Potential Toxicity of Nanomaterials

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Nanoparticles (manufactured) or ultrafine particles (naturally produced in the environment; often from transportation sources) are particles with at least one dimension $\leq$ 0.1 microns (100 nanometers [nm]). Inhalation of potentially injurious particles small enough to travel to deep lung (fine particles - $\leq$ 2.5 microns [2,500 nm] or ultrafine- (nano-) particles) [7, 15] can have different outcomes. For example, one outcome might be that they cause no biomedical consequences. Alternatively, ultrafine or nanoparticles can cause and heal minor injuries in relatively healthy lung epithelial cells (LEC). Or, they may lead to chronic degenerative lung disease or even to lung cancer if the LEC are injured, or become injured [10a, 15, 17].

Induction and release of the cytokines interleukins 6 and 8 (IL6 and IL8) by airway cells in vitro has been used extensively to assess relative toxicity of occupational particles and particles occurring in nature [19], as soil particles and as particles originating from transportation vehicles. TRP channels (transmembrane receptors) established as primary sensors of thermal stimuli in the peripheral nervous system of mammals [1] are associated with pores and the release of cytokines such as IL-6 and IL-8 (Sabnis et al., 2008) [16]. Nano sized particles of the same nominal composition are useful for the induction of IL-6 and IL-8. The manufactured pure oxides were less potent than natural $PM_{2.5}$ particles whose source was soil or the positive controls. The potency for inducing IL-6 secretion by BEAS-2B did not correlate well with the generation of reactive oxygen species (ROS), possibly because they absorbed IL-6 on the high surface area silica and titanium dioxide particles. Measured IL-6 increased when BSA or fetal calf sera was added to the incubation mixture, suggesting that there was non-specific binding of IL-6 to surfaces > 1 order of magnitude above that of plastic on which they were cultured.

Particle artifacts from high surface areas need to be considered in experiments of this type. However, the study suggests that manufactured metal oxide nanoparticles are not highly toxic to lung cells compared to other environmental nanoparticles [19].

Exposure to air polluted with particles less than 2.5 micrometers (2,500 nm) in size was associated with epidemiologically adverse cardiopulmonary health consequences in humans [6]. Six healthy volunteers exposed to fine and ultrafine but non toxic magnesium oxide (MgO) particles. Exposure levels were: mean +/- standard deviation for exposure was ~ 4,138 +/- 2163 x mg/m^3. Ninety-eight (98%) percent of the particles were fine (< 2.5 micrometers, 2,500 nm) and 28% were ultrafine (< 100 nm). No differences were noted from controls, as indicated by bronchoalveolar lavage (BAL) inflammatory cell concentrations, interleukin concentrations (IL-1, IL-6, IL-8), tumor necrosis factor, pulmonary function or peripheral blood neutrophil concentrations [6].

In animal studies, rats exposed *in vivo* to very high levels (1.5-2 g/m^3) of nanoactive magnesium oxide, titanium dioxide or FAST ACT (combined MgO and TiO_2) for ~4 hours had no reductions of body weight by 3-14 days after initiation of exposure. They had no clinical signs or macroscopically visible pathologic changes on examination at necropsy at 14 days after initiation of exposure. Based on this evidence, manufactured magnesium, titanium or combined magnesium and titanium nanoparticles had no "in life" effect, at very high doses for short exposure times [5].

MgO particles, at least, may have been cleared relatively rapidly. In studies at Kansas State University [13], we have shown that non-toxic magnesium oxide (MgO), relatively insoluble in distilled water, is much more rapidly soluble in LEC tissue culture fluid (Hanks Balanced Salt Solution (HBSS) or Dulbecco's Modified Eagles Medium (DMEM); distilled water peak ~2-4 days; HBSS peak $\leq$ 1-3 hours). Peak dissolution speed and amount both increased ~ 2 fold (amount) or ~2 to 5+ fold (speed) with lung stimulant fluid containing bicarbonate relative to distilled water. Speed and amount of solubility were related directly to the bicarbonate concentration (a major human body buffer) in the dissolving media. Most likely, a more soluble carbonate compound (magnesium carbonate trihydrate; nesquehonite) was formed. It should be

noted that solubility in these studies was defined as material that remained after micro-centrifugation at 13,200 x gravity for 20 minutes. Measurement of particle size by laser diffraction showed reduction in particle size from 100-1,000 nm (un-centrifuged) to ~40-100 nm (centrifuged) consistent in similar particle distributions with $\geq$ 99% removal as soluble material [13-14], within the reproducibility of the metal measurements.

