ULTRAFINE PARTICLES: AGGRAVATING EXPOSURE FACTOR?

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ABSTRACT

The carcinogenic potential of chromium (VI) has been the subject of numerous environmental and occupational studies, from which many regulatory efforts emanated. Besides the regulation of levels of known toxic substances (e.g. Cr (VI)), the classification of dusts for regulatory purposes has thus far depended on broad definitions of particle size, mainly used to distinguish only between inhalable and respirable particles. In the past, ultrafine particles (less than 0.1 µm diameter) ($PM_{0.1}$) were often regarded as a non-issue, because it was believed that significant doses would not deposit in the lungs.

A growing body of scientific data is indicating that ultrafine particles are more toxic than their coarse counterparts. Increased toxicity can be related to the unique dosimetric aspects of the deposition and disposition of inhaled ultrafine particles. Secondly, ultrafine particles have a larger surface area per given mass. The increased surface area can act as a carrier for co-pollutants, specifically transition metals (including chromium) that could form a coat on the particle surfaces during particle formation. This suggests that the presence of "inert" ultrafine particles in the environment may enhance the potential for exposure to reactive airborne compounds, including Cr (VI).

These factors are particularly important when information on occupational epidemiology is interpreted. It is desirable to consider the particle size distribution, for example when occupational exposure to hexavalent chromium compounds is assessed. Since ultrafine particles are more toxic than coarse particles with the same chemical composition, it could be speculated that cancer slope factors might be related to particle size distribution. This is relevant not only to carcinogenic chromium compounds, but also basically for any particle-associated toxicants. In general, the probability of developing health effects associated with occupational toxicants is likely to be higher when these are associated with the ultrafine particle range.

1. INTRODUCTION

Particulate matter may be classified by size as "coarse" particles less than 10 µm aerodynamic diameter (PM_{10}), "fine" particles less than 2.5 µm diameter ($PM_{2.5}$) and "ultrafine" particles less than 0.1 µm diameter ($PM_{0.1}$). Most particles larger than 10 µm in aerodynamic diameter are deposited in the nose or oral pharynx and cannot penetrate to tissues distal to the larynx. Recent data have shown that very fine particles (0.01 µm and smaller) are also trapped relatively efficiently in the upper airways by diffusion (Brownian deposition). Particles that penetrate beyond the upper airways are available to be deposited in the bronchial region and the deeper-lying airways. Therefore, the alveolar region has significant deposition efficiencies for particles smaller than 5 µm and larger than 0.003 µm [1].

The epidemiology of particulate matter effects on the respiratory system has received considerable attention, but there is a lack of specific studies on the health effects of environmental and occupational exposure to ultrafine particles. The mechanisms of observed health effects have not been clearly delineated. Direct effects on tissue by toxic components residing on the surface of particles are important, but the cellular components of the immune system and the biochemical signalling and effect molecules are obviously also involved. A central theme in cellular studies of the effects of ultrafine particles is the stimulation of the inflammatory response. If particles of mineral origin are deposited in the alveolar region, the release of fibrogenic factors is stimulated. These reactions result in the pathological manifestations noted in exposed individuals.

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The respiratory tract is a well-known target of the systemic effects of chromium inhalation. Effects involve mainly inflammatory responses, while fibrosis has also been described. Health effects in humans include rhinorrhea (runny nose), coughing, wheezing, decreased lung function and even asthmatic reactions. The latter is confined to sensitised individuals exposed to elevated concentrations. Animal inhalation studies have described several impacts of exposure on the numbers, functions and reactive-oxygen production profiles of immunological and lymphoreticular cells in bronchoalveolar lavage fluid. Acute and intermediate-duration exposure to moderate levels of chromium(III) and/or chromium(VI) compounds generally caused mild irritation, accumulation of macrophages, inflammation, and impaired lung function. Reversible fibrosis was described after 30 or 90 days of exposure to 0.1 mg/m^3 chromium(VI) (sodium dichromate) in rats [2].

