects and mechanisms

To shed light on the plausibility of epidemiological findings, experimental studies have examined how PM may induce acute health effects in voluntary individuals in exposure chambers, acute and chronic effects in animal studies and biological responses in cultured cells. These experimental studies confirmed that PM may affect the respiratory

system.<sup>36,37</sup> Recent studies have also shown effects on the cardiovascular system in humans and animals.<sup>36–38</sup>

#### *Respiratory disease and mechanisms*

Inflammation appears to be a major determinant in PM-induced health effects. Alveolar macrophages (AM) and pulmonary epithelial cells constitute the first line of defense against inhaled noxious compounds, and seem to play a central role in the onset of inflammatory reactions upon PM exposure. These responses involve the production and release of a range of signaling molecules, such as cytokines, chemokines, leukotrienes/prostaglandins, and adhesion molecules, which operate in a complex network between epithelial cells, AM and other immune cells, including neutrophils, eosinophils and T cells.<sup>39–41</sup>

Depending on the cytokines and/or chemokines released as a result of PM exposure, different classes of immune cells will be recruited.<sup>40,42</sup> The differential attraction of immune cells is associated with different diseases. In allergic asthma, the attraction of eosinophils involves different mediators to those that attract neutrophils in non-allergic inflammation-dependent diseases.<sup>43</sup> The recruited immune cells may release cytokines, reactive oxygen species (ROS), lipid mediators and toxic proteases, which may contribute to epithelial damage. This may, in turn, lead to an increased release of cytokines and chemokines from the epithelium, subsequently increasing and/or prolonging the inflammatory reaction, overwhelming the defense mechanisms and possibly progressing to chronic inflammation.

Several studies have shown that PM may be cytotoxic and induce both apoptotic and necrotic cell death. Although the importance of cell death in PM-induced disease is less clear, cell death may also be involved in the inflammation and development of both acute health effects and chronic lung diseases.<sup>44–47</sup> These processes may occur in parallel to a remodeling of the lung tissue, with increased proliferation of fibroblasts and myofibroblasts, which is of great importance during the development of disease.<sup>48</sup>

With respect to lung cancer, animal studies have supported epidemiological evidence that different types of PM, in particular, diesel exhaust particles (DEP), induce cancer development in humans.<sup>49,50</sup> The mechanisms include direct interaction with the DNA of metabolized particle components and oxidative stress products, and the formation of adducts and mutations.<sup>51</sup> In addition, inflammatory mediators may directly contribute to cancer progression through mitogenic, angiogenic and mitogenic

actions.<sup>52</sup> The relative importance of these mechanisms in PM-induced lung cancer has not been clarified, but presumably several processes act in concert.

#### *Cardiovascular disease and mechanisms*

A number of hypotheses have been forwarded to explain how PM might affect the cardiovascular system, as reviewed by others.<sup>38,53–55</sup> In brief, some evidence suggests that ultrafine particles, soluble components from the particles, such as metals and organic substances, or inflammatory substances from the lung, may enter the circulatory system and act on the first encountered target organ, the heart. One possibility is that inhaled particles, particle-related components or induced substances may stimulate the autonomic nervous system. Some lines of experimental evidence suggest that PM may have an influence on heart rate variability, which could be associated with higher risk of cardiac arrhythmias.<sup>56,57</sup> Such an association has also been supported by epidemiological and panel studies.<sup>58–60</sup> Other evidence points to PM-induced changes of haematological/coagulation processes in the blood,<sup>58,61,62</sup> which may lead to the development of myocardial infarction. Several epidemiological studies have shown an association between increased levels of inflammatory markers, such as interleukin (IL)-6 and C-reactive proteins (CRP), and coagulation factors, such as fibrinogen, with coronary heart disease and mortality.<sup>63–65</sup> A less examined possibility for the development of cardiovascular disease is that PM may induce a hypertrophic response in the myocardium. Cardiac hypertrophy is known to be a major risk factor for heart failure.<sup>66</sup> Inflammation may also be involved in the hypertrophic response of the heart, in addition to the atherosclerotic response of the endothelium.<sup>67,68</sup> Thus, the role of inflammatory responses seems very important in various aspects of development of cardiovascular disease. However, the critical pathophysiological pathways have not yet been identified.

#### **Importance of size in particle-elicited effects**

Particle size and correlated parameters, number and surface area, have been a key issue in the understanding of particle effects. Particle size determines, to a great extent, inhalation, deposition and elimination of particles. The coarse PM may reach the larger airways, but also, to some extent, the smallest airways and alveoli. A larger part of the fine and ultrafine particles deposits in the latter regions of the lungs, though ultrafines have a substantially

higher deposition efficiency. The smallest fraction, 10 nm or below, is mostly deposited in the upper airways. Thus, inhalation and deposition of these particle size fractions indicate that the size distribution of the aerosol may have an impact on the localization and type of health effects elicited.

### *Epidemiological studies*

In most epidemiological studies, PM<sub>10</sub> or black smoke (BS; black combustion particles generally of small size) have been measured, but several studies also display data on PM<sub>2.5</sub> and some on PM<sub>0.1</sub>, particle number concentrations, or OC/EC ratio. Several US short-term studies have indicated that PM<sub>2.5</sub> was associated with higher risk estimates than larger size fractions, indicating that combustion-dominated particle fractions with smaller average size and considerably larger surface area, but also different composition, may have more impact on mortality and morbidity than larger particles.<sup>69–71</sup> However, other studies suggest that acute exposure to coarse PM may be significantly associated with mortality and morbidity.<sup>72–75</sup>

Studies on the effects of long-term increases in air pollution showed associations with PM<sub>2.5</sub>, whereas larger size fractions did not show significant associations.<sup>32</sup> The risk estimates for increases in mortality in relation to long-term increases in PM<sub>2.5</sub> were considerably greater than for short-term increases.<sup>27</sup>

