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REVIEW ARTICLE

Dosimetry and toxicology of inhaled ultrafine particles

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Abstract

Both epidemiological and toxicological studies indicate that inhalation and subsequent deposition of airborne particles into the lungs have adverse health effects. Recently, the ultrafine particle (UfP) fraction (diameter < 100 nm) has received particular attention, as their small size may lead to more toxic properties. In this study we summarize the current knowledge on the dosimetry of inhaled particles (including UfPs) with a focus on recent data on translocation of UfPs into secondary target organs (such as brain and heart) suggesting that the lifetime dose of ambient UfPs in secondary target organs is about 10^{11} particles. Furthermore, we highlight the main pathways of particle induced toxicity and the reasons for the potentially higher toxicity of UfPs. Finally, we discuss recent evidence indicating that (BET) surface area is the single most relevant dose metric for the toxicity of UfPs, which has important implications for regulatory measures on the toxicity of ambient and engineered particles.

Keywords: *Oxidative stress; lung; ROS; nanoparticle toxicity; BET surface area; particle toxicity*

Introduction

'*All things are poison and nothing is without poison, only the dose permits something not to be poisonous.*' As already stated by Paracelsus, the medieval physician, substances, which are typically considered poisonous, may be harmless, while benign materials may become toxic depending on the administered dose. Hence, the concepts of dose and toxicity are intimately linked for all inhaled substances, including ambient particulate matter (PM).

Epidemiological studies have repeatedly suggested that enhanced ambient PM levels result in increased morbidity and mortality (Peters et al. 1997, Stone et al. 2007). Toxicological studies show that particle induced oxidative stress may lead to pulmonary or even systemic inflammation (Donaldson & Tran 2002), which ultimately may promote the progression of atherosclerosis and precipitate acute cardiovascular responses ranging from increased blood pressure to myocardial infarction (Delfino et al. 2005).

Airborne PM can enter the organism through the lungs, skin or retina. As the lung is actively ventilated (respiration) and has by far the largest surface area (~140 m^2) (Gehr et al. 1978), the lung presents the main entrance way for airborne PM into the human organism. The accumulated particle dose in the lungs is determined not only by the amount of particles deposited onto the walls (epithelium) of the respiratory tract, but by the effectiveness of the lungs' natural defence system (clearance mechanisms). The former mainly depends on the physicochemical properties of the particles and on the physiological parameters such as lung morphology and respiratory conditions. The latter relates to the fate of these particles within the organism, i.e. whether they are dissolved by body fluids, metabolized, eliminated or isolated from the organism, or translocated to other organs besides the organ of intake (Kreyling et al. 2006a, Oberdörster et al. 2005). Ultimately, the combined effects of particle deposition into and clearance from the lungs, particle processing and transport within the organism, and the toxicity of the particles determine the

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particle related health impact. While mass-based maximum exposure levels allowed for various types of PM are already established in several countries (including US and Europe), alternative dose metrics such as particle number or surface area have recently been put forward, in part due to their higher sensitivity to the potentially more toxic ultrafine particle (UfP) fraction of PM (Peters et al. 1997, Stoeger et al. 2009), which comprises the PM segment with particle diameter below 100 nm.

In this mini-review we summarize the state of knowledge on the dosimetry of (ultrafine) particles in the lungs, including particle lung deposition, clearance mechanisms and translocation into secondary organs (e.g. brain and heart), we provide an overview over the reasons for the potentially higher toxicity of UfPs, and we highlight the role of (BET) surface area as a powerful dose metric for the toxicity of different types of ultrafine soot particles. In the following discussion PM refers to UfPs unless stated otherwise.

Dosimetry of inhaled particles

The dosimetry of inhaled particles describes the temporal variability of the particle dose accumulating in the lung (or one of its compartments, e.g. tracheobronchial, alveolar) or other organs. The organ-specific particle burden is determined by the sources and sinks of particles for a given organ.

Lung deposition

The 'source' of particles in the lungs is the deposition of inhaled particles onto the lung epithelium, which depends on the morphology of the lungs, the respiratory conditions and the physicochemical properties of the particles. A detailed presentation of these aspects is presented in mini reviews by Kim and Hofmann and their co-workers (this issue). In brief, constrained by lung deposition measurements (Beckett et al. 2005, Heyder et al. 1986, Jaques & Kim 2000) various computer models have been developed (mostly for spherical particles) allowing the calculation of both regional and total particle deposition for an average lung morphology at different respiratory conditions and particle characteristics, where the latter typically comprise the geometric diameter and bulk density (Asgharian et al. 2004, Hofmann 1996, ICRP 1994). Some deposition models also account for changes in particle size and density due to hygroscopicity, i.e. the ability of the particles to absorb water under the high relative humidity conditions encountered in the lungs (Ferron et al. 1988). For non-spherical particles (such as ultrafine chain-like soot particles), the effect of particle shape can be incorporated into deposition models for spherical particles (ICRP 1994) by replacing the geometric diameter and bulk density by the readily measured mobility diameter and effective density (ratio of particle mass and mobility-based volume assuming particle sphericity), respectively (Schmid et al. 2007). In contrast to larger particles, UfPs are mainly deposited due to diffusion not due to inertial impaction, gravitational sedimentation or interception.

