

REVIEW

Mechanisms of particulate matter toxicity

S. SALVI and S. T. HOLGATE

Department of Medicine, Southampton General Hospital, Southampton, UK

Introduction

The human lung with a surface area of 40–120 m² is constantly exposed to between 10 000 and 20 000 L of ambient air daily, which consists of a wide range and a large number of non-biological particles produced by human inhabitation. The lung's defence mechanisms are usually adequate to deal with a large number of particles deposited on to its surface. In brief, these constitute mechanical removal and biochemical neutralization. Larger particles deposited on the conducting areas such as the trachea and bronchi are propelled upwards by the mucociliary clearance mechanisms into the throat and finally swallowed, while smaller particles which reach the oxygen absorbing area of the lung are removed by scavenging cells called macrophages, which either carry the phagocytosed particles up to the airways towards the mucociliary clearance system or are transported through the alveolar wall into the lungs' internal clearance channels, the lymphatic vessels which drain into the lymph nodes, where they might remain in an inert state, or be transported into the blood. Particles present in the ambient air contain transition metals capable of generating reactive oxygen molecules that produce an oxidant-mediated damage to cells lining the mucous membrane. These oxidizing substances are rapidly neutralized by antioxidants which are secreted by epithelial cells lining the respiratory tract or which passively diffuse from the plasma into the respiratory lining fluid.

Although the healthy lung is capable of dealing effectively with a large number of particles deposited on to its surface, these defence mechanisms may be overwhelmed by either particle number overload or by the inherent toxicity of the particle. In patients compromised with lung functions such as defective mucociliary clearance or abnormal immune function, even a small particle load may be sufficient to produce harmful effects on the lung. Recently, there have been increasing concerns over the adverse health effects of these ambient non-biological particles. Over 60 epidemiological studies conducted in different geographical locations of the world have demonstrated a consistent association between ambient levels of particles with various

health outcome indices, including mortality, exacerbations of asthma, chronic bronchitis, respiratory tract infections, ischaemic heart diseases and strokes [1–4]. Similarly, there has been an increasing understanding into the underlying biological mechanisms of toxicity of particles over recent years, which has helped to strengthen the association between particulate matter and health as causal, as has been suggested in epidemiological studies. This article reviews the literature of the various mechanisms of particulate matter toxicity.

Particle deposition studies

Exposure concentration of respirable particles is central to determining the dose deposited in the lung. Large numbers of particles deposit in the lung following exposure to high airborne mass concentrations. One of the major factors which determines the toxicity of inhaled particles is their dosimetry characteristics, which include particle deposition, clearance, retention, translocation and dissolution within the different regions of the lung, factors which are important for their persistence within the lung [5]. The dose of inhaled particles and the deposition site within the lung vary widely and are mainly determined by their aerodynamic size and shape, breathing pattern and lung structure [6]. Generally, as the particle size and breathing rates increase, particles deposit more in the proximal areas, whereas enhanced distal deposition takes place with small-sized particles and slow breathing rates [7,8]. The ambient particulate cloud comprises particles of different sizes ranging from a few nanometres to several tens of micrometers, and amongst these, particles below 10 µm in diameter have the potential to reach and be deposited in the alveoli, while those greater than 10 µm are likely to land in proximal airways and be removed by the ciliary activity. Within the conducting airways, particle deposition tends to be greatest at the carinae, especially those particles > 1 µm in diameter [9]. Previous studies in humans using radioactive particles of varying sizes have demonstrated that particles with a mass median diameter of 2.5 µm undergo 83% total lung deposition, while particles of 8.2 and 11.5 µm diameters undergo 49 and 31% deposition, respectively, suggesting an inverse relationship between particle size and total lung deposition [10]. Similarly, smaller-sized particles tend to be retained in

Correspondence: S. Salvi, University Medicine, Level D Centre Block, Southampton General Hospital, Southampton SO16 6YD, UK.

the lung for a longer duration (77% retention for particles $2.5\text{ }\mu\text{m}$ in diameter, compared with 15% retention for particles of diameter $8.2\text{ }\mu\text{m}$ after 24 h) [11]. Recent studies using Single Photon Emission Computed Tomography (SPECT) with MRI have demonstrated greater total lung deposition for particles of mass median diameter $1.8\text{ }\mu\text{m}$, compared with particles of size $6.8\text{ }\mu\text{m}$ [12]. Particles smaller than $2\text{ }\mu\text{m}$ in diameter show a clear tendency to achieve greater peripheral deposition than those greater than $2\text{ }\mu\text{m}$ in diameter. Interestingly, even in the presence of moderate to severe airway obstruction, such as bronchial asthma, particles smaller than $2\text{ }\mu\text{m}$ are capable of reaching high levels of peripheral deposition when compared with coarser particles [13]. Overall lung deposition has been shown to be increased in patients with obstructed airways and abnormal geometry [14]. A 30% reduction in airway cross-sectional area results in a deposition increase in the bifurcating airways by more than 100% [15]. Regional deposition dose, or local tissue burdens, can be significantly different among individuals, even if total lung deposition values are comparable. It is therefore possible that particle burdens could reach the threshold limits at local lung regions under exposure conditions that are normally acceptable, particularly in individuals with compromised lungs [15].