A recent review of the literature did not reveal major differences between the impact of coarse and fine fractions on morbidity after short-term increases in exposure.<sup>76</sup> The data indicate that coarse PM is associated with hospital admissions for respiratory and cardiovascular disease. The studies included in the review comprised mainly particles of wind-blown dust, soil erosion and volcanic ash. In addition, a study on road dust in Stockholm showed that coarse PM was associated with exacerbation of respiratory disease, but not cardiovascular disease, in contrast to fine PM (Forsberg, personal communication). Though mineral components (quartz, asbestos) have been associated with the development of cancer in workplace environments, there is no evidence, at present, that ambient coarse PM is associated with the occurrence of lung cancer. In contrast, fine PM consisting of combustion particles has been associated with lung cancer development in humans.<sup>32</sup> Although some studies have indicated the importance of fine traffic-derived particles in allergy, compared to coal combustion or industry-derived particles, other studies could not corroborate these findings.<sup>28–30</sup>

It has been suggested that ultrafine particles are to blame for the adverse effects of PM. Some studies have observed associations between ultrafine particles and increased cardiovascular mortality and morbidity. However, it seems that PM<sub>2.5</sub> was equally or more strongly associated with cardio-respiratory symptoms, mortality or lung permeability than ultrafines.<sup>77–79</sup> With regard to both mortality and asthma medication, studies indicate that fine and ultrafine PM may be associated with immediate and delayed effects, respectively, which might indicate independent effects of the two size fractions.<sup>80,81</sup> Ultrafine particles appeared to be more important to the reduction in lung function, measured as peak expiratory flow, in adult asthmatics than other PM metrics.<sup>82,83</sup>

The association of fine and ultrafine PM with cardiovascular effects has been investigated in some studies, with respect to particle number and mass. The results have not been convincing for a predominant role of ultrafines in mortality or morbidity. In one study, the 1.0–0.1  $\mu\text{m}$  PM fraction seemed to be more consistently associated with increased probability for myocardial ischemia than mass or number of ultrafine particles.<sup>84</sup> Heart muscle repolarization, as determined by electrocardiac measurement, was approximately as strongly associated with PM<sub>2.5</sub> as with other particle metrics, such as particle number.<sup>85</sup>

### *Experimental studies*

In experimental studies, both ambient PM and different types of model particles have been used to investigate the importance of size and surface area. Instillation of polystyrene particles of different sizes into rat lungs, has shown that smaller particles induce stronger inflammatory responses than larger particles.<sup>86</sup> The ability to induce inflammation was proportional to particle surface area, suggesting that the larger surface area determines the higher inflammatory potential of small particles. Only ultrafine polystyrene particles induced significant increases in intracellular calcium concentrations *in vitro* in macrophages and IL-8 gene expression in epithelial cells, in contrast to particles of other sizes.<sup>86</sup> The effects of different fine and ultrafine polystyrene particles have also been tested with respect to pulmonary microvascular permeability.<sup>87</sup> Both studies showed that ultrafine polystyrene particles were more potent than fine particles, and that the adverse pulmonary effects were related to the total surface area and number of particles, rather than to the instilled mass. These findings are supported by *in vivo* studies on carbon black and titanium dioxide (TiO<sub>2</sub>) particles of different size fractions,

showing that surface area provides the best correlation with biological effects.<sup>88,89</sup> Notably, ultrafine carbon black has been reported to induce stronger pulmonary inflammation than ultrafine TiO<sub>2</sub>.<sup>90</sup> Moreover, a recent study on fine and coarse particles showed that differences in surface area could only account for differences in biological reactivity of particles of the same rock species, but not for differences between rock species.<sup>91</sup> These results emphasize that surface area and surface reactivity are two separate factors controlling the biological effects of particles.

Another important consideration is that lung deposition of inhaled particles does not show a straightforward correlation with size (as previously discussed). An inhalation study of particles of varying sizes showed that pulmonary toxicity was related to the mass of particles deposited in the lungs, which followed the order: 33 > 673 > 170 > 1495 (nm particles).<sup>92</sup> Thus, results from studies using cell cultures or tracheal instillation, may oversimplify the importance of particle size and surface area compared to inhalation studies.

Interestingly, a range of experimental studies on the *in vivo* and *in vitro* toxicity and inflammatory potential of coarse, fine and ultrafine ambient PM suggests that the coarse fraction is the most potent, even at the same mass.<sup>16–18,93–98</sup> In many cases, a higher endotoxin content in the coarse fraction could explain these observations, but some of the studies also suggest that endotoxin was not the dominant factor eliciting these effects (as discussed later). The important message from these studies is that the different size-fractions of outdoor PM may contain qualitatively different constituents, and that this may be far more important for the biological effects than the size of the particles.

As with non-allergic inflammatory reactions, studies on model particle-induced allergic sensitization indicate that this ability is also driven by the particle surface, so that smaller particles are more potent than larger particles when administered at the same mass.<sup>99</sup> However, as discussed above, the causal relationship between particle size/surface area and biological effects is less clear when studying the effects of complex outdoor PM-fractions of varying composition instead of pure model particles. Mouse footpad injections of coarse and fine PM sampled from different European cities, showed that the fine particles had a stronger adjuvant effect on ovalbumin-induced IgE.<sup>100</sup> However, no systematic differences were found between fine and coarse particles on other ovalbumin-induced effects (number of lymph node cells, expression of cell surface

receptors and *ex vivo* cytokine production).<sup>101</sup> Moreover, no significant difference between these fine and coarse particles could be detected in a respiratory allergy mouse model, with the exception that coarse particles were more potent to elicit histopathological lesions.<sup>102</sup>

It has been suggested that ultrafine particles may elicit some unique toxic effects due to their small size. Most notably, it has been speculated that ultrafines may translocate from the lungs into the systemic circulation leading to cardiovascular effects. Studies with radioactively-labeled particles or ultrafine carbon particles containing the stable isotope <sup>13</sup>C, show that some of the inhaled or instilled ultrafine particles enter the circulation and reach extrapulmonary organs.<sup>103–106</sup> However, the importance of this translocation of ultrafines into the circulation in comparison to other potential causes of PM-induced cardiovascular effects, such as pulmonary inflammation or autonomic nervous stimulation, remains to be determined.