For toxicological and pharmacological studies, the alveolar region is of particular interest, as in this region the lungs are most permeable (air–blood barrier is only about 2 μm thick) in order to facilitate gas exchange between the blood and the inhaled air (Gehr et al. 1978). In this regime both ultrafine and fine particles have high deposition efficiencies of 30–60% (between 20 and 40 nm) and 20–30% (between 1 and 3 μm) of the inhaled particles respectively (ICRP 1994). Because the number and mass of typical urban particle size distributions is dominated by ultrafine and supermicron particles, respectively, the former and latter size segment dominate the lung deposited particle number and mass (in the alveoli), respectively. On the other hand, there are two almost equally important modes at about 20 and 140 nm for the deposited particle surface area, the toxicologically most relevant dose parameter (as discussed below) (Figure 1). This deposition characteristic is almost independent of activity level (sleep and heavy exercise) resulting in an about 50% contribution of UfPs (<100 nm) to the total deposited surface area (Figure 1). Hence, there is a substantial contribution of urban UfPs to the toxicologically most relevant lung-deposited particle surface area, which is aggravated by the reduced clearing efficiency of UfPs from the alveolar region as discussed below. It is important to note that the deposited hourly surface dose (total particle surface area deposited in the alveoli over 1 h) is much larger during heavy exercise (320 mm^2) than during sleep (45 mm^2)

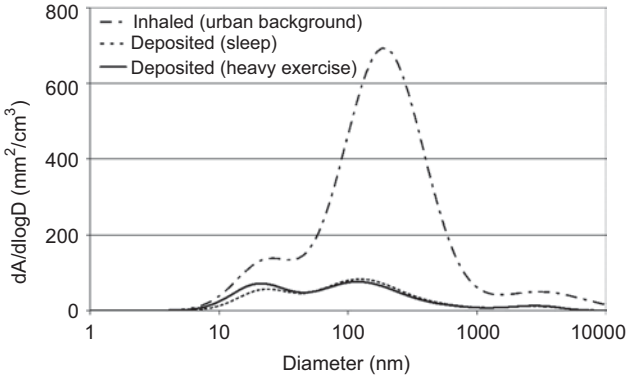

Figure 1. Size-resolved particle surface area deposited (per breath) in the alveolar region during sleep and heavy exercise (ICRP 1994) for a typical urban background aerosol distribution (inhaled) (Baron & Willeke 2001, p. 103). For both activity levels, UfPs (<100 nm) contribute about 50% to the deposited surface area.

due to the vastly different ventilation rates of 3.0 and 0.45 $m^3 h^{-1}$, respectively.

Clearance mechanisms and retention

Once the particles are deposited onto the walls (epithelium) of the respiratory tract they come into contact with the surfactant layer and with either mucous (in the airways) or serous lining fluid (in the alveoli). Particle compounds which are either lipid or water soluble will be dissolved and rapidly diluted, bound to proteins, often metabolized in the lining fluid and eventually transferred to the blood and lymphatic circulation, undergoing further metabolization or excretion via kidney and urine. Although dissolved parts of PM have the potential to reach any organ (Patton et al. 2004), their toxic potential is typically considered low due to the high degree of dilution in the body fluids. Therefore, particle toxicity is mainly related to the insoluble part of the particles, which may lead to high doses of toxins on a highly localized scale (e.g. individual cells) not only in the lungs, but also in other organs due to the potential of systemic particle translocation especially for UfPs as will be discussed below. Henceforth, the term 'particles' will refer to the insoluble part of the particles unless stated otherwise.

Airways

The residence times of the particles in the lungs is limited by several effective defence (clearance) mechanisms, which act as a particle sink. The walls of the upper parts of the lungs (airways) are covered with a protective mucus layer, which is transported by beating cilia towards the larynx from where it is swallowed into the gastrointestinal tract (GIT) for excretion. Mucociliary clearance will remove all particles larger than about 6 μm from the lungs within 1–2 day. For UfPs with enhanced mobility, penetration through the mucus into periciliary spaces and into the bronchial epithelium significantly reduces the mucociliary removal efficiency to about 25% for UfPs (Kreyling et al. 2006b, Möller et al. 2008). The remaining particle fraction is eliminated at a much slower rate by other clearance mechanisms, such as uptake by phagocytic or epithelial cells (Geiser et al. 2005).