Most particles obtained from the ambient atmosphere of urban settings lie in the size range between 0.05 and $0.2\text{ }\mu\text{m}$ [5]. Their chemical composition varies considerably, but the ultrafine fraction (particles $\leq 2.5\text{ }\mu\text{m}$ in mass median diameter, called $\text{PM}_{2.5}$) consists of about half carbon and half salts, mainly ammonium sulphate and ammonium nitrate [16]. Amongst the various sources, diesel exhaust is the single most major contributor to particulate matter in most urban areas world-wide, accounting for up to 90% of the total particle mass in some major cities such as London, mainly because diesel vehicles generate up to 100–150 times more particles than those discharged from catalyst-equipped petrol vehicles of similar performances [17]. The mass median diameter of diesel exhaust particles is approximately 0.2 with over 90% being < 1 [18]. Recent electron microscopy studies suggest that over 80% of the number of diesel exhaust particles are in the size range around $0.1\text{ }\mu\text{m}$ [19], adsorbed onto which are an estimated 18 000 different high molecular weight organic compounds [20]. Studies carried out at Birmingham University have demonstrated that particles in this size range have around 50% lung deposition [5].

Experimental studies in rats have shown that ultrafine particles sustain more lung injury and pathology than those exposed to the same deposited mass of fine respirable particles of the same material [21,22]. It has therefore been suggested that particle numbers and size appear to be more important than the mass in producing a biological

response [23]. This mainly appears to be due to the large surface area available on smaller-sized particles. The mass of a $1\text{-}\mu\text{m}$ particle is equivalent to the mass of 1000 $0.1\text{-}\mu\text{m}$ particles, however, the surface area of the same mass of a $0.1\text{-}\mu\text{m}$ particle is 10 times that of a $1\text{-}\mu\text{m}$ particle. Therefore a 10% increase in PM_{10} would represent a very large percentage change of both the number of particles and their surface area [5]. Therefore, as particles get smaller, their surface area for the same mass becomes greater, and hence their capacity to carry toxic substances and free radicals increases. More free radicals are detected in ultrafine particulate samples compared with coarser samples of the same substance [22,24] and hence the differences in their ability to cause lung inflammation can be explained on the basis of different amounts of free radical activity.

Although their contribution to particle mass is quite small, ultrafine particles are typically present in the air in very high numbers. For a given weight of ambient particles, the large majority of the mass resides in the fraction greater than $1\text{--}2\text{ }\mu\text{m}$ in diameter, but the greatest number of particles by far will be below this size. Fixed site particulate monitors which record the total mass of suspended particles in the ambient air, measured as PM_{10} (particles with a mass median diameter $\leq 10\text{ }\mu\text{m}$) disproportionately represents the largest-sized particles, and may therefore not be effectively measuring the most important particulate size fraction associated with adverse health effects [25,26]. Similarly, area sampling data from fixed site monitors may be of limited value in the investigation of the biological effects of exposure to PM_{10} , since some studies have demonstrated that the levels measured by personal PM_{10} monitors measure values on average up to 2–5 times higher than those recorded at fixed site monitors [27,28].

Laboratory-based studies

In vivo animal exposure studies

A number of animal models have been developed to investigate the toxicological mechanisms of particles. These have generally involved instillation of suspensions of particles collected from ambient air or particles of specific composition, such as diesel particles, residual oil fly ash, carbon particles or titanium dioxide. Following deposition in the lung, alveolar macrophages rapidly phagocytose particles in an opsonin-independent manner, using scavenger-type receptors [29] and migrate towards the bronchoalveolar junction, where they tend to accumulate and aggregate [30–33]. Large numbers of ultrafine particles, however, pose a substantial burden for the macrophage phagocytic system and result in increased numbers of particles coming in contact with the respiratory epithelium. Earlier studies in animals exposed to high levels of particles

generated from diesel exhaust have reported damage to alveolar macrophage membranes and depressed phagocytic activity followed by damage to ciliated epithelial cells lining the trachea and large airways [34–36]. Morrow [37] demonstrated that impairment of clearance by macrophages begins when the particles occupy 6% of the macrophage volume and is completely inhibited at 60% of macrophage volume, while Oberdorster *et al.* [21] demonstrated impairment of clearance at 2.6% macrophage volume, especially with ultrafine particles when the particle retention time in the lung increases eightfold. Oxidative damage mediated by particles then leads to activation of macrophages with the subsequent release of potent pro-inflammatory mediators such as IL-1, IL-6 and TNF α [38,39]. Instillation of fine carbon particles in the lungs of rabbits stimulates the bone marrow to release neutrophils into the peripheral blood and shorten their transit time to the lung [40], an effect mediated by the production and release of inflammatory mediators from activated alveolar macrophages.

One of the earliest changes of lung toxicity in guinea pigs mediated by particles is damage to capillary endothelial cells and type II pneumocytes of the alveolar epithelium, which leads to alveolar oedema and a subsequent cellular inflammatory response involving neutrophils [41]. In guinea pigs scanning electron microscopy has demonstrated that the particulate component of diesel exhaust causes alveolar destruction in a concentration- and duration-dependant manner [42]. Rodents chronically exposed to particles produce increased levels of cytoplasmic and lysosomal enzymes and total proteins, suggesting cellular injury, which is followed by increased synthesis and deposition of collagen [43,44] producing thickening of the alveolar septa and blood vessels and the development of interstitial fibrosis [31,45–48].