#### *Consistency considerations*

Experimental studies with uniform model particles, such as polystyrene, carbon black and TiO<sub>2</sub>, clearly show that smaller particles are more potent than larger particles at equal mass due to their larger surface area, and that particle surface area (or number) correlates better with biological responses than particle mass. These findings also show that particles *per se* may be toxic, in particular ultrafines, because of their large surface to mass ratio and a reactivity of the surface. Interestingly, experimental studies of coarse, fine and ultrafine PM sampled from outdoor air appear to indicate that the larger particles are equally or even more potent than the finer particles. This may appear contradictory to reports from studies with pure model particles, but the findings from experimental PM-studies rather reflect that the importance of size and surface area does not over-ride the importance of particle composition, implying that the coarse fractions tend to contain more toxic and/or inflammatory compounds than the fine and ultrafine fractions. Epidemiological studies show considerably more evidence for a role of PM<sub>2.5</sub> than PM<sub>10–2.5</sub> in mortality, whereas both the coarse and fine fractions appear to be involved in morbidity caused by air pollution. Thus, experimental and epidemiological findings are somewhat conflicting with respect to the importance of PM<sub>2.5</sub>. However, there seems to be some consistency in that the coarse fraction may have an adverse effect on public health. The information from epidemiological studies on the importance of ultrafine particles is limited because of the limited

availability of exposure data. This may be due to measurement and exposure classification problems. However, the available evidence does not suggest that ultrafine particles play a predominant role, despite their much larger surface area. Thus, there seems to be evidence for the assumption that size alone is not the critical determinant of PM-induced health effects.

### Importance of particle-associated metals

Several years ago, WHO published air quality guidelines on metals as air pollutants.<sup>107</sup> More recent information about the different metals has resulted in metals being scrutinized as important constituents of PM. In brief, transition metals, such as iron and copper, are believed to contribute to particle-induced formation of ROS through the Fenton reaction, and have, therefore, been considered important for particle toxicity.<sup>108–110</sup> Other metals, such as zinc, may trigger effects more directly by interacting with cellular proteins.<sup>111,112</sup>

#### *Epidemiological studies*

In most epidemiological studies, the metal content of the PM has not been analysed. However, in some special locations, the PM-sources are known to be rich in metals, for instance in areas with metallurgic industries. In the Utah Valley area, air pollution changes during a transient closure of a steel mill were associated with changes in mortality and morbidity. PM levels and mortality and morbidity declined during the closure of the mill, but increased again when the mill was reopened.<sup>113</sup> The changes in health effects were not fully accounted for by PM mass. Though metals were not modelled in the epidemiological study, it seems likely that the number of metal particles and amount of metals in particles was considerably reduced during the closure period. The mortality risk estimates in the Utah Valley studies appeared to be in the upper range compared to other investigations, in which exposure to traffic dominates.<sup>114</sup>

In a study of Canadian cities, Burnett *et al.*,<sup>115</sup> found that iron, nickel and zinc, in addition to sulphates, were associated with increased mortality. The associations of these constituents were even better than for total mass, indicating that they were better predictors for mortality than mass. However, the larger variation in the metal-associated effects than those found for mass also indicated that there were other important contributing components in the mixture.

A cross-sectional study in Eastern Germany showed higher lifetime prevalence of respiratory

disorders and allergic sensitization in children living near industrial sites compared to children without such exposure.<sup>116</sup> A decline in pollution reduced respiratory symptoms in children.<sup>117</sup> Later analyses revealed higher levels of particles and higher metal content near the industrial site compared to the more rural area.<sup>118</sup> Though metal composition in these studies was not modeled, the subsequent experimental results suggested that a reduction in effects might be achieved by the reduction in particle-associated metal exposure (as discussed below).

In a European multi-centre study, acute respiratory health effects seemed associated with PM<sub>10</sub> exposure. The significance of this association was lost in two-pollutant models, whereas the effect estimates for iron and silicon remained unchanged, indicating their importance.<sup>119</sup> An analysis of the Six Cities data, including elemental composition, revealed the importance of nickel, lead and sulphur on the increased mortality of long-term increased air pollution.<sup>35</sup>

#### *Experimental studies*

Experimental studies with PM filter extracts sampled in Utah Valley support the epidemiological findings. *In vivo* instillation studies on humans and rats have shown that PM collected while the steel mill was open induced pulmonary injury and inflammation, whereas PM sampled during the period of closure, did not induce pulmonary injury or inflammation. Analysis indicated that a higher metal content (iron, copper, nickel, lead, and zinc among others) in the extracts from the active periods of the steel mill may explain the higher biological activity of these extracts as opposed to the extract from the closed period.<sup>113,120</sup> Consistent with the results above, *in vitro* studies on lung cells also showed that the extracts from the active periods of the steel mill had a higher biological activity than the extract from the closed period, such as higher capacity for oxygen radical formation and induction of cytokine expression. The oxidative capacity and the cytokine expression appeared to be related to the metal content.<sup>121</sup> A similar higher inflammatory effect of metal-rich particles (with high levels of zinc, copper, nickel and cadmium) compared to PM<sub>2.5</sub> with lower metal content has also been reported in a study from former Eastern Germany.<sup>118</sup> For other cellular responses, such as cytotoxicity and phagocytic function of AM, the metal content may not be equally important.<sup>121,122</sup>

In order to investigate the importance of metals, the Utah Valley extracts were treated with a metal-

chelator. Untreated extract induced increased IL-8 release from airway epithelial cells, whereas the chelator-treated extract did not induce IL-8.<sup>123</sup> Moreover, *in vivo* exposure of rats showed that untreated extract induced more lung toxicity than the chelator-treated extract. Similarly, chelator treatment has been shown to attenuate particle-induced effects in other studies. A combination of deferoxamine and ferrozine inhibited the activation of NF- $\kappa$ B by PM10 and a water-soluble PM10 fraction, suggesting an important role of iron.<sup>124</sup> Deferoxamine and antioxidants have been shown to inhibit cytokine expression in human bronchial epithelial cells exposed to residual oil fly ash (ROFA) particles containing vanadium, nickel and iron.<sup>7</sup> Vanadium-containing compounds, but not nickel or iron sulfates, mimicked the *in vitro* effect of the ROFA particles.<sup>7</sup> Moreover, soluble vanadium, nickel and iron appeared to be the causative factors for ROFA-induced inflammatory reactions and cytotoxicity in rat lungs.<sup>6</sup>