Alveoli

In the deeper region of the lungs (alveolar region) a major fraction of the deposited particles will be cleared by macrophages, specialized defence cells, which recognize and internalize non-soluble particles via phagocytic uptake within less than a day after deposition. Upon internalization by macrophages the particles are either disintegrated or, if this is not possible, isolated from the organism with subsequent accumulation in the lymphatic system of the lung. Only a minor fraction of the phagocytized particles is transported up to the airways with subsequent mucociliary clearance. While macrophages respond within a few hours to almost all particles larger than 200 nm, the recognition mechanism becomes significantly less effective (~20%) for UfPs (Tabata & Ikada 1988). The remaining fraction of UfPs is retained long term in the epithelium and interstitial spaces (Brown et al. 2002, Möller et al. 2008, Wiebert et al. 2006) and recent animal studies suggest that a fraction of these UfPs is transported back to the luminal side of the epithelium (e.g. via the lymphatic system) with subsequent clearance via the mucociliary escalator (Semmler-Behnke et al. 2007).

Translocation and accumulation in secondary organs

Particles retained in the lungs may react with ions, chelating molecules and proteins, which may result in functional changes of the latter as well as coating or complexing of the particles. Thereby the particles may specifically bind to selected proteins and follow protein-mediated transport processes (Kim & Malik 2003), carrying them from the epithelial surface of the lungs into the circulation from where they can reach so-called secondary target organs such as liver, spleen, heart, brain and central nervous system (Kreyling et al. 2002b, Oberdörster et al. 2002). The particle size-dependent pathways (Patton et al. 2004) may be paracellular across tight junctions between adjacent epithelial cells or transcellular by transcytosis as shown by Heckel and colleagues (Heckel et al. 2004).

Translocation into secondary organs is most effective for small particles, such as UfPs. Measurements in animal models have shown that the translocation from the lungs into other secondary organs (24 h after exposure) is about 1 and 0.3% for inhaled 15 and 80 nm Ir particles, respectively (Kreyling et al. 2002a, Semmler et al. 2004). A similar translocated fraction of 1–2% was reported for 50 and 200 nm polystyrene particles (Chen et al. 2006). Animal models also indicate that small amounts of the translocated particles are retained long-term in secondary target organs. About 0.1% of inhaled 15 nm iridium particles were retained in secondary organs as long as 6 months after a single 1-h inhalation of 15 nm ^{192}Ir particles by healthy, adult WKY rats (Semmler et al. 2004). Direct evidence for ambient particle translocation in humans is only available for occupational exposure scenarios. Classical pathology was able to locate asbestos and coal particles in secondary organs (e.g. liver) of heavily exposed workers (Auerbach et al. 1980, LeFevre et al. 1982). In addition, there is increasing evidence that the residence time of particles in the circulation and subsequent uptake by secondary organs may be enhanced by deliberate surface modifications (Borm et al. 2006).

Based on the long-term measurements in rats (Semmler et al. 2004) we can estimate the accumulation

of UfPs in secondary target organs as 10^{11} particles during the lifetime of man (~100 years), where we assumed that (1) the average concentration of UfPs in ambient air is 10^4 particles per cm^3 (10^{10} m^{-3}), (2) the inhaled air volume per day is 10 m^3, (3) the lung deposited particle fraction in the alveolar regime is 0.3, (4) the insoluble particle fraction is 0.1 and (5) the translocated fraction is 0.001 (obtained for rodents by Kreyling et al. 2007). Although the particle number dose accumulating in secondary target organs is about four orders of magnitude below the lung dose, it may not be negligible especially when considering chronic diseases or carcinogenic processes (Beeson et al. 1998, Peters et al. 2006). As a caveat we note that extrapolation of the translocation data from rodents to man requires great caution due to interspecies differences.

Toxicology

Epidemiological studies have repeatedly described correlations between the level of ambient PM and increased morbidity and mortality in adults and children (Salvi 2007, Peters et al. 1997). Particularly convincing evidence was provided by a combination of epidemiological and toxicological studies related to the temporary (1 year) shut down of a steel mill in the Utah Valley. This shut down led to a substantial reduction of metal-rich ambient PM accompanied by reduced morbidity and mortality rates in the region around the steel mill (Pope et al. 1992, Ransom & Pope 1992). These epidemiological results were corroborated by toxicological studies, which associated ambient PM during operation of the steel mill with pulmonary injury, neutrophilic inflammation and increased airway responsiveness (Dye et al. 2001, Ghio & Devlin 2001).