A growing body of scientific data is indicating that ultrafine particles are more toxic than their coarse counterparts. Increased toxicity can be related to the unique dosimetric aspects of the deposition and disposition of inhaled ultrafine particles. Secondly, ultrafine particles have a larger surface area per given mass. The increased surface area can act as a carrier for co-pollutants, specifically transition metals (including chromium) that could form a coat on the particle surfaces during particle formation. This suggests that ultrafine particles in the environment may enhance the potential for exposure to reactive airborne compounds, including chromium. Chromium in the environment is known to occur as particle-bound chromium or chromium dissolved in droplets [2]. It should also be considered that relatively pure chromium compounds, such as the oxides, might also occur in ultrafine form. The physiological mechanisms that are involved in ultrafine particle effects and the impact of current knowledge on the perspective on occupational and environmental management practices are highlighted in this manuscript.

2. PATHOGENIC CHARACTERISTICS OF ULTRAFINE PARTICLES

2.1 Introduction

In general, the scientific literature indicates that ultrafine particles are more toxic to the respiratory system than coarse particles. The increased toxicity of ultrafine particles can be related firstly to their larger surface area per given mass. The increased surface area can act as a carrier for co-pollutants such as gases and chemicals, specifically transition metals that could form a coat on the particle surfaces during their formation [3]. Increased surface areas have also been associated with increased inflammatory responses, probably related to increased reactive oxygen species generation, independent of transition metal exposure [4].

Secondly, dosimetric aspects of the deposition and disposition of inhaled ultrafine particles differ from those of larger particles. Several plausible mechanisms have been proposed for both the initial pulmonary injury and the consequent systemic effects following ultrafine particle exposure. These mechanisms involve physical, chemical and biological characteristics of particulate matter.

2.2 Implications of the greater surface area per unit mass

A variety of studies have indicated that ultrafine particles are typically more pathogenic than larger particles of the same mineral type, even when the mineral involved, for example titanium dioxide or carbon black, is an insoluble dust of low toxicity [5]. Indeed, the concept is emerging that surface area is the dose measure that predicts pulmonary response, rather than mass [6]. The greater toxicity associated with greater surface area per unit mass, has been attributed to increased interaction between the lung tissue and the toxic components residing on the surface of the particles [7]. In the ultrafine hypothesis, Oberdörster and colleagues suggested that particles smaller than 0.02 μm might elicit a strong and persistent pulmonary inflammation [8-9]. This hypothesis has been extended by other researchers and theorises that the very large surface of absorption offered by this particle size may carry toxicants into the deep lung, and may cause inflammation, adhesion molecule expression, altered blood coagulation and cardiac electrical aberrations as mechanisms of disease [10-12].

Results of experimental studies with ultrafine particles composed of low-toxicity materials such as polystyrene have also shown an increased inflammatory response with increased total surface area, without any contribution from other factors such as co-pollutants or transition metals. This suggests that surface area drives inflammation in the short term and that ultrafine particles cause a greater response because of the greater surface area they possess [4].

2.3 The number of particles

Some epidemiological studies have found good associations between the number of ultrafine particles per unit volume of air and the occurrence of respiratory effects [13]. Oberdörster *et al*., [8-9] have proposed that ultrafine particles, even at small mass concentrations, may have serious negative health effects because of their high number per volume. It has also been suggested that high numbers of ultrafine particles in the alveolar region may overwhelm the alveolar macrophages, resulting in decreased clearing efficiency of ultrafines from the alveoli [3, 14].

2.4 Surface properties and chemical composition

The health effects observed after ultrafine particle exposure have been attributed to chemical agents that may be delivered to the airways and alveoli through the deposition of particles. These include acidity, hydrogen peroxides, nitrates, sulphates, organic carbon and acid aldehydes [15-16]. Recent analyses have reported water-soluble species, including calcium, sodium, ammonium ion, nitrate, and sulphate in the ultrafine atmospheric particles. Other substances detected in the ultrafine range are potassium, iron, copper, zinc, and strontium [17]. Reactive metal ions produce oxidative lung injury due to the formation of reactive oxygen and/or reactive nitrogen species [15-16]. Metals that have been proposed to play a role include nickel[18], vanadium and copper [19].

The complexity of the surface chemistry also impacts on the bioreactivity of ultrafine particles. Murphy *et al*. [20] have shown that ultrafine particles with diverse surface chemical constituents attached more easily to the extracellular matrix than particles with uniform surface chemical compositions. Another indication of the importance of surface properties is the finding that hydrophobic and hydrophilic particles of the same size elicit pulmonary-inflammatory responses of different strengths, although conflicting relationships have been described in the literature [3, 21].