Several experimental studies suggest a role of metals in PM-induced cardiovascular effects. Long-term inhalation studies have shown that zinc-containing PM may cause myocardial injury in rats.<sup>125</sup> Copper, zinc and vanadium have been shown to induce a range of different cardiovascular effects, including increased expression of different cytokines and stress proteins, reduction in spontaneous beat rate, vasoconstriction and vasodilation.<sup>126–128</sup> Notably, the effects may be triggered through a complex interplay between different metals. Campen *et al.*,<sup>129</sup> reported that nickel and vanadium may interact synergistically to cause immediate and delayed cardiovascular effects. Nickel-exposure was found to cause delayed bradycardia, hypothermia and arrhythmogenesis, whereas vanadium did not cause any significant delayed effects alone, but enhanced the effect of nickel.<sup>129</sup> In contrast, vanadium, but not nickel or iron exposure, resulted in immediate responses on the same cardiovascular parameters. Moreover, nickel was also found to exacerbate the immediate effects of vanadium, whereas iron attenuated the vanadium-induced effects.<sup>130</sup>

Metal-rich particles have also been found to enhance allergic responses to ovalbumin and house dust mite,<sup>131–133</sup> and to induce the increased release of allergy-related cytokines, eosinophil recruitment and airway hyper-responsiveness in mice.<sup>134</sup> Moreover, metal ions, such as aluminium, cadmium, nickel and strontium ions, have been found to enhance IL-4 release and degranulation of mast cells.<sup>135</sup> Thus, there seems to be some support for

the idea that soluble metals from PM may be involved in allergic responses.

Although a range of experimental studies has indicated that metals are among the causative components in PM-induced effects, the relationship may not be straightforward. Cellular ROS generation in polymorphonuclear leukocytes was significantly correlated with insoluble silicon, iron, manganese, titanium and cobalt, but not with soluble transition metals, and deferoxamine treatment did not affect the ROS generation.<sup>136</sup> Investigation of coarse and fine PM from four European cities showed that the coarse fractions, in general, contained more metals, such as iron and copper, and also induced more cytokine release than the fine fractions. However, the variation in metal content could not account for the variation in cytokine release induced by the different coarse fractions.<sup>18</sup> Studies on mineral particles as well as soil-derived PM2.5 have also failed to find a correlation between metal content and biological effects.<sup>91,137–139</sup> The addition of transition metals to ultrafine carbon black has revealed contradicting results *in vivo* and *in vitro*. Iron and copper increased carbon black-generated ROS in a cell-free system, and increased pulmonary inflammation in rats, but did not increase particle-induced intracellular ROS production or TNF- $\alpha$  release in cultured macrophages.<sup>140</sup> Thus, although metals may be involved in PM-induced inflammatory responses, they may not be indispensable for PM-induced effects.

#### Consistency considerations

Though the evidence from epidemiological studies is scarce, the studies seem to indicate some importance for the presence of metals in air pollution-associated mortality. This seems to be consistent with the experimental studies showing that metals play important roles in both pulmonary inflammation and cardiovascular effects induced by PM. Experimental studies also suggest that metals from PM may be involved in allergic responses. However, despite some indications, epidemiological studies have not established a sufficient connection between the metal content of PM and allergic disorders. None of the study approaches has been able to pinpoint a specific metal or group of metals culpable for the health effects of PM. However, vanadium, zinc, iron, copper and nickel stand out as potentially more important than other metals. Also, the results do not exclude the importance of other particle components.

## Importance of particle-associated secondary sulphates

Sulphates constitute an important part of the fine PM. For a long time, these particles have been regarded as harmful constituents of PM.

### *Epidemiological studies*

US studies showed associations between sulphates and increased mortality and morbidity.<sup>31,32</sup> Reanalyses of these data confirmed these associations, but did not rule out the possibility that the sulphates serve as a surrogate for some other component they strongly correlate with.<sup>141,142</sup> In addition, European analyses have indicated a role for sulphates in the health effects of air pollution.<sup>34</sup>

### *Experimental studies*

The experimental studies on secondary inorganic aerosols have been thoroughly reviewed by Schlesinger and Cassee.<sup>143</sup> The authors concluded that these compounds, which are dominated by sulphates and nitrates, have little biological reactivity at environmentally relevant concentrations. The health risk of exposure to secondary sulphates, in particular, was later reviewed by Grahame and Schlesinger,<sup>4</sup> who drew the same conclusion that secondary sulphates are unlikely to cause the adverse health effects reported from epidemiological studies. However, both reviews acknowledge that there are limitations in the existing data, and that more studies may be needed to precisely clarify any role of these compounds to PM-induced health effects.

### *Consistency considerations*

In a recent review, Grahame and Schlesinger,<sup>4</sup> came up with alternative explanations for the observed associations in epidemiological studies, pointing to studies that show the importance of traffic-related air pollutants or industrial emissions. The argument was that most sampling monitors in studies showing the effects of sulphates, were more representative for background concentrations. Investigations focusing on site-specific monitoring of exposure indicate the importance of traffic exhaust emissions. However, a recent study of diabetic patients whose exposure was estimated by a local air pollution monitor, showed associations between sulphates and other PM metrics and vascular symptoms.<sup>144</sup>

Grahame and Schlesinger emphasize the variations in sulphate or PM<sub>2.5</sub> exposure between cities in the Six Cities Study and their associated health risks, which they suggest to be more consistent with specific local emissions than regional sulphate

concentrations. In addition, in the American Cancer Study, subpopulations with lower education levels seemed more at risk for health effects than other groups, which might be related to exposure to higher levels of pollution from traffic. Their arguments seem to be in line with the observed similarly increased risk of health effects in different US regions, despite variations in air pollution composition, including sulphates. However, Forsberg *et al.*,<sup>145</sup> found increased risks of health effects in areas dominated by long-range transboundary and regional air pollution, including sulphates. A specific role of sulphates was not investigated in this case. The possibilities pointed out by Grahame and Schlesinger to explain the discrepancies between different approaches remain to be investigated.