It is commonly hypothesized that the toxicity of ambient PM and UfPs is related to the generation of reactive oxygen species (ROS), a main cause of cellular oxidative stress, which even at low doses triggers intracellular signalling processes, such as the activation of redox-sensitive transcription factors like NF-κB and AP1 (Cho et al. 2006, Rahman et al. 2006) as seen from Figure 2. In urban environments combustion-derived UfPs such as soot particles dominate PM number concentrations and contribute significantly to the total surface area of PM. Typically, soot particles consist of an inert (biopersistent) carbon core coated with potentially toxic pollutants consisting of hundreds of organic chemicals and many transition metals (Lighty et al. 2000). For combustion-derived UfPs, two main pathways of particle-induced ROS formation have been identified (Figure 2): (i) catalytic reactivity of the surface of the inert particle matrix (including the bioavailability of redox-cycling catalysts like transition metals or quinones), and (ii) metabolic generation of reactive byproducts during cellular detoxification of organic compounds especially polyaromatic hydrocarbons (PAHs) (Bonvallot et al. 2001, Stoeger et al. 2009). Once inflammatory processes are initiated ROS formation can be aggravated by stimulating inflammatory cells to undergo oxidative burst activity or to upregulate inducible nitric oxide synthase and cause nitric oxide production (Porter et al. 2007). For large particles (>500 nm), an additional mechanism is related to the enzymatic production of ROS upon particle phagocytosis, which is crucial for the killing of bacteria (Fubini & Hubbard 2003). Furthermore, since UfPs are known to translocate across epithelial–endothelial barriers into the blood and lymphatic circulation, from where they might reach sensitive (secondary) target organs, particle toxicity might not be limited to lung cells, but may cause direct extrapulmonary effects. In healthy individuals the potential of inhaled particles to induce oxidative injury is constrained by endogenous antioxidant defences (Li et al. 2003, Xia et al. 2006). Impairment of these antioxidant defences may in part explain the enhanced vulnerability of asthmatics and COPD patients to air pollutants, causing exacerbation (Kelly et al. 1999, Li et al. 2003).

Identification of a single physicochemical dose parameter (e.g. particle number, surface area or mass) for the toxicity of ambient PM may seem a daunting task in light of the various different toxicological pathways listed above and the diverse physicochemical nature

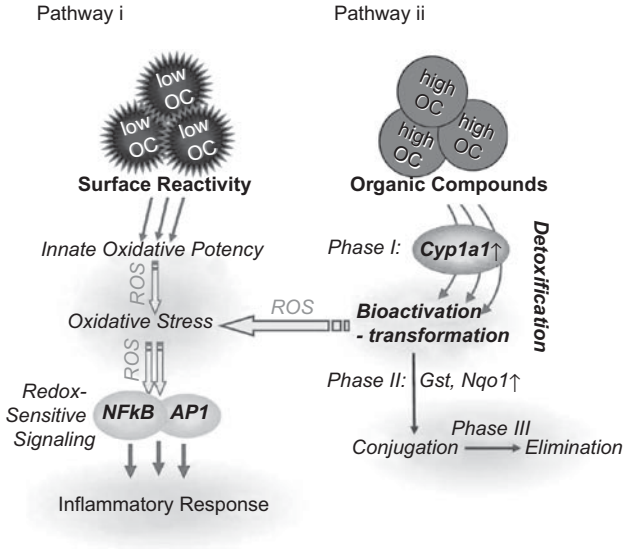

Figure 2. The oxidative stress paradigm for inflammatory response (toxicity) induced by soot particles includes two main toxicological pathways: (i) the particles' innate oxidative surface reactivity causes cellular oxidative stress directly (without metabolic activity) and (ii) detoxification of the bioavailable polyaromatic organic compounds (PAHs) induces the expression of the CYP1A1 protein, which causes (indirect) cellular oxidative stress by metabolic activation of PAHs. Pathway i is typical for pure carbon particles with low organics content (low OC), whereas pathway ii is characteristic for soot particles with high levels of organics (high OC) especially bioactive PAHs.