Guinea pigs and mice exposed to diesel particles following allergen challenge demonstrate increased nasal mucosal hyperresponsiveness to histamine, with enhanced sneezing and increased nasal secretions [49,50], which is associated with increase in vascular permeability, stiffness of lung parenchyma and airway resistance and a reduction in lung volumes and lung compliance [51]. In a mouse model of allergic airways disease, with features similar to human asthma, intratracheal instillation of fine particulate matter obtained from ambient urban atmosphere has demonstrated to increase airway hyperreactivity associated with increase lavage eosinophil numbers and IL-4 levels [52]. Similarly, instillation of diesel particles in mice has demonstrated aggravation of allergen-induced airway inflammation, characterized by an eightfold increase in IL-5 levels in bronchoalveolar lavage fluid, a 35-fold increase in eosinophilic infiltration and a sixfold increase in lymphocytes in the lung tissue, along with increase in the numbers of goblet

cells in the bronchial epithelium [52,53]. Increased levels of mRNA for IL-2, IL-4 and GM-CSF have also been reported in the airway tissue following inhalation of diesel particles [52]. Co-administration of diesel particles with allergen increases the levels of IL-4, a TH2-type cytokine along with a concomitant decrease in IFN γ , a TH1-type cytokine in the mediastinal lymph nodes of mice [54,55]. Recently, the carbon core of diesel particles has been shown to have a significant adjuvant effect on the local immune-mediated inflammatory response as well as on the systemic-specific IgE response to allergen [56], while polycyclic aromatic hydrocarbons have been shown to promote greater IgE-epsilon (ϵ) RNA transcription and translation [57]. Particles generated from diesel exhaust have an adjuvant activity on the production of IgE and IgG1 antibodies against dust mite allergen in mice immunized intranasally with mite allergen [58]. Diesel particles have also been shown to adsorb allergens from grass pollen onto their surface and therefore act as potential carriers to increase their deposition in the respiratory tract [59]. This would have the effect of greatly increasing the allergen dose that reaches the lung and at the same time enhancing its antigenicity [60].

In vitro cell culture studies

Airway epithelial cells and macrophages are the initial target cells for particle interaction and deposition, and are therefore the cells most likely to be affected by the toxic effects of particles. Most *in vitro* particle exposure studies have therefore focused their work on these cells. Particles have been shown to produce dose-dependant membrane damage to epithelial cells [61,62]. Primary human airway epithelial cells when exposed to particles obtained from diesel exhaust *in vitro* actively phagocytose them followed by increased production of several pro-inflammatory cytokines such as IL-6, IL-8 and GM-CSF [61–64]. This suggests that epithelial cells can perform phagocytic functions as do macrophages and neutrophils, and also act as initiators of inflammatory responses by secreting various inflammatory mediators. Release of these pro-inflammatory cytokines appears to be more pronounced in epithelial cells obtained from mild asthmatic subjects compared with healthy subjects [65]. Similarly, particles collected from ambient urban air have been demonstrated to activate the transcription factor NF κ B and enhance the expression of IL-6, IL-8 and ICAM-1 in secondary-transformed human airway epithelial cells [66]. Residual oil fly ash, an important contributor to urban ambient particulate matter, when exposed to human airway epithelial cells *in vitro* induces the production of increased levels of IL-6, IL-8 and TNF α , as well as mRNA coding for these cytokines, which is inhibited by the inclusion of either a metal chelator or free radical scavenger, suggesting that metals present in the

particles produce an oxidative stress, which may be responsible for the production and release of inflammatory mediators [67]. Emission source and ambient particles contain significant concentrations of first row transition metals which have the capacity to support electron exchange and catalyse free radical production [68,69].

Diesel exhaust particles upregulate the expression of histamine H-1 receptor mRNA in human airway epithelial and endothelial cells *in vitro* and enhance histamine-induced increase in IL-8 and GM-CSF production [70]. Upregulation of histamine H-1 receptors is an important factor associated with nasal and bronchial hyperresponsiveness, features characteristic of allergic rhinitis and bronchial asthma and may therefore explain the increased incidence of asthma and rhinitis observed during particulate pollution episodes.

Peripheral blood mononuclear cells obtained from atopic subjects and cultured with particles obtained from diesel exhaust *in vitro* increase IgE levels which is associated with increased numbers of IgE secreting B cells [71]. Similarly, purified B cells when cultured with extracts from diesel exhaust particles demonstrate increased IgE production in the presence of costimulatory molecules such as IL-4 and CD40 [57]. It has been suggested that polyaromatic hydrocarbons present in diesel particles promote greater mRNA transcription and translation via acting on aryl hydrocarbon receptors present on B lymphocytes [57].

Human alveolar macrophages obtained from healthy human subjects following acute short-term exposure to diesel particulates show greatly impaired phagocytosis of yeast particles *in vitro* [72]. Exposure of particles obtained from ambient air to freshly isolated human alveolar macrophages for 24 h induces apoptosis and changes their phenotype towards a higher immune-active state capable of releasing a large number of inflammatory mediators [73]. Similarly, diesel particles when incubated with alveolar macrophages obtained from mice stimulate the release of TNF α and IL-6 [39].