Presently, the results from epidemiological and experimental investigations do not seem consistent with each other. Different types of epidemiological studies indicate a role for sulphates in PM-induced health effects. The experimental studies indicate that sulphates only elicit effects at concentrations far higher than environmentally relevant.

## Importance of particle-associated soluble organic compounds

Organic compounds constitute a considerable part of particle mass. They are a very diverse mixture of volatile and non-volatile components, such as benzene, xylene and PAH. In air, the compounds may undergo chemical modifications that may alter their ability to induce biological effects.<sup>146</sup> This may indicate particle component heterogeneity depending on the time and chemical reactions in the atmosphere.

### *Epidemiological studies*

Investigations in areas with a predominant source leading to the emission of particles with organic components are infrequent, but those that exist do not reveal major differences to studies which show associations of general urban air combustion-dominated PM with lung cancer.<sup>32</sup> In addition, smoke from residential heating with coal or wood showed increases in mortality/morbidity similar to those of general urban air exposure.<sup>147,148</sup>

The most prominent effect of PAH is the increased risk of lung cancer. For women using insufficiently ventilated stoves, PAH in wood smoke is estimated to increase their risk of developing lung cancer.<sup>149</sup> There are no studies describing cancer development in the general population in relation to measured PAH concentrations in the outdoor air. One review, that included a meta-analysis, dealt exclusively

with occupational studies.<sup>150</sup> The analysis of occupational studies showed a significant increase in the risk of contracting lung cancer with increased exposure to benzo(a)pyrene (B[a]P) as a PAH marker. Two studies show an increased risk of contracting lung cancer in occupational settings with diesel exhaust. PAH were not measured in these studies.<sup>151,152</sup> A review on children revealed an increase in biomarkers of DNA damage in blood cells in areas with high air pollution, compared to subjects in areas with lower pollution. However, none of the results were related to PAH levels in PM.<sup>153</sup> There is insufficient evidence to link DEP and PAH to asthma or allergy.<sup>154</sup>

Relationships between more volatile compounds (benzene) in ambient air and health effects have been reported, but the compounds have generally been regarded as indicators for traffic pollution.<sup>155</sup> In addition, benzene has been linked to cancer. A study on childhood leukemia found a positive association between exposure to benzene and the risk of childhood leukaemia.<sup>156</sup> Air pollution containing ultra-fine particles and high levels of benzene was associated with increased oxidative DNA damage.<sup>157</sup>

#### *Experimental studies*

Several experimental studies have attributed the biological effects of combustion particles, in particular DEP, to adsorbed organic compounds. First and foremost, a range of PAH and other organic compounds found in DEP and other combustion particles are well-known carcinogens, and their effects have been thoroughly described in the literature. In accordance with this, organic compounds extracted from combustion particles have been shown to form DNA adducts, induce mutations and inhibit gap junction intercellular communication.<sup>158–162</sup> Moreover, genotoxicity, mutagenicity, and tumorigenicity have been shown for organic extracts from outdoor PM.<sup>163–167</sup> Thus, there is substantial experimental evidence for a possible role of soluble organic compounds in PM-induced carcinogenesis. A differential potential of the various PAH components in inducing carcinogenesis is well established. B[a]P, a potent carcinogen, has frequently been chosen as a model compound to which the potential of the other members of the group is often related.

Organic compounds also appear to play an important role in PM-induced cytotoxicity. Organics, such as PAH, have been shown to induce both apoptotic and anti-apoptotic signals.<sup>168</sup> DEP induced apoptosis in macrophages through ROS generation, with subsequent activation of caspase cascades, loss of membrane integrity and DNA

damage.<sup>169</sup> Organic extracts from DEP were able to induce apoptosis, whereas residual DEP – with organic constituents extracted – neither induced apoptosis nor ROS generation. Fractionation of different organic constituents of DEP has shown that the quinone-rich polar fraction was more potent than the aromatic fraction in inducing ROS generation, decreasing membrane potential, with loss of mitochondrial membrane mass and apoptosis in macrophages, whereas the aliphatic fraction had no effect on cell viability.<sup>170</sup> DEP-induced cytotoxicity has also been attributed to the particle core of DEP and not the soluble organic compounds,<sup>171</sup> suggesting cell-specific differences in responses to DEP and its constituents.

Experiments with peripheral blood mononuclear cells (PBMCs) showed that exposure with organic DEP extracts increased the production and release of the chemokines IL-8 and RANTES, whereas MCP-1 was down-regulated.<sup>172</sup> Ohtoshi *et al.*,<sup>173</sup> showed that DEP induced the release of GM-CSF and IL-8 from human airway epithelial cells. Charcoal and graphite, used as models for the carbon core of DEP, did not affect GM-CSF or IL-8 release, whereas B[a]P, one of the important aromatic hydrocarbons contained in DEP, showed a stimulatory effect on the cytokine release.<sup>173</sup> In accordance with this, DEP-induced GM-CSF release from human airway epithelial cells was mainly caused by adsorbed organic compounds, although the residual particles also caused some effects.<sup>174,175</sup> Notably, these studies report that organic DEP extracts induced IL-8 release three to six times higher than the background levels (and similar or lower for RANTES and GM-CSF). In comparison, PM and ROFA particles have been shown to induce more than 20 times increase in IL-8,<sup>16,121</sup> whereas metals, such as zinc and vanadium, have been reported to induce more than 10 and 20 times increases, respectively.<sup>7,176</sup> Thus, it may appear that although the organic compounds may induce cytokine release, they may not be particularly potent.