Under certain circumstances, the electric charge on ultrafine particles can also determine the particle deposition pattern. Experimental studies conducted in vivo with humans and with airway models have all shown an increase in respiratory tract deposition due to particle charge. However, enhanced deposition by electrical forces was noted only if the charge on the particle exceeded a threshold value, which depended on the particle size as well as the airway size [22-23].

2.5 The unique deposition of inhaled ultrafine particles

Ultrafine particles may exist as singlet particles or as aggregates. In the form of aggregates their deposition characteristics can change, as the aggregates would have a greater aerodynamic diameter than the singlet particles. The aerodynamic characteristics of the aggregates would, however, vary depending on their compactness. If the aggregate is more open with chains and extensions then, like thistledown, it will have greater aerodynamic resistance and the likelihood of settling will be less [6]. According to the International Commission on Radiological Protection (ICRP) model for particle deposition in the human respiratory tract, alveolar deposition is highest for inhaled singlet (as opposed to aggregated) ultrafine particles of around 20 nm (0.020 μm) diameter [24].

It was previously believed that ultrafine particles would not deposit in significant concentrations in the alveolar region, due to a mechanism referred to as Brownian diffusion. However, this impression has been shown to be questionable. Several studies have indicated effects in laboratory animals exposed to ultrafine particles that could not be repeated in exposures to non-ultrafine particles of the same chemical composition in the same study [6]. This may be indirect evidence, but it is difficult to imagine health effects without particle-to-cell contact in the epithelial interface of the lung. Some recent studies present direct evidence that inhaled ultrafine particles are deposited in the lungs [25] and that the experimental deposition is consistent with deposition model predictions. Jaques and Kim [26] reported and inverse association between particle sizes and lung deposition, that is, inhalation of decreasing ultrafine particle sizes result in increased total lung deposition. Brown *et al*., [27] was able to demonstrate deposition of technetium-99m-labelled particles in volunteers and average 24-hour lung retention of deposited particles of 80 to 90 per cent.

3. MECHANISMS OF PARTICLE-INDUCED RESPIRATORY HEALTH EFFECTS

3.1 General

The greater pathogenicity of ultrafine particles can be explained through experimental observations of more intense inflammatory responses, increased fibrogenic potential, and increased oxidant-generating abilities [5, 8-10, 28-31]. The chemical, cellular, physiological and biochemical mechanisms that underlie these effects are discussed below.

3.2 Immunological mechanisms

Studies in rats have indicated that ultrafine carbon black particles show greater pro-inflammatory effects compared to fine particles [29, 32]. Alveolar macrophages, lymphocytes and neutrophils can be stimulated to release a variety of cytokines including platelet activating factor and tumour necrosis factor [33-35]. Airway epithelial cells have been shown to act directly as immune effector cells by releasing cytokines, such as interleukin-8 [36], granulocyte-macrophage colony stimulating factor [37] and platelet activating factor [38]. The releases of these factors mediate the inflammatory and fibrogenic response of the lungs to the inhalation of ultrafine particles.

3.3 Reactive oxygen species generation

Ultrafine particle exposure induces oxidative stress as a result of reactive oxygen species production [4, 29]. Increased generation of oxidant species, e.g. the hydroxyl radical, is an important finding, since these are known to have pro-inflammatory effects and to result in tissue damage [15, 18]. According to the transition metal hypothesis, mentioned previously, reactive metal ions that occur on the surface or in particles produce an oxidative lung injury due to the formation of reactive oxygen and/or reactive nitrogen species [15-16]. However, reactive oxygen generation have also been shown to occur independent of transition metal exposure [32]. MacNee and Donaldson [39] reviewed the issue and concluded that preliminary data *in vitro* and *in vivo* suggest that local and systemic oxidative stress occur in response to ultrafine particles and that the effects of such oxidative stress on pro-inflammatory gene regulation and changes in blood coagulation may result in the adverse effects of particulate air pollution.

3.4 Differential gene expression

Chemically identical dusts of differing size can produce quite different patterns of gene expression in the airway wall. MacNee and Donaldson [39] concluded that ultrafine particles stimulate both local and systemic oxidative stress that impacts on pro-inflammatory gene regulation, resulting in the adverse effects of particulate air pollution.