Diesel particles when inoculated with allergen in mouse bone marrow-derived cultured mast cells have shown to greatly increase the production of IL-4 and IL-6 [74]. *In vitro* exposure of ambient particles collected from different urban settings to phagocytic cells demonstrates induction of oxidative stress which correlates with the iron content of the particles [75], while PM_{2.5} collected from the ambient air induces the expression of stress-related genes such as heat shock proteins hsp70, the magnitude of which correlates with the amount of soluble copper in the particulate preparations [76]. It is therefore suggested that the lung dose of bioavailable transition metal and not instilled particulate mass is the primary determinant of the acute inflammatory response for both the ambient and combustion source particulate matter samples [77]. Recently, particles from

diesel exhaust have also shown to activate the alternative pathway of the complement system and increase the production of biologically active degradation products of complement proteins such as C3a [78].

Human in vivo studies

The marked effect of inhaled particles on the human airways was highlighted in the 1990s by the finding that healthy human volunteers when exposed to diesel exhaust particles for 1 h demonstrated an increase in the number of alveolar macrophages, neutrophils and T lymphocytes in the broncho-alveolar lavage fluid [72]. Subsequently, diesel particles have been shown to produce an acute cellular and mediator inflammatory response in the airway tissue and peripheral blood of healthy human subjects [79]. These inflammatory indices included peripheral blood neutrophilia and thrombocytosis, increase in histamine, fibronectin, neutrophils, and B lymphocytes in the airway lumen and an increase in the number of inflammatory cells (neutrophils, lymphocytes and mast cells) in the bronchial tissue. This was mediated by an upregulation in the expression of adhesion molecules on the capillary endothelial cells and leucocyte cell surfaces. Similarly, diesel particles have also been shown to increase gene transcription and the expression of IL-8 protein in the human bronchial epithelium [80].

Nasal instillation of diesel particles in atopic human subjects has been shown to produce a 25-fold increase in IgE mRNA and a fivefold increase in IgE levels in nasal lavage [81]. When these particles are co-administered with allergen in atopic rhinitic subjects they produce a 50-fold increase in allergen-specific IgE, when compared with instillation of allergen alone [82]. Diesel particles also significantly increase the number of proliferating IgE-secreting B cells in the nasal mucosa [81], and recently it has been demonstrated that the combination of nasal mucosal stimulation with diesel exhaust particles and ragweed allergen is capable of driving an *in vivo* isotype switch to IgE in humans with ragweed allergy [83]. These results suggest that diesel particles induce a qualitative and quantitative increase in IgE levels, and can therefore contribute to the enhancement of mucosal allergy.

Apart from the physical property of ultrafine size, particle toxicity has also been linked to its pH [84] and metal content [77]. A large number of transition metals appear to be adsorbed onto the surface of particles, which are capable of generating reactive oxygen intermediate species from various airway cells. These in turn activate redox-sensitive transcription factors such as NF κ B, AP-1 and AP-2 which induce gene transcription of a large number of pro-inflammatory molecules such as cytokines, cell adhesion molecules and inflammatory mediator receptors [85]. These mechanisms may underlie the inflammatory effects of particles.

Particles and cardiovascular system

Ambient particles have been associated with cardiovascular mortality and morbidity, especially among the elderly population [2,86]. Seaton *et al.* [87] have suggested that low-grade inflammation caused by ultrafine particles deposited in the alveoli might lead to increased coagulability and that the altered blood rheology might therefore be a part of the pathological mechanism linking particulate pollution with cardiovascular mortality and morbidity. Several haematological factors, including plasma viscosity, fibrinogen, factor VII and plasminogen activator inhibitor occurring as a consequence of inflammatory reactions are predictive of cardiovascular disease. To support this hypothesis, Peters *et al.* [88] have recently demonstrated increased plasma viscosity in a large population sample which correlated with particulate matter levels in the ambient atmosphere. Similarly, instillation of ultrafine carbon black in the lungs of rats has been demonstrated to increase the levels of coagulation factor VII [89]. A 1-h exposure to diesel exhaust particles in healthy human volunteers in chamber studies has demonstrated an increase in the number of circulating platelets and neutrophils in the peripheral blood, along with an acute cellular and mediator inflammatory response in the airways [79]. An increase in the number of leucocytes and platelets in the systemic circulation, even without apparent signs of cell activation, may impair the flow of blood cells through the microcirculation and increase the haemodynamic resistance to flow in many organs [90]. There is increasing evidence to suggest that neutrophils play a key role in reducing myocardial function as part of the systemic response to inflammation [91]. Release of IL-6 from macrophages following particle phagocytosis, as has been demonstrated *in vitro* [39], could stimulate hepatocytes to secrete fibrinogen [92] and increase blood viscosity. Similar but less-severe systemic responses to ultrafine particle-associated lung inflammation could result in chronic activation of circulating neutrophils, which could in turn lead to cardiovascular damage in patients with already compromised myocardial function.