of ambient PM (Lighty et al. 2000, Putaud et al. 2004). Therefore, recent studies have attempted to characterize the toxicity of ambient particles based on their oxidative potency (or oxidative potential) using *in vitro* screening systems, which involve the depletion of antioxidants (e.g. ascorbate (vitamin C), reduced glutathione) in the presence of ambient PM (Mudway et al. 2004, Stoeger et al. 2009) mimicking the *in vivo* antioxidant defence system of the lungs. Ultimately, the predictive capacity of the oxidative potency (or any other dose parameter) for particle toxicity has to be verified by comparison with an *in vivo* toxicity parameter such as the influx of polymorphonuclear leucocytes (PMNs) into the lungs of rodents 24 h after intratracheal instillation of particles (Stoeger et al. 2006), which has been shown be an indicator of early inflammatory responses (Oberdörster 2000). The measured PMN dose-response curves can be converted into *in vivo* inflammatory efficacy, which is defined as a characteristic PMN level (here: 20% PMNs in the bronchoalveolar lavage (BAL) fluid) divided by the particle mass causing this effect (Stoeger et al. 2009).

Stoeger and co-workers investigated the relationship between inflammatory efficacy (in mice), oxidative potency and various physicochemical parameters for six types of ultrafine soot particles in mice consisting of a carbon core surrounded by an organic coating with widely varying primary particle diameter (10–50 nm), organic content (OC; 1-20%) and specific BET surface area (43-800 $mm^2/\mu g = m^2/g$) (Stoeger et al. 2006, Stoeger et al. 2009). BET surface area is likely to be particularly useful for toxicological studies, as it represents the surface area, which is accessible for molecular adsorption (as described by Brunauer, Emmett and Teller (Brunauer et al. 1938)) and hence is closely related to the catalytic reactivity (or oxidative potency) of PM. Thus, the good correlation between oxidative potency and BET surface area shown in Figure 3A is not surprising (Stoeger et al. 2009), but the appearance of two distinct subgroups deserves attention. Particles with high amounts of OC (or PAHs as determined by thermoanalytical speciation (Matuschek et al. 2007)) displayed a 5-fold lower surface-specific oxidative potency (slope) than low PAH particles (no PAHs were detectable) presumably due to shielding of the highly reactive carbon core by organic coating. If oxidative potency were the only relevant inflammatory mechanism (pathway i in Figure 2), the inflammatory efficacy of high PAH particles should be mitigated compared with low PAH particles. However, Figure 3B shows excellent linear correlation between *in vivo* inflammatory efficacy and BET surface area irrespective of PAH level. This indicates that the shielding of the carbon core for high PAH particles is compensated by another process, which only occurs *in vivo*. Because for high PAH particles, both the *CYP1a1* gene was expressed (1.2–3.9-fold induction) and the cytochrome P450 1A1 (CYP1A1) protein was detected in the lungs, ROS formation due to CYP1A1 enzyme-mediated biotransformation of bioavailable organics (PAHs) is a likely candidate for a second pathway (pathway ii in Figure 2), which only occurs *in vivo*, as it requires metabolic activity. Hence, oxidative potency alone cannot adequately describe the *in vivo* toxicity (inflammatory efficacy) of soot particles, if PAH-rich particles are involved. On the other hand, BET surface area is an excellent predictor for *in vivo* particle toxicity irrespective of the physicochemical composition of the soot particles (Figure 3B) due to the compensatory nature of organics-related shielding of the reactive carbon core and biotransformation of PAHs for high PAH particles. From the slope of Figure 3B (0.006% PMN per mm^2) it is evident that the characteristic level of 20% PMN influx into the lungs of mice is induced by 33 cm^2 of soot particles. In

