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Phospholipid lung surfactant and nanoparticle surface toxicity: Lessons from diesel soots and silicate dusts

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Abstract

Because of their small size, the specific surface areas of nanoparticulate materials (NP), described as particles having at least one dimension smaller than 100 nm, can be large compared with micrometer-sized respirable particles. This high specific surface area or nanostructural surface properties may affect NP toxicity in comparison with micrometer-sized respirable particles of the same overall composition. Respirable particles depositing on the deep lung surfaces of the respiratory bronchioles or alveoli will contact pulmonary surfactants in the surface hypophase. Diesel exhaust ultrafine particles and respirable silicate micrometer-sized insoluble particles can adsorb components of that surfactant onto the particle surfaces, conditioning the particles surfaces and affecting their *in vitro* expression of cytotoxicity or genotoxicity. Those effects can be particle surface composition-specific. Effects of particle surface conditioning by a primary component of phospholipid pulmonary surfactant, diacyl phosphatidyl choline, are reviewed for *in vitro* expression of genotoxicity by diesel exhaust particles and of cytotoxicity by respirable quartz and aluminosilicate kaolin clay particles. Those effects suggest methods and cautions for assaying and interpreting NP properties and biological activities.

Concerns for health hazard from nanoparticulate exposures

Research has demonstrated the importance of parameters such as size and number in determining the toxicity of insoluble particles with nanometer dimensions, or nanoscale structures (Oberdörster et al., 1995, 2004; Driscoll, 1996; Donaldson et al., 2000; Oberdörster 2000; Tran et al., 2000). Nanostructured materials including nanoparticles (NP) are defined as having at least one dimension smaller than 100 nm (Maynard & Kuempel, 2005). There also is concern for surface property effects on NP-induced toxicity or disease risk. This is due to the large specific surface area, i.e., surface area per unit mass, of NP associated with their small size, and because surface area and surface properties can strongly affect the toxicity or disease risk

associated with respirable micrometer-sized particles. Therefore, while such possible effects are under investigation, NP as administered for cellular or animal model bioassay ideally should not be altered in size, morphology, aggregation and surface properties from their condition upon deposition in the lung after workplace or environmental inhalation exposure. As part of this, a critical concern is the conditioning of NP that will occur upon the initial deposition of particles upon the aqueous hypophase environmental interface of the lung, e.g., by adsorption of and dispersion in biomolecular components of lung surfactant or serum.

NP may differ on a mass basis from larger particles of the same composition for expression of toxicity and for biological transport and bioavailability because of higher specific surface area of NP. Materials deemed low in toxicity as larger particles may exhibit toxic effects as NP. Greater toxicity is reported for ultrafine carbon black, TiO_2 and latex particles compared to larger low-toxicity low-solubility particles of the same material (Donaldson et al., 2000); a tenfold increase in inflammation observed for the same mass of ultrafine versus fine particles was attributed to increased oxidative activities of the ultrafine particles. A set of dusts of low toxicity when in the micrometer size range including TiO_2, talc, carbon black, and photocopier toner, and particles with some toxicity including coal mine dust and diesel exhaust particulate material (DPM), were found to have comparable toxicity on a surface area basis for lung tumor induction in a rat model; and that toxicity increased strongly with increase in dose measured as surface area (Maynard & Kuempel, 2005). However, in some cases there is a strong mineral specific component of toxicity not resolved by surface area normalization of dose; for example, fine-sized crystalline silica, e.g., quartz dust, is much more active than TiO_2 for pulmonary inflammation in an animal model (Oberdörster et al., 1994). Differing degrees of inflammation and lung injury upon ultrafine NiO, Co_3O_4, TiO_2 and carbon black instillation in rat lung have been reported (Dick et al., 2003). Degree of lung injury was found to correlate to the particle's ability to generate surface free radicals and to cause oxidant damage. Surface area, chemical composition and surface reactivity were all deemed important factors in particle toxicity. Exacerbated pulmonary inflammation may be a means by which airborne pollutant matter (PM) exerts its toxicity (Tao et al., 2003). The smallest PM, below 2.5 μm, was most consistently associated with toxicity; the toxicity was attributed to oxidative stress caused by reactive oxygen species associated with metal, semi-quinone, lipopolysaccharide, or hydrocarbon constituents of ultrafine particles.

NP also may be able to cross the cell membrane and enter the bloodstream from the lungs (Ferin & Oberdörster, 1992; Oberdörster et al., 1992; Geiser et al., 2005). This general cell-penetrating ability is known, and even exploited, in the field of in vivo imaging. NP with special fluorescent, magnetic or optical properties such as "quantum dots" and magnetic resonance imaging contrast agents are functionalized with biocompatible coatings such as peptides, polysaccharides or other polymers and then directed within cells to permit selective signaling from specific cell components (Michalet et al., 2005; Sadeghiani et al., 2005). This ability to cross cell membranes has been pursued to provide functionalizing agents to transport peptides and DNA fragments into cells, e.g., through the endothelial tight-junction blood–brain barrier (Pantarotto et al., 2003, 2004a, b; Lu et al., 2005; Zhi et al., 2005). Such an uncommon effect is reported in studies of a variety of inorganic NP (Peters, et al., 2004). Cytotoxicity is a concern in new applications of NP, and safe exposure levels must be determined before these agents can be used in medical procedures. The majority of NP surveyed (TiO_2, SiO_2, and Co) were internalized into human epithelial cells, though most did not show cytotoxic effects. A pro-inflammatory stimulation and impairment of proliferative activity was observed for nano-Co and nano-SiO_2 particles, which was speculated to lead to a chronic inflammatory response and subsequent development of granulomas.

Carbonaceous materials represent a major class of NP, and a wide range of toxicity may result from variations in their shape, size, and complex chemical composition. The spherical nanoparticulate soot fullerene (C_{60}) was intentionally produced in 1985 by laser ablation of graphite targets. Limited toxicity studies of fullerene indicate this material was toxic to fish in aqueous systems, where fullerenes were found to pass the blood–brain barrier and cause brain damage (Oberdorster, 2004). The discovery of fullerenes has led to