Summary

The ambient atmosphere contains a large number of non-biological particles produced by human inhabitation. Following inhalation, ultrafine particles deposit in large numbers in the conducting and gas exchange areas of the lung. By virtue of their extremely small size, they have a large surface area capable of carrying large numbers of toxic substances and free radicals on their surface. This provides the opportunity for surface chemistry of the particles to have a profound biological effect on the cells which come in contact with them. Alveolar macrophages rapidly phagocytose particles

which deposit in the airways, however, their phagocytic capacity decreases when the particle load is high. This results in their prolonged retention in the lung and increased interaction with epithelial and other cells [93]. Free radicals generated via transition metals associated with particles lead to oxidative damage and activation of macrophages resulting in the release of several pro-inflammatory mediators, which initiate an acute cellular and mediator inflammatory response in the airways. Epithelial cells on coming in contact with ultrafine particles actively phagocytose them, followed by increased production and release of a large number of pro-inflammatory cytokines, which further amplify the inflammatory response. Particles generated from diesel exhaust on reaching the airway submucosa produce a qualitative and quantitative increase in IgE levels, which further trigger an allergic inflammatory response. Ultrafine particles which penetrate the interstitium make contact with interstitial macrophages and other sensitive cell populations and release inflammatory mediators which can enter the blood stream to produce a low-grade systemic inflammatory response, with effects on blood platelets and clotting factors, thereby increasing plasma viscosity. This could lead to cardiovascular dysfunction, especially in patients with an already compromised cardiovascular status.

References

- 1 Kaiser J. Showdown over Clean Air Science. *Sci* 1997; 277:466–9.
- 2 Dockery DW, Pope CA III, Xu X *et al.* An association between air pollution and mortality in six US cities. *NEJM* 1993; 329:1753–9.
- 3 Schwartz J. Air pollution and daily mortality. A review and meta analysis. *Environ Res* 1994; 64:36–52.
- 4 Pope CA III, Thun MJ, Namboodiri MM, Dockery DW, Evans JS, Speizer FE. Particulate air pollution as a predictor of mortality in a prospective study of US adults. *Am J Respir Crit Care Med* 1995; 151:669–74.
- 5 COMEAP (Committee on the Medical Effects of Air Pollutants). The nature of airborne particles. Non biological particles and health. Department of Health, Holgate ST, *et al.* 1995:9–28.
- 6 Fleming JS, Halson P, Conway J *et al.* Three dimensional description of pulmonary deposition of inhaled aerosols using data from multi modality imaging. *J Nuclear Med* 1996; 37:873–7.
- 7 Heyder JL, Gebhart J, Rudolf G, Schiller CF, Stahlhofen W. Deposition of particles in the human respiratory tract in the size range 0.005–15 μm . *J Aerosol Sci* 1986; 17:811–25.
- 8 Iwasa T, Wasserman K, Taplin GV. Lung Scintigraphy and pulmonary function studies in obstructive airway diseases. *Am Rev Respir Dis* 1970; 102:161–72.
- 9 Kim CS, Fisher DM, Lutz DJ, Gerrity TR. Particle deposition in bifurcating airway models with varying airway geometry. *J Aerosol Sci* 1994; 25:567–81.