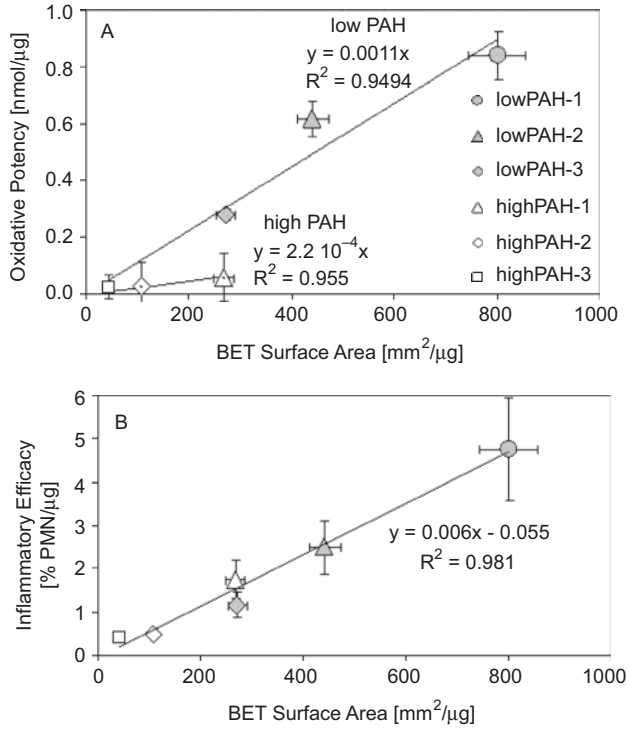

Figure 3. The *in vitro* oxidative potency and *in vivo* inflammatory efficacy (PMN influx into the lungs of mice) for six types of ultrafine soot particles, three types with low and three with high levels of bioactivated PAHs. (A) Both subgroups show excellent linear correlation between oxidative potency and BET surface area, but the surface specific oxidative potency (slope) for high PAH particles is 5-fold lower than that for low PAH particles. This can be explained by the presence of an organic coating (for high PAH particles) which shields the reactive carbon core. (B) The inflammatory efficacy correlates well with BET surface area irrespective of PAH level. This indicates that the shielding of the carbon core by organics (high PAH) is compensated by the additional inflammatory efficacy due to bioactivation of PAHs (metabolic activity). In this representation mass is only a scaling factor, as both the abscissa and ordinate are normalized to mass. Thus, these graphs depict the relationship between the BET surface area of the particles and their (A) oxidative potency and (B) inflammatory capacity.

contrast, neither particle mass nor OC showed a similarly good correlation with inflammation (Stoeger et al. 2006) and the relatively good correlation with particle number is likely to be incidental mainly due to the narrow range of primary particle diameters of the investigated particles (Stoeger et al. 2007). A similarly high predictive capacity of BET surface area for particle toxicity was also found for other particle types such as TiO_2 and polystyrene (Brown et al. 2001, Oberdörster et al. 2005).

These results support the following mechanistic scheme for soot particle-induced inflammation depicted in figure 2. On the one hand, particles with high innate oxidative potency (linked to low PAH or low OC) generate direct oxidative stress by particle–cell interaction, which in turn activates redox-sensitive transcription of proinflammatory genes (pathway i). On the other hand, particles with high amounts of bioavailable organic compounds, like PAHs, induce detoxification pathways leading to biotransformation and bioactivation of PAHs with associated ROS formation (pathway ii). The combined effect on *in vivo* inflammatory response in the lungs of mice correlates best with the BET surface area of the particles.

Summary

For urban environments, ultrafine particles (mainly soot) contribute significantly (~50%) to the lung-deposited particle surface area and hence to the adverse health effects of ambient PM. UfPs have an enhanced toxicity potential due to (1) high surface-to-mass ratio (high reactivity), (2) prolonged residence time in the lungs as a result of mitigated clearance efficiency and (3) low but possibly not negligible translocation rates across epithelial–endothelial barriers into the blood and lymphatic circulation, from where UfPs might reach vulnerable (secondary) target organs (e.g. heart, liver and brain).

BET surface area is emerging as the single most relevant dose parameter for particle toxicity both in the ultrafine (<100 nm) and fine (100–2500 nm) size range. For various types of soot particles, BET surface area is an excellent predictor for *in vivo* particle toxicity (here: inflammatory efficacy determined from PMN influx into the lungs) irrespective of their physicochemical characteristics (Figure 3B) due to the compensatory nature of organics-related shielding of the reactive carbon core and biotransformation of PAHs. Although similar results were reported for a limited number of other particle types, further studies are needed to confirm the predictive capacity of BET surface area for particle toxicity. Identification of the (most) relevant dose parameter is the basis for much needed regulatory measures on the toxicity of not only ambient but also engineered particles.

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References

Asgharian B, Menache MG, Miller FJ. (2004). Modeling age-related particle deposition in humans. *J Aerosol Med* 17:213–24.

Auerbach O, et al. (1980). Presence of asbestos bodies in organs other than the lung. *Chest* 77:133–7.

Baron PA, Willeke K. (2001). *Aerosol Measurement*. New York: Wiley Interscience.

Beckett WS, et al. (2005). Comparing inhaled ultrafine versus fine zinc oxide particles in healthy adults: a human inhalation study. *Am J Respir Critical Care Med* 171:1129–35.

Beeson WL, Abbey DE, Knutsen SF. (1998). Long-term concentrations of ambient air pollutants and incident lung cancer in California adults: results from the AHSMOG study. Adventist Health Study on Smog. *Environ Health Perspect* 106:813–23.

Bonvallot V, et al. (2001). Organic compounds from diesel exhaust particles elicit a proinflammatory response in human airway epithelial cells and induce cytochrome p450 1A1 expression. *Am J Respir Cell Mol Biol* 25:515–21.