Indications of a link between traffic emissions and the development of allergy, has led to a series of experimental studies on the effect of DEP and organic DEP constituents. DEP and organic DEP extracts, including pyrene and B[a]P, have been shown to elicit adjuvant activity on IgE production in mice immunized with pollen allergen.<sup>177,178</sup> Similarly, DEP has also been shown to have an adjuvant activity on IgE and IgG production in mice immunized with ovalbumin. Interestingly, both organic DEP constituents, residual DEP and carbon black displayed an adjuvant effect on IgE/IgG production to ovalbumin, suggesting that both the

adsorbed organic constituents of DEP and the non-soluble carbon core were involved in the observed effects.<sup>179,180</sup>

*In vitro* studies support these *in vivo* findings, and thus strengthen the evidence for a role of organic DEP constituents in allergic responses. PAH-extracts from DEP were shown to enhance IgE production by different immune cells.<sup>181</sup> Organic DEP extracts also stimulate IL-4 release. Pyrene was found to induce IL-4 production by primary human T cells.<sup>182</sup> Organic DEP extracts induced IL-4 production and histamine release from human basophils, but were not found to synergistically increase allergen-induced IL-4 production or histamine release.<sup>183</sup> Extracts of DEP have also been found to favour T-helper 2 (Th2) cell recruitment by immune cells from allergic patients, by differentially regulating the Th2-recruiting chemokine MDC and the Th1 recruiting chemokine IP-10.<sup>184</sup> Recent studies also suggest that organic DEP constituents may stimulate the antigen-presenting activity of monocytes.<sup>185</sup>

#### Consistency considerations

Presently, there is insufficient epidemiological evidence for a role of organic components in PM-induced diseases in the general population. The epidemiological evidence for the carcinogenic effects of organic compounds comes mainly from occupational studies. Most likely, the exposure concentrations have been much higher than for the general population, although the exposure period may have been shorter. These studies are consistent with the DNA-damaging, mutagenic and carcinogenic effects of organic PM-components in experimental studies. Although it remains to be investigated whether the concentrations of soluble organic components in PM, and, in particular those from DEP or wood combustion PM, are sufficient to cause similar effects in the general population, an increase in lung cancer has been associated with PM. This would be consistent with a role for organic compounds as causative agent, but does not exclude the involvement of other components. Experimental studies suggest that organic compounds may also be involved in a range of biological effects of DEP exposure, including cytotoxicity, inflammation and allergic reactions, suggesting that organic compounds may contribute to a range of PM-induced diseases. Presently, there is little experimental information on the contribution of specific organic compounds in PM effects, as most studies have only used extracts of the organic fraction. Moreover, there is still little information on the relative importance of the organic fraction in outdoor sampled PM in these responses compared to other PM constituents.

In particular, the experimental observations of a potential role of organic compounds in DEP-induced allergic or non-allergic inflammation warrant further investigation.

#### Importance of particle-associated biological components

Particles may contain different biological components, such as endotoxins, beta-glucans and mould spores. These biological components are ubiquitous and occur in both indoor and outdoor PM. Endotoxin levels vary considerably with season, temperature and humidity.<sup>186,187</sup>

#### Epidemiological studies

Several studies show associations between endotoxin exposure and respiratory morbidity in occupational settings, with an apparent protective effect on sensitization and allergy.<sup>188,189</sup> Studies of endotoxin in outdoor PM are scarce, whereas several studies have examined health effects in relation to endotoxin levels indoors.<sup>186</sup> Tavernier *et al.*,<sup>190</sup> found that endotoxin was a risk factor for asthma development, but was not associated with PM<sub>2.5</sub>, mite allergen, environmental tobacco smoke or dampness. Similarly, Montealegre *et al.*,<sup>191</sup> observed that endotoxins, in contrast to cat and mite allergen, were associated with the induction of asthma symptoms in a hot climate region.

Indoor moulds and mould allergens have been associated with allergic sensitization in children.<sup>192</sup> Also, indoor mite and pet allergens, and outdoor allergens, including mould allergens, were associated with asthma exacerbations, but again this was not related to any particle metrics.<sup>14,193</sup> A study from California indicated that pollen associated with particle metrics had effects on asthmatics.<sup>154</sup>

#### Experimental studies

A range of studies has shown that microbial constituents may play an important role in PM-induced inflammation. Bacterial endotoxins are well-known inflammatory agents and have been widely used to investigate inflammatory responses in various cell systems. A series of studies have shown that the more potent induction of cytokines and chemokines by ambient PM<sub>10-2.5</sub> than PM<sub>2.5</sub>, is related to a higher endotoxin content in the coarse fraction.<sup>16,17,93-98</sup>

Broncho-alveolar lavage fluid from rats instilled with PM from a rural and an industrial location in Germany, showed evidence of neutrophilic inflammation, increased TNF- $\alpha$  release, and depletion of glutathione, that was associated with PM endotoxin

content.<sup>96</sup> Exposure of human AM and airway epithelial cells to PM, sampled in Chapel Hill, USA, showed that PM<sub>10-2.5</sub> induced a stronger pro-inflammatory response than fine and ultrafine PM. Cytokine release induced by all Chapel Hill PM fractions was attenuated by the use of blockers preventing endotoxin binding, hence suggesting a crucial role of endotoxins in these PM samples.<sup>17,97,98</sup> Moreover, components of Gram-positive bacteria induced higher IL-8 release from airway epithelial cells than Gram-negative, showing that the type of bacterial constituents may affect PM inflammatory potential.<sup>97</sup> Studies using Mexico City PM on monocytes also suggest that the endotoxin content was important to PM-induced pro-inflammatory reactions, but that PM-induced cytotoxicity was mediated through endotoxin-independent mechanisms.<sup>94</sup> Studies with dust from nine sites in western US also indicate that endotoxins are not involved in PM-induced cell death.<sup>194</sup>

Although the evidence appears to support a role of the microbial content in PM-induced inflammatory reactions, other studies have failed to find a correlation with endotoxin.<sup>18,124,194</sup> Veranth *et al.*,<sup>194</sup> showed that the tested particles caused much higher IL-6 release than lipopolysaccharides (LPS), or surrogate particles mixed with LPS. In a recent study of PM sampled from four European cities during three different seasons, the coarse particles were found to induce more cytokine release than fine particles.<sup>18</sup> Despite a higher concentration of endotoxin in PM<sub>10-2.5</sub> than PM<sub>2.5</sub>, variations in endotoxin content did not reflect variation in cytokine release induced by the different coarse fractions. Moreover, the addition of polymyxin B did not affect PM-induced cytokine release. Thus, it seems probable that PM may also induce pro-inflammatory reactions through endotoxin-independent mechanisms.