- 10 Anderson M, Svartengren M, Philipson K, Camner P. Regional human lung deposition studied by repeated investigations. *J Aerosol Sci* 1988; 19:1121–4.
- 11 Svartengren M, Linnman L, Philipson K, Camner P. Regional deposition in human lung of 2.5 μm particles. *Exp Lung Res* 1987; 12:265–79.
- 12 Conway JH, Fleming JS, Holgate ST. Review of three dimensional imaging of inhaled aerosols. *Eur Respir Rev* 1997; 7:180–3.
- 13 Conway JH, Moore E, Fleming JS, Holgate ST. Three dimensional imaging of lung deposition from nebuliser in moderate/severe asthmatics. *Am J Respir Crit Care Med* 1998; 157:A456.
- 14 Kim CS, Kang TC. Comparative measurement of lung deposition of inhaled fine particles in normal subjects and patients with obstructive airway diseases. *Am J Respir Crit Care Med* 1997; 155:899–905.
- 15 Kim CS, Hu SC. Regional deposition of inhaled particles in human lungs: comparison between men and women. *Am J Physiol* 1998; 84:1834–44.
- 16 Chin JC, Liu CS, Cassamassi J et al. A neighbourhood scale study of PM_{10} source in Rubidox. *California Atm Environ* 1992; 24:693–706.
- 17 Salvi S, Frew AJ, Holgate ST. Is diesel a cause for increasing allergies? *Clin Exp Allergy* 1999; 29:4–8.
- 18 Health Effects Institute. Introduction. Diesel exhaust: A Critical analysis of emissions, exposure and health effects. A special Report on the Institute's Diesel Working Group, Nauss KM and The HEI Diesel Working Group 1995:13–61.
- 19 Kerminen VM, Makela TE, Ojanen C, Hillamo RE et al. Characterisation of the particulate phase in the exhaust from a diesel car. *Environ Sci Technol* 1997; 31:1883–9.
- 20 Weisenberger B. Health effects of diesel emissions. An update. *J Soc Occup Med* 1984; 47:90–2.
- 21 Oberdorster G, Ferin J, Lehnert BE. Correlation between particle size, in-vivo particle persistence and lung injury. *Environ Health Perspect* 1994; 102 (Suppl. 5):173–9.
- 22 Donaldson K, Li XY, MacNee W. Ultrafine (nanometer) particle mediated lung injury. *J Aerosol Sci* 1998; 29:553–60.
- 23 Peters A, Wichmann HE, Tuch T, Heinrich J, Heyder J. Respiratory effects are associated with the number of ultrafine particles. *Am J Respir Crit Care Med* 1997; 155:1376–83.
- 24 Zhang QW, Kusaka Y, Sato K, Nakakuki K, Kohyama N, Donaldson K. Differences in the extent of inflammation caused by intratracheal exposure to three ultrafine metals: Role of free radicals. *J Toxicol Environ Health* 1998; 53:423–38.
- 25 Batterbee JL, Rose NL, Long X. A continuous high resolution record of urban airborne particulates suitable for retrospective microscopical analysis. *Atmospheric Environ* 1997; 31:171–81.
- 26 Wilson WE, Suh HH. Fine particles and coarse particles. Concentration relationships relevant to epidemiological studies. *J Air Waste Manag Asso* 1997; 47:1238–49.
- 27 Lioy PJ, Waldmann JM. The personal, indoor and outdoor concentrations of PM_{10} measured in an industrial community during the winter. *Atmospheric Environ* 1990; 24:57–66.
- 28 Watt MD, Goddel J, Cherrie A, Seaton A. Individual exposure to particulate air pollution and its relevance to thresholds for health effects: a study of traffic wardens. *Occup Environ Med* 1995; 52:790–2.
- 29 Kobzik L. Lung macrophage uptake of unopsonized environmental particulates. *J Immunol* 1995; 155:367–76.
- 30 Mauderly JL, Bice DE, Carpenter RL et al. Effects of inhaled nitrogen dioxide and diesel exhaust on developing lung. Research Report No. 8. Health Effects Institute, Cambridge, MA, 1987.
- 31 Mauderly JL, Snipes MB, Barr EB et al. Pulmonary toxicity of inhaled diesel exhaust and carbon black in chronically exposed rats. Report No. 68, Health Effects Institute. Cambridge, MA, 1994.
- 32 Heinrich U, Mohr U, Fuchst R, Brockmeyer C. Investigation of a potential cotumorogenic effect of the dioxides of nitrogen and sulphur and of diesel engine exhaust on the respiratory tract of Syrian golden hamsters. Research Report No. 29. Health Effects Institute, Cambridge, MA, 1989.
- 33 Strom KA, Garg BD, Johnson JT, D'Arcy JB, Smiler KL. Inhaled particle retention in rats receiving low exposures of diesel exhaust. *J Toxicol Environ Health* 1990; 29:377–98.
- 34 Ishinishi N, Kuwabara N, Nagase S, Suzuki T, Ishiwata S, Kohno T. Long term inhalation studies on effects of exhaust from heavy and light duty diesel engines on F344 rats. In: Ishinishi N, Koizumi A, McClellan RO et al. eds. *Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust* New York, NY: Elsevier Science Publishing Co., 1986:329–48.
- 35 Mauderly JL, McClellan RO, Mokler BV, Redman HC. Pulmonary responses of Fischer 344 rats and CD-1 mice to exposure to diesel exhaust (Abstr.). *Toxicologist* 1982; 2:50.
- 36 Castranova V, Bowman L, Reasor MJ, Lewis T, Tucker J, Miles PR. The response of rat alveolar macrophages to chronic inhalation of coal dust and/or diesel exhaust. *Environ Res* 1985; 36:405–19.
- 37 Morrow PE. Possible mechanisms to explain dust overloading of the lungs. *Fundamental Appl Toxicol* 1988; 10:369–84.
- 38 Yang HM, Ma JYC, Castranova V, Ma JKH. Effects of diesel exhaust particles on the release of IL-1 and $\text{TNF}\alpha$ from rat alveolar macrophages. *Exp Lung Res* 1997; 23:269–84.
- 39 Terashima T, Amakawa K, Matsumaru A et al. Diesel exhaust particles stimulate alveolar macrophages to release $\text{TNF}\alpha$ and IL-6, but not GM-CSF. *Am J Respir Crit Care Med* 1998; 157:A880.
- 40 Terashima T, Wiggs B, English D, Hogg JC, van Eeden SK. Phagocytosis of small carbon particles (PM_{10}) by alveolar macrophages stimulates the release of PMN's from bone marrow. *Am J Respir Crit Care Med* 1997; 155:1441–7.
- 41 Ichinose T, Furuyama A, Sagai M. Biological effects of diesel exhaust particles. 2. Acute toxicity of DEP introduced into lung by intratracheal instillation. *Toxicol* 1995; 99:153–67.
- 42 Nagai A, Kakuta Y, Ozawa Y et al. Alveolar destruction in guinea pigs chronically exposed to diesel engine exhaust — A light microscopic and electron microscopic morphometry study. *Am J Respir Crit Care Med* 1996; 153:724–30.
- 43 Hendersen RF, Pickrell JA, Jones RK et al. Response of rodents to inhaled diluted diesel exhaust: biochemical and cytological