Borm PJA, et al. (2006). The potential risks of nanomaterials: a review carried out for ECETOC. *Particle Fibre Toxicol* 3.

Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. (2001). Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicol Appl Pharmacol* 175:191–9.

Brown JS, Zeman KL, Bennett WD. (2002). Ultrafine particle deposition and clearance in the healthy and obstructed lung. *Am J Respir Critical Care Med* 166:1240–7.

Brunauer S, Emmett PH, Teller E. (1938). Adsorption of gases in multimolecular layers. *J Am Chem Soc* 60:309–19.

Chen J, et al. (2006). Quantification of extrapulmonary translocation of intratracheal-instilled particles in vivo in rats: effect of lipopolysaccharide. *Toxicology* 222:195–201.

Cho HY, Reddy SP, Kleeberger SR. (2006). Nrf2 defends the lung from oxidative stress. *Antioxid Redox Signal* 8:76–87.

Delfino RJ, Sioutas C, Malik S. (2005). Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health. *Environ Health Perspect* 113:934–46.

Donaldson K, Tran CL. (2002). Inflammation caused by particles and fibers. *Inhal Toxicol* 14:5–27.

Dye JA, et al. (2001). Acute pulmonary toxicity of particulate matter filter extracts in rats: coherence with epidemiologic studies in Utah Valley residents. *Environ Health Perspect* 109 (Suppl. 3):395–403.

Ferron GA, Kreyling WG, Haider B. (1988). Inhalation of salt aerosol particles. II. Growth and deposition in the human respiratory tract. *J Aerosol Sci* 19:611–31.

Fubini B, Hubbard A. (2003). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. *Free Radic Biol Med* 34:1507–16.

Gehr P, Bachofen M, Weibel ER. (1978). The normal human lung: ultrastructure and morphometric estimation of diffusion capacity. *Respir Physiol* 32:121–40.

Geiser M, et al. (2005). Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ Health Perspect* 113:1555–60.

Ghio AJ, and Devlin RB. (2001). Inflammatory lung injury after bronchial instillation of air pollution particles. *Am J Respir Crit Care Med* 164:704–8.

Heckel K, Kiefmann R, Dorger M, Stoeckelhuber M, Goetz AE. (2004). Colloidal gold particles as a new in vivo marker of early acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 287:L867–78.

Heyder J, Gebhart J, Rudolf G, Schiller CF, Stahlhofen W. (1986). Deposition of particles in the human respiratory tract in the size range 0.005–15 microns. *J Aerosol Sci* 17:811–25.

Hofmann W. (1996). Modeling techniques for inhaled particle deposition: the state of the art. *J Aerosol Med* 9:369–88.

ICRP (1994). International Commission on Radiological Protection (ICRP), Human respiratory tract model for radiological protection: a report of a task group of the ICRP, ICRP publication 66; Annals of the ICRP. Oxford, UK: Elsevier Science Ltd, p. 1–482.

Jaques PA, Kim CS. (2000). Measurement of total lung deposition of inhaled ultrafine particles in healthy men and women. *Inhalat Toxicol* 12:715–31.

Kelly FK, Mudway J, Blomberg A, Frew A, Sandstrom T. (1999). Altered lung antioxidant status in patients with mild asthma. *Lancet* 354:482–3.

Kim KJ, Malik AB. (2003). Protein transport across the lung epithelial barrier. *Am J Physiol Lung Cell Mol Physiol* 284:28–32.

Kreyling W, Moeller W, Semmler-Behnke M, Oberdörster G. (2007). Particle dosimetry: deposition and clearance from the respiratory tract and translocation towards extra-pulmonary sites. In: Donaldson K, Borm P, editors. *Particle Toxicology*. Boca Raton, FL: CRC Press. 47–74.

Kreyling WG, Semmler-Behnke M, Moeller W. (2006a). Health implications of nanoparticles. *J Nanoparticle Res* 8:543–62.

Kreyling WG, Semmler-Behnke M, Möller W. (2006b). Ultrafine particle – lung interactions: does size matter? *J Aerosol Med* 19:74–83.

Kreyling WG, et al. (2002a). Minute translocation of inhaled ultrafine insoluble iridium particles from lung epithelium into extrapulmonary tissues. *Ann Occup Hyg* 46 (Suppl. 1): S223–6.

Kreyling WG, et al. (2002b). Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J Toxicol Environ Health Part A* 65:1513–30.

LeFevre ME, Green FH, Joel DD, Laqueur W. (1982). Frequency of black pigment in livers and spleens of coal workers: correlation with pulmonary pathology and occupational information. *Hum Pathol* 13:1121–6.