Analysis of PM composition has documented that particles may also act as allergen carriers.<sup>15</sup> Despite several studies exploring the importance of PM in allergy and asthma development, such as the adjuvant properties of particles (also discussed under soluble organic components), the extent to which particle-bound allergens may be involved in these processes is unclear.

#### Consistency considerations

Mould, mite and pet allergens are involved in allergic sensitization. However, these effects are often not related to particle metrics and, therefore, it is difficult to relate these results to experimental findings that include particles. The epidemiological evidence is insufficient to conclude whether bacter-

ial components enhance allergic sensitization or not, but there is more evidence for a role in the exacerbation of asthma. As previously discussed, inflammation is believed to be a key factor in PM-induced morbidity and mortality. Whereas a range of experimental studies have suggested endotoxins as one of the main inflammatory constituents of PM, epidemiological investigations have, so far, not considered the importance of endotoxins in relation to PM effects.

#### Importance of crustal particles

The constituents of the coarse fraction with its crustal components have mainly been described in some detail in occupational settings. In ambient air epidemiological studies, such data are scarce.

#### Epidemiological studies

In a recent study by Forsberg *et al.* (personal communication), an increase in hospital admission for asthma, but not for cardiovascular disease, was reported to be associated with the coarse PM fraction in contrast to the fine fraction. The coarse fraction was dominated by mineral particles due to road pavement abrasion and resuspension. A Finnish study from Helsinki with comparable PM composition showed an association of the coarse fraction with the prevalence of cough.<sup>195</sup> Additionally, soil erosion and volcanic ash particles were associated with increases in morbidity and mortality.<sup>196,197</sup> Volcanic ash may contain minerals similar to those found in crushed rock used in asphalt, including quartz, pyroxene, mica and feldspar. The content of these minerals in the ash has not been related to health effects. In a study from Phoenix (USA), wind-blown sand was associated with mortality.<sup>74</sup> Sand may consist of quartz and, thus, may potentially be hazardous. Studies of dust storms in Washington State (USA), revealed associations between high levels of particle exposure and respiratory symptoms.<sup>198,199</sup> In contrast, another study of dust storms did not find significant effects of coarse particles that were presumed to contain mineral components.<sup>69</sup> In summary, this might indicate that the importance of the crustal particles for respiratory effects depends on the composition of PM.

#### Experimental studies

Decades with experimental studies on quartz and asbestos have thoroughly established the carcinogenic, inflammatory and cytotoxic potential of mineral particles. Studies on silica (SiO<sub>2</sub>) suggest that crystallinity is an important factor to the toxicity of mineral particles, since crystalline silica

(quartz) displays higher biological activity than amorphous silica.<sup>200</sup> Notably, there is only limited information on the effect of minerals, other than quartz and asbestos. However, additionally, more complex mineral composition, generated from stone used in road pavement, may cause inflammatory responses both *in vivo* and *in vitro*.<sup>201,202</sup> Presently, which minerals make some stone particles more potent than others remains to be identified, but it appears that feldspar minerals have a low ability to induce inflammatory reactions.<sup>91</sup> Moreover, mica particles, thin sheet-like minerals, which are commonly found in spring road dust in Finland, have been shown to induce cytokine release from macrophages.<sup>203</sup>

#### *Consistency considerations*

Epidemiological studies in which crustal particles dominate, generally show that these particles may elicit adverse effects on respiratory morbidity. This is consistent with experimental data, showing the potential of mineral particles to induce the release of pro-inflammatory cytokines. The majority of experimental studies have been performed with quartz and asbestos, which might not be representative of the crustal particles from wind-blown dusts or road abrasion particles. Thus, despite epidemiological data associating health effects with exposure to mineral-rich PM, there is presently a lack of experimental studies exploring the relative importance of mineral components in ambient PM.

#### **Certain limitations in epidemiological and experimental data**

Most epidemiological studies have shown statistically significant associations between PM of different sizes and health effects. However, little is known about which constituents or properties of PM may be causative for the observed effects. With respect to size, many studies have attempted to estimate exposure from one or a few monitoring stations, often placed in an urban background area. This may not capture the considerable city variations in PM size, and reduces the possibility of correct assessment of the effect of different size fractions. This may lead to underestimation of the effect of these size fractions. The use of geographical information systems in modeling address-based exposure or even personal exposure may improve the exposure characterization. Some recent studies have corroborated the importance of improved characterization.<sup>33,204,205</sup> In particular, for ultrafine particles, the optimal means to measure exposure remains to be determined. Some studies have focused on

particle number, others on the mass of ultrafines. Also, the OC/EC measurements seem to correlate with measurements of ultrafines. Specific surface area or primary particle size has rarely been assessed. Since ultrafine particles tend to aggregate with distance from the source, a more precise exposure characterization may show a more important role of ultrafines than presently indicated from epidemiological studies.

With respect to the importance of different components of PM, the data are even less clear than for the size fractions. The epidemiological investigations depend on the existence of contrasts in effect and exposure. PM components show a widely varying inter-correlation, so that the attribution of effects to specific components may become difficult. In addition, most epidemiological studies may not have been designed to elucidate the importance of different components. Given the experimental results of different components exerting similar effects, and the difficulty in determining contrasting exposures for various components, the epidemiological assessment may not be useful in elucidating the importance of different components beyond the assessment of contribution by different sources. Thus, the studies do not provide sufficient evidence alone with respect to which components might be most important to regulate in order to reduce adverse health effects.

A consistency between epidemiological and experimental results would strengthen the conclusions. However, the experimental findings also vary, and drawing conclusions from the combined results of epidemiological and experimental studies may be difficult. This may be due to the frequently different end points studied, the much higher concentrations used in experimental studies, and the scarcity of sensitive models.