- changes in bronchoalveolar lavage fluid and in lung tissue. *Fundamental Appl Toxicol* 1988; 11:546–67.
- 44 Mauderly JL, Bice DE, Cheng YS et al. Influence of experimental pulmonary emphysema on toxicological effects from inhaled nitrogen dioxide and diesel exhaust. Research Report No. 30. Health Effects Institute, Cambridge, MA: 1989.
 - 45 Barnhart MI, Chen S, Salley SO, Puro H. Ultrastructure and morphometry of guinea pigs chronically exposed to diesel engine exhaust: Six months experience. *J Appl Toxicol* 1981; 1:88–103.
 - 46 White HJ, Garg BD. Early pulmonary response of the rat lung to inhalation of high concentration of diesel particles. *J Appl Toxicol* 1981; 1:104–10.
 - 47 Mauderly JL, Jones RF, Henderson RF et al. Relationship of lung structural and functional changes to accumulation of diesel exhaust particles. In: Dodgson J, McCallum RO, Bailey MR, Fisher DR, eds. *Inhaled Particles VI*. Oxford: Pergamon Press, 1988:659–69.
 - 48 Lal SB, Das N, Das BP, Gulati K. Biochemical and histopathological changes in respiratory system of rats following exposure to diesel exhaust. *Indian J Exp Biol* 1998; 36:55–9.
 - 49 Kobayashi T, Ito T. Diesel exhaust particulates induce nasal mucosal hyperresponsiveness to inhaled histamine aerosol. *Fundamental Appl Toxicol* 1995; 27:195–202.
 - 50 Kobayashi T. Exposure to diesel exhaust enhances nasal allergic reaction induced by repeated antigen administration in guinea pigs. *Am J Respir Crit Care Med* 1998; 157:A151.
 - 51 Schlesinger RB. Toxicological evidence for health effects from inhaled particulate pollution — does it support the human experience. *Inhalation Toxicol* 1995; 7:99–109.
 - 52 Gavett SH, Kelly MM, Madison SL, Dreher KL. Fine ambient air particulate matter increases lavage eosinophil numbers and IL-4 in allergic mice. *Am J Respir Crit Care Med* 1998; 157:A881.
 - 53 Takano H, Yoshikawa T, Ichinose T, Miyabara Y, Imaoko K, Sagai M. Diesel exhaust particles enhance Ag induced airway inflammation and local cytokine expression in mice. *Am J Resp Crit Care Med* 1997; 156:36–42.
 - 54 Fujimaki H, Nohara O, Ichinose T, Watanabe N, Saito S. IL-4 production in mediastinal lymph node cells in mice intratracheally instilled with diesel exhaust particulates and antigen. *Toxicol* 1994; 92:261–8.
 - 55 Fujimaki H, Saneyoshi K, Nohara O, Shiraishi F, Imai T. Intranasal instillation of diesel exhaust particles and antigen in mice modulated cytokine production in cervical lymph node cells. *Int Arch Allergy Immunol* 1995; 108:268–73.
 - 56 Lovik M, Hogreth AK, Gaarder PI, Hageman R, Eide I. Diesel exhaust particles and carbon black have adjuvant activity on the local lymph node response and systemic IgE production to ovalbumin. *Toxicol* 1997; 121:165–78.
 - 57 Takenaka H, Zhang KE, Diaz-Sanchez D, Tsien A, Saxon A. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: Direct effects on B cell IgE production. *J Allergy Clin Immunol* 1995; 95:103–15.
 - 58 Suzuki T, Kanoh T, Ishimori M, Ikeda S, Ohkuni H. Adjuvant activity of DEP in production of anti IgE and anti IgG₁ antibodies to mite allergen in mice. *J Clin Lab Immunol* 1996; 48:187–99.
 - 59 Knox RB, Suphioglu C, Taylor P et al. Major grass pollen Lolp1 binds to DEP's: Implications for asthma and air pollution. *Clin Exp Allergy* 1997; 27:246–51.
 - 60 Frew AJ, Salvi SS. Diesel exhaust particles and respiratory allergy. *Clin Exp Allergy* 1997; 27:188–204.
 - 61 Bayram H, Devalia JL, Sapsford RJ et al. The effect of diesel exhaust particles on cell function and release of inflammatory mediators from human bronchial epithelial cells in-vitro. *Am J Respir Cell Mol Biol* 1998; 18:441–8.
 - 62 Steerenberg PA, Zonnenberg JAJ, Dormans JAMA et al. Diesel exhaust particles induced release of IL-6 and IL-8 by (primed) human bronchial epithelial cells (BEAS- 2B) in-vitro. *Exp Lung Res* 1998; 24:85–100.
 - 63 Boland S, Baeza S, Fournier T, Aubier M, Marano F. Effects of diesel exhaust particles on the viability and morphology of airway epithelial cells in culture. *Am J Respir Crit Care Med* 1998; 157:A155.
 - 64 Ohtoshi T, Takizawa H, Okazaki H et al. Diesel exhaust particles stimulate human airway epithelial cells to produce cytokines relevant to airway inflammation in vitro. *J Allergy Clin Immunol* 1998; 101:778–85.
 - 65 Davies RJ, Bayram H, Abdelaziz MM, Khair OA, Sapsford RJ, Devalia JL. Effect of diesel exhaust particles on release of inflammatory mediators from bronchial epithelial cells of atopic asthmatic subjects and non atopic non asthmatic subjects. *Am J Respir Crit Care Med* 1998; 157:A743.
 - 66 Kennedy T, Ghio AJ, Reed W et al. Copper dependant inflammation and NF κ B activation by particulate air pollution. *Am J Respir Cell Mol Biol* 1998; 19:366–78.
 - 67 Carter JD, Ghio AJ, Samet JM, Devlin RB. Cytokine production by human airway epithelial cells after exposure to an air pollution particle is metal dependant. *Toxicol Appl Pharmacol* 1997; 146:180–8.
 - 68 Pritchard RJ, Ghio AJ, Lehman JR et al. Oxidant generation and lung injury after particulate air pollutant exposure increase with concentrations of associated metals. *Inhalation Toxicol* 1996; 8:457–77.
 - 69 Gilmour PS, Brown DM, Lindsay TG, Beswick PH, MacNee W, Donaldson K. Adverse health effects of PM₁₀ particles: involvement of iron generation of hydroxyl radical. *Occup Environ Med* 1996; 53:817–22.
 - 70 Terada N, Hamano N, Maesako K et al. Diesel exhaust particulates upregulate histamine receptor mRNA and increase histamine induced IL-8 and GM-CSF production in nasal epithelial cells and endothelial cells. *Clin Exp Allergy* 1999; 29:52–9.
 - 71 Pflutzner W, Thomas P, Przybilla B. Influence of suspended diesel exhaust particles (DEP) in number and activity of IgE secreting cells in vitro. *J Allergy Clin Immunol* 1995; 95:335 (Abstr.).
 - 72 Rudell B, Sandstrom T, Stjernberg N, Heldman KB. Controlled diesel exhaust exposure in an exposure chamber: pulmonary effects investigated with bronchoalveolar lavage. *J Aerosol Sci* 1990; 21:S411–S414.
 - 73 Holian A, Hamilton RF, Morandi MT, Brown SD, Li L. Urban particle-induced apoptosis and phenotype shifts in human