3.5 Interaction of gaseous and particulate pollutants in the respiratory tract.

Biological responses to the inhalation of polluted atmospheres may depend upon the interplay between individual materials. Schlesinger has already proposed in 1995 that characterising effects from exposure to mixtures of air pollutants is necessary for adequate quantification of health risks. Mixtures may act synergistically, resulting in more-than-additive effects, or interactions may be antagonistic, resulting in dampened effects [40]. The occurrence and type of interaction depends on numerous factors, including the biological endpoint and the specific exposure conditions, such as concentration, duration, and the physico-chemical characteristics of the exposure atmosphere. Proposed mechanisms of interaction include physical adsorption, chemical reactions in the exposure atmosphere, or on a particle surface, and alteration of the pulmonary environment.

An example of synergism resulting from gas adsorption onto the particle surfaces is the exacerbation of the fibrogenicity of quartz by adsorbed NO_2, presented in an early animal study by Shevchenko [41]. With regard to alterations of the pulmonary environment, Last [42] has hypothesised that synergism between oxidant gases and acidic sulphate particles would result from a shift in the local micro-environmental pH of the lung following deposition of the acidic particles, enhancing the effects of the co-inhaled gas by producing a change in their reactivity or residence time of reactants, such as free radicals, involved in oxidant-induced tissue injury. Under specific conditions, however, antagonism could also result [40].

3.6 Inhibited disposal by macrophages

Ultrafine particles are probably more easily deposited in the lungs, as explained previously. In addition, the fate of ultrafine particles after their deposition may be very different from that of larger particles. Preliminary studies showed that decreasing particle size, down to the ultrafine size, was associated with decreased efficiency of phagocytosis by macrophages. It appears that deposited ultrafine particles are not as readily phagocytised by alveolar macrophages as are larger particles [3].

Some studies demonstrated a direct deleterious effect of pre-exposure to ultrafine particles (about 20 nm in diameter) on the subsequent phagocytotic abilities of macrophages (measured with 2 μm diameter indicator beads). This effect was not observed with fine particle (about 200 nm diameter) exposure [6]. It therefore seems that the adverse effects of ultrafine particles may be mediated in part by their ability to inhibit phagocytosis. Inhibition of phagocytosis would allow increased interaction between ultrafine particles and alveolar epithelial cells. Ultrafine particles could consequently penetrate much more rapidly to interstitial sites, possibly including the endothelium and even entering the blood circulation[3, 6].

4. MECHANISMS OF PARTICLE-INDUCED SYSTEMIC HEALTH EFFECTS

4.1 Particle penetration into the circulation

Recently, the hypothesis has evolved that ultrafine particles have a greater ability to penetrate the pulmonary interstitium [7]. The ultrafine hypothesis advanced by Oberdörster *et al*. [9] suggests that ultrafine particles penetrate through the alveolar wall into the blood circulation and is deposited in the cardiac tissue, where it causes cardiac arrhythmia and death. Experimental animal studies indicated the penetration of ultrafine particles into the circulation and its deposition in several organs, including the heart, in relatively small percentages of the total inhaled dose [3]. Nemmar *et al.* [43] described the clearance of denatured albumin from the lung into the blood circulation in hamsters and [44] described the translocation of platinum particles (13 nm diameter) to the liver in rats.

Oberdörster *et al.* [45] also demonstrated effective translocation of ultrafine elemental carbon particles to the liver of rats by 1 day after inhalation exposure. Translocation pathways included direct input into the blood compartment from ultrafine carbon particles deposited throughout the respiratory tract. However, it was concluded that input from ultrafine particles present in the gastrointestinal tract needs to be considered as well and that translocation to blood and extrapulmonary tissues may well be different between ultrafine carbon and other insoluble (metal) ultrafine particles. A recent human study showed translocation of inhaled isotope-labelled ultrafine carbon aerosols to the liver [46].