In addition, variations in experimental conditions hamper the comparability of reported findings. Experimental studies show that a number of PM constituents may induce similar effects. Few experimental investigations have focused on the relative ability of different PM constituents to induce health effects. Due to the lack of standardization of exposure conditions, cell types, etc., it is hard to determine which components contribute the most to a given response. Thus, there seems to be a critical need for comparative studies on different PM constituents. Moreover, the knowledge on cooperative actions of different components is also scarce. While some studies have suggested synergistic effects, other studies have shown that antagonistic effects may occur between different PM components. Most notably, transition metals have

been shown to enhance the effect of particles.<sup>140,206,207</sup> DEP exposure has been shown to attenuate the inflammatory response to endotoxin.<sup>98,208,209</sup> However, the importance of combined exposure to different PM-components is far from elucidated and needs to be determined.

During the last years, there has been an ever increasing focus on the role of ultrafines in PM-induced health effects. This has been based on experimental studies showing that PM effects are closely linked with total surface area, and the fact that ultrafine particles constitute a relatively large part of the total surface and number of particles in PM<sub>10</sub> compared to their contribution of mass. A predominant role of ultrafine particles in the adverse effects of ambient PM does not seem to be sufficiently supported by the few available epidemiological studies. There are also some caveats appearing from the experimental research on ultrafines. First, although these studies advocate the importance of the particle surface in driving PM toxicity, exposures to different particle sizes are still made on a per mass basis and often with a limited number of concentrations, instead of exposing to equal amount of particle surface area. This hampers comparisons of effects among particles of different size or composition. Second, there seems to be a lack of studies that have attempted to elucidate whether the greater effect of ultrafines than fine or coarse particles (on a per mass basis) may compensate for the considerable larger mass of fine and coarse particles found in PM<sub>10</sub>. Answering this would provide important information on the toxicity of ultrafine particles.

## Concluding remarks

Current air quality standards and abatement strategies are based on the total mass of suspended particles, thereby treating all particulates as equally toxic. Such an assumption hardly fits with basic toxicological principles. Based on experimental studies with model particles, it has been argued that particle surface area or total number of particles would be a better measure, since these metrics are more strongly correlated with effects than particle mass. However, studies on PM sampled from outdoor air do not necessarily support this assumption, since the coarse fraction has often been shown to be the most inflammatory fraction. Using particle number or surface area would still not discriminate sufficiently between particles of different sources and with different composition. Indeed, there seems to be increasing support for the idea that the chemical characteristics are important for

the adverse health effects of ambient PM. Thus, there has been a call for more specific exposure metrics which account for the composition or origin of ambient PM.<sup>4,145</sup> To comply with this requires the identification of the hazardous and non-hazardous PM components. Presently, the available evidence seems insufficient for drawing general conclusions on this matter. In particular, there is a lack of epidemiological data on specific PM components. There are also limitations in the ability to compare experimental data, especially on the relative effects of different constituents, and how different compounds may interact to elicit adverse effects.

The experimental investigations reviewed in this paper suggest that a range of PM components, despite widely different physico-chemical properties, may induce similar cellular responses. Thus, it seems possible that some PM constituents, to a certain extent, may be substituted with other compounds without considerable effects on the overall activity of the PM matrix, as suggested by others.<sup>210</sup> In particular, this seems to be the case for inflammatory reactions which are believed to be central in the development of a range of PM-induced diseases in the pulmonary and cardiovascular systems. This would explain why some epidemiological studies from different regions with PM from different sources and with different compositions, may end up with risk estimates within a similar range. Such a scenario also suggests that epidemiological studies may have difficulties with identifying the critical components of PM. Though different PM constituents may induce qualitatively similar effects, it seems likely that the relative ability to induce a given response (ie, the quantitative effect) would vary among different components, and that some components are more important than others for specific health effects. Such an assumption would also be in agreement with the heterogeneity in risk estimates reported from multi-centre studies.

Despite weaknesses in the analytic approaches, some conclusions may be drawn from the combined data. There seems to be some consistency between epidemiological and experimental data with respect to size. Coarse particles have an effect that should not be neglected. The magnitude of the effect may differ with the type of coarse particle. However, the importance of the different PM size-fractions may be more a question of which compounds they contain, rather than the actual particle sizes. Notably, experimental studies suggest that endotoxins are the predominant inflammatory compound in PM from many (but not all) areas. Given the importance of inflammatory processes in PM-induced disease, the importance of endotoxins in epidemiological

studies warrants more investigation. However, more data are also needed to discriminate between different types of coarse particles, among them minerals. Regarding the hypothesis of a predominant role of ultrafine particles, this issue also warrants further investigation.

The consistency between epidemiological and experimental findings for specific PM components appears most convincing for metals, which seem to be important to the development of both pulmonary and cardiovascular disease. The identification of specific metals is less clear, but zinc, copper, vanadium, iron and nickel may potentially be more important than other metals. Metals may also be involved in PM-induced allergic sensitization, but the epidemiological evidence for this is scarce. Soluble organic compounds appear to be implicated in PM-induced allergy and cancer, but the data from epidemiological studies are insufficient for any conclusions. Experimental studies also suggest that organic compounds from PM may cause inflammatory reactions, but their relative contribution com-

pared to other PM constituents is unclear and needs to be determined. The results of studies with secondary inorganic compounds are inconsistent between epidemiological and experimental studies.

Despite a considerable amount of research over the last decades, we have only just begun to understand how PM elicits adverse health effects. Identifying the key components or sources responsible for these effects is not a small task. Both epidemiological and experimental studies are needed to further clarify the issues indicated in this review. Also, uncertainties regarding exposure concentrations and models must be overcome to bridge the gaps between *in vitro*, *in vivo* and epidemiological investigations. To increase the pace in scientific advances, improvements in study designs should be considered. In particular, there is a need for better exposure assessments in epidemiological investigations, whereas experimental data would benefit from an improved comparability of studies. Combined experimental and epidemiological investigations may help answer some of the unresolved issues.

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