- alveolar macrophages. *Environ Health Perspect* 1998; 106:127–32.
- 74 Saneyoshi K, Nohara O, Imai T, Shiraishi F, Moriyama H, Fujimaki H. IL-4 and IL-6 production of bone marrow derived mast cells is enhanced by treatment with environmental pollutants. *Int Arch Allergy Immunol* 1997; 114:237–45.
- 75 Gallagher JE, Prahalad A, Inmon J, Lewtas J, Ghio A, Becker S. Characterization of air pollution particles to elemental analysis and cellular oxidant generation. *Am J Respir Crit Care Med* 1998; 157:A141.
- 76 Vincent R, Goegan P, Johnson G, Brook J. Effects of ambient particles on xenobiotic, metal and stress inducible promoter cat fusion genes in HepG2 cells. *Am J Respir Crit Care Med* 1995; 151:A267.
- 77 Costa DL, Dreher KL. Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in health and compromised animal models. *Environ Health Perspect* 1997; 105:1053–60.
- 78 Kanemitsu H, Nagasawa S, Sagai M, Mori Y. Complement activation by diesel exhaust particles. *Biol Phar Bull* 1998; 21:129–32.
- 79 Salvi S, Blomberg A, Rudell B et al. Acute inflammatory responses in the airways and peripheral blood following short term exposure to diesel exhaust in healthy human volunteers. *Am J Respir Crit Care Med* 1997; 155:425 (Abstr.).
- 80 Salvi S, Blomberg A, Semper A et al. Upregulation of IL-8 mRNA following acute exposure to diesel exhaust in the bronchial tissue of healthy human subjects. *Am J Respir Crit Care Med* 1998; 157:A697 (Abstr.).
- 81 Diaz-Sanchez D, Dotson AR, Takenaka H, Saxon A. Diesel exhaust particles induce local IgE production *in vivo* and alter the pattern of IgE mRNA isoform. *J Clin Invest* 1994; 94:1417–25.
- 82 Diaz-Sanchez D, Tsien A, Fleming J, Saxon A. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human *in vivo* nasal ragweed specific IgE and skews cytokine production to a Th2 type phenotype. *J Immunol* 1997; 158:2406–13.
- 83 Fujieda S, Diaz-Sanchez D, Saxon A. Combined nasal challenge with diesel exhaust particles and allergen induces *in vivo* IgE isotype switching. *Am J Respir Cell Mol Biol* 1998; 19:507–12.
- 84 Hattis D, Wasson JM, Page GS, Stern B, Franklin CA. Acid particles and tracheobronchial region of the respiratory system. An 'irritative-signalling' model for possible health effects. *JAPCA* 1987; 37:1060–6.
- 85 Meyer H, Palil HK, Bauerle PA. Regulation of the transcription factors NFkB and AP-1 by redox changes. *Chemico-Biol Interacts* 1994; 91:91–100.
- 86 Poloniecki JD, Atkinson RW, Pone de Leon A, Anderson HR. Daily time series for cardiovascular hospital admissions and previous day's air pollution in London. *Occup Environ Med* 1997; 54:534–40.
- 87 Seaton A, MacNee W, Donaldson K, Godden D. Particulate air pollution and acute health effects. *Lancet* 1995; 345:176–8.
- 88 Peters A, Doring A, Wichmann HE, Koenig W. Increased plasma viscosity during an air pollution episode: a link to mortality. *Lancet* 1997; 349:1582–7.
- 89 Proceedings of the Royal Microscopical Society. Particulate aerosols. 1998; 33:211–2.
- 90 Sutton DW, Schmidtschonbein GW. Elevation of organ resistance due to leukocyte perfusion. *Am J Physiol* 1992; 262:H1646–H1650.
- 91 Herbertson MJ, Werner HA, Godard CM et al. Antitumor necrosis factor alpha prevents decreased ventricular contractility in endotoxemic pigs. *Am J Respir Crit Care Med* 1995; 152:480–8.
- 92 Akira S, Kishimoto T. IL-6 and TNF α in acute phase response to viral infection. *Immunol Rev* 1992; 127:25–50.
- 93 MacNee W, Li XY, Gilmour PS, Donaldson K. Proinflammatory effect of particulate air pollution (PM₁₀) *in-vivo* and *in-vitro*. *Ann Occup Hyg* 1997; 41 (Suppl. 1):7–13.