4.2 Ultrafine particles and cardiovascular effects

It has been suggested that the induction of airway inflammation, expression of leukocyte and endothelial adhesion molecules in blood, the alteration of blood coagulability and alteration in cardiac electrical activity may be involved in the exacerbation of underlying cardiorespiratory disease. It was hypothesised that airway inflammation may activate endothelium and circulating leukocytes, and induce a systemic acute phase response with transient hypercoagulability [11]. MacNee and Donaldson [39] reviewed the issue and concluded that preliminary data *in vitro* and *in vivo* suggest that both local and systemic oxidative stress occur in response to ultrafine particles and that the effects of such oxidative stress on changes in blood coagulation may result in the adverse effects of particulate air pollution on the cardiovascular system. The hypothesis of systemic effects is supported by a study by Donaldson *et al.* [6] on carbon black (11 nm primary particle size) that reported global oxidative stress in the plasma and increased plasma factor VII, which is an independent risk factor for cardiovascular disease.

The cytokine hypothesis involves the release of secondary messengers (cytokines) by various pulmonary cells into the circulation, ultimately resulting in particulate-matter induced health effects [47]. Cytokines, including tumour necrosis factor and interleukins, have been linked to cardiac arrhythmias and increased levels of platelet-activating factor, a clotting factor implicated in atherosclerosis and cardiac thrombosis. The principal objection to the cytokine hypothesis as applied to cardiac and circulatory effects, is the short half-lives of cytokines in the blood [48].

The vagal nerve hypothesis involves the response of the autonomic nervous system to pulmonary irritants. The C-fibres in the lung and other receptors detect the initial irritation of airways and alveoli by particulate matter and the afferent arm of the vagus nerve transmits the signal to the respiratory centres. Signalling in this nerve is associated with measures of autonomic dysfunction, such as increased heart rate and decreased heart rate variability. This, in turn, is associated with adverse coronary events and death [48].

5. HOST SUSCEPTIBILITY FACTORS

Factors such as advanced age, specific disease states, pre-existing inflammation in the lungs and sensitisation due to pre-exposures to sensitising agents appear to influence the susceptibility of individuals to the health effects of ultrafine particles. The implications of susceptibility is potentially so important that very low concentrations may cause noticeable effects in susceptible persons, while less sensitive individuals may not respond at all [6].

Individuals with compromised respiratory tract function due to pre-existing disease states (e.g. emphysema and early stages of respiratory tract infection with gram negative bacteria) are more sensitive than healthy individuals. Aged individuals also seem to be at a higher risk of oxidative stress-induced lung injury [3, 49]. A theoretical model of deposition of 20 nm particles in human alveoli showed increased deposition and retention of nanoparticles in pathological alveoli, that is, alveoli with higher stiffness of the alveolar wall [50]. This would contribute to the increased sensitivity noticed in individuals with pre-existing disease states. Other authors have also concluded that pathological changes in the lungs, such as the airway narrowing found in chronic obstructive pulmonary diseases (COPD) and asthma cause an increase in the efficiency of deposition of ultrafine particles [6, 27, 51].

6. CONCLUSION

Experimental studies indicate that ultrafine particles are more toxic than coarse particles with the same chemical composition. This greater toxicity has been linked to increased surface areas and increased particle numbers at the same mass concentrations. Adsorbed chemicals on the surface may also play a role. Physiological considerations such as increased alveolar deposition of ultrafine particles and decreased efficiency of phagocytosis by macrophages are also influential in the health outcomes of ultrafine particle exposure.

Ultrafine particles have respiratory as well as systemic effects and several hypotheses for the mechanisms governing these effects have been forwarded. The biochemical, cytological and physiological reactions that are triggered by interactions between ultrafine particles and pulmonary cells are being delineated by experimental studies. This should develop a clearer picture of the molecular mechanisms underlying the pathological manifestations of inflammation. Regulation of exposure to ultrafine particles should consider the host susceptibility of the receptor population, since important factors that influence the outcome of exposure have been identified.

These factors are particularly important when information on occupational epidemiology is interpreted. It is desirable to consider the particle size distribution, for example when occupational exposure to hexavalent chromium compounds is assessed. Since ultrafine particles are more toxic than coarse particles with the same chemical composition, it could be speculated that cancer slope factors might be related to particle size distribution. This is relevant not only to carcinogenic chromium compounds, but also basically for any particle-associated toxicants. In general, the probability of developing health effects associated with occupational toxicants is likely to be higher when these are associated with ultrafine particles.

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