Li N, Hao MQ, Phalen RF, Hinds WC, Nel AE. (2003). Particulate air pollutants and asthma – a paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin Immunol* 109:250–65.

Lighty JS, Veranth JM, Sarofim AF. (2000). Combustion aerosols: factors governing their size and composition and implications to human health. *J Air Waste Manage Assoc* 50:1565–1618.

Matuschek G, Karg E, Schroeppel A, Schulz H, Schmid O. (2007). Chemical investigation of eight different types of carbonaceous particles using thermoanalytical techniques. *Environ Sci Technol* 41:8406–11.

Möller W, et al. (2008). Deposition, retention and translocation of ultrafine particles from the central airways and lung periphery. *Am J Respir Crit Care Med* 177:426–32.

Mudway IS, et al. (2004). An *in vitro* and *in vivo* investigation of the effects of diesel exhaust on human airway lining fluid antioxidants. *Arch Biochem Biophys* 423:200–12.

Oberdörster G. (2000). Toxicology of ultrafine particles: *in vivo* studies. *Phil Trans Math Phys Eng Sci (Ser A)* 2000:2719–40.

Oberdörster G, Oberdörster E, Oberdörster J. (2005). Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113:823–39.

Oberdörster G, et al. (2002). Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J Toxicol Environ Health Part A* 65:1531–43.

Patton JS, Fishburn CS, Weers JG. (2004). The lungs as a portal of entry for systemic drug delivery. *Proc Am Thorac Soc* 1:338–44.

Peters A, et al. (2006). Translocation and potential neurological effects of fine and ultrafine particles a critical update. *Particle Fibre Toxicol* 3:13.

Peters A, Wichmann H-E, Tuch T, Heinrich J, Heyder J. (1997). Respiratory effects are associated with the number of ultrafine particles. *Am J Respir Crit Care Med* 155:1376–83.

Pope CA 3rd, Schwartz J, Ransom MR. (1992). Daily mortality and PM_{10} pollution in Utah Valley. *Arch Environ Health* 47:211–17.

Porter DW, Leonard SS, Castranova V. (2007). Particles and cellular oxidative and and nitrosative stress. In: Donaldson K and Borm P, editors. *Particle Toxicology*. Boca Raton, FL: CRC Press. p. 119–38.

Putaud JP, et al. (2004). European aerosol phenomenology. 2: Chemical characteristics of particulate matter at kerbside, urban, rural and background sites in Europe. *Atmos Environ* 38:2579–95.

Rahman I, Yang SR, Biswas SK. (2006). Current concepts of redox signaling in the lungs. *Antioxid Redox Signal* 8:681–9.

Ransom MR, Pope CA 3rd. (1992). Elementary school absences and PM_{10} pollution in Utah Valley. *Environ Res* 58:204–19.

Salvi S. (2007). Health effects of ambient air pollution in children. *Paediatr Respir Rev* 8:275–80.

Schmid O, Karg E, Hagen DE, Whitefield PD, Ferron G. (2007). On the effective density of non-spherical particles as derived from combined measurements of aerodynamic and mobility equivalent size. *J Aerosol Sci* 38:431–43.

Semmler-Behnke M, et al. (2007). Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent re-entrainment onto airways epithelia. *Environ Health Perspect* 115:728–33.

Semmler M. et al. (2004). Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhalat Toxicol* 16:453–9.

Stoeger T, et al. (2006). Instillation of six different ultrafine carbon particles indicates a surface area threshold dose for acute lung inflammation in mice. *Environ Health Perspect* 114:328–32.

Stoeger T, Schmid O, Takenaka S, Schulz H. (2007). Inflammatory response to TiO2 and Carbonaceous particles scales best with BET surface area. *Environ Health Perspect* 115:A290–1.

Stoeger T, et al. (2009). Deducing *in vivo* toxicity of combustion-derived nanoparticles from a cell-free oxidative potency assay and metabolic activation of organic compounds. *Environ Health Perspect* 117:54–60.

Stone V, Johnston H, Clift MJ. (2007). Air pollution, ultrafine and nanoparticle toxicology: cellular and molecular interactions. *IEEE Trans Nanobiosci* 6:331–40.

Tabata Y, Ikada Y. (1988). Effect of the size and surface charge of polymer microspheres on their phagocytosis by macrophage. *Biomaterials* 9:356–62.

Wiebert P, et al. (2006). Negligible clearance of ultrafine particles retained in healthy and affected human lungs. *Eur Respir J* 28:286–90.

Xia T, Kovochich M, Nel A. (2006). The role of reactive oxygen species and oxidative stress in mediating particulate matter injury. *Clin Occup Environ Med* 5:817–36.