Contrast this to titanium dioxide particles. Such particles had minimal solubility (1.5-2.1 ppm $\pm$ ~ 10-25%) at high (5X) relative and at low (1X) concentrations from distilled water, or media containing bicarbonate at different concentrations. Concentrations were significant above with a $p < 0.01$) 2 times out of 3; but, with a $p > 0.05$ 1 time out of 3, when corrected for 3 comparisons (Games, 1977). Measurement of particle size by laser diffraction showed reduction in particle size from 300-2,000 nm (un-centrifuged) to ~20-300 nm (centrifuged) consistent in similar particle distributions with $\geq$ 95% removal [14] as soluble material, which was within the reproducibility of the metal measurements. Carbonaceous particles which are relatively insoluble and would also be expected to persist. If sufficiently small these particles would be expected to go to the interstitial space and remain as a potentially injurious source. Alternatively, if they were not, they would most likely be cleared. Titanium dioxide particles have a tendency to aggregate in biological fluids [2, 8, 15]. Thus, TiO_2 particles have some probability of being phagocytized.

Lung responses are influenced by repeated injury, or persistence of ultrafine particles or nanoparticles. For example, mice inhaling urban traffic particles that were organic and would be expected to persist. When air was filtered successively by bag-, JFL-90- and HEPA-filters concentrations were 2.9 $\mu g/m^3$ showed no "in life" changes as indicated by no changes in lung and body weights, and lung/body weight ratios; respiratory physiology (pressure-volume curve) measurements; histopathology changes and morphometry changes [9]. When airborne particles were not filtered, concentrations were 16.8 $\mu g/m^3$. Comparisons were among unexposed controls, prenatal, post-natal or both (combined prenatal and post natal) exposures. Combined exposures had no differences in body weight, among the four groups. Although both levels are taken to be acceptable or nearly so by US-EPA standards ($PM_{2.5} < 15$ mg/m^3), post natal and combined groups had trends toward reduced lung/body weight ratios at 90 days exposure and

significantly reduced surface to volume ratios when compared to pre natal (before birth) exposures or unexposed controls. Reduced surface to volume ratios in lungs may have reduced their ability to exchange oxygen into the capillaries. Thus, lungs of the mice exposed to higher levels had structural and histopathology changes relative to prenatal or unexposed controls [9]. Mice in the filtered air chamber had no detectable lung responses, suggesting the probable utility of HEPA masks.

Although nanoparticles commonly have a dimension of < 100 nm, on suspension in media agglomerates of nanoparticles are more common structures [10]. The authors exposed mice to relatively dispersed single walled carbon nanotubes (DSWCNT; mean diameter 0.69 micrometers, 690 nm; aggregated to small fine particles; ~10 μg/mouse lung; ~300 μg/kg mouse) by pharyngeal aspiration. Lung sections in sacrificed mice demonstrated transient inflammatory and neutrophil phases that resolved and were similar to that observed with larger agglomerates. There were no granulomatous lesions or epithelioid macrophages, but the average thickness of connective tissue in alveolar regions stained by Sirius red (collagen special stain) in cryo fixed sections increased from ~0.1 to 0.5 at 7 days- and 0.9 at 1-month-post exposure. Health and respiratory physiology appeared unchanged as indicated by body weight, lung volume, alveolar surface area, alveolar gas volume and alveolar tissue volume. Quantum dots attached to DSCWNT were not detected in alveolar macrophages. Quantum dots alone in exposures were avidly phagocytized by such macrophages. No differences were seen when compared with unlabelled DSWCNT. Gold-labelled- and quantum dot labeled-DSWCNT were rapidly incorporated into pulmonary interstitium, but didn't produce granulomatous lesions [10].

Less dispersed CNT can result in deposits ~ 15 micrometers (15,000 nm). Once in the lungs, the action of surfactant and protein may reduce the interactions between nanotubes and water and allow dispersion of these agglomerates. The dispersive action of alveolar lining fluid on nanoparticles has been demonstrated in vitro [10, 16a]. Gold and quantum dot labeling of less dispersed DSWCNT demonstrated that a significant fraction of aspirated DSWCNT material may exist at < 1 micrometer (1,000 nm) and can rapidly migrate into the alveolar interstitial spaces. These small aggregates were not a target for macrophage activity. The only detectable

alteration was an increase in interstitial collagen as indicated by Sirius red staining of cryo-fixed tissue at 1 month after exposure. Carbon nanotubes were seen in lung tissue indicating their persistence [10].

Although total lung collagen was not measured, lung sections seemed to have very little persisting inflammation, less intense than animals in an earlier study who inhaled 50 nanocuries (nCi; 1,500 roentgen equivalent to man [rems]) of $^{238}PuO_2$ with diffuse interstitial pulmonary fibrosis similar to that shown in Mercer et al., 2008 spontaneously by 288 days after exposure (Pickrell et al., 1983) [11]. Similar plutonium exposures involved 8-12% of the projected histologic section's area with diffuse alveolar thickening [3]. Thus, our 1.5 to 2 fold increases in total lung collagen are ~ equivalent in extent to the diffuse alveolar thickening seen in collagen special stains of Mercer et al. [10]. If equivalent responses can be expected in human lungs following exposure to nanoparticles, this is good news, because they are probably negligible or reversible. Taken together data suggests that we may need to look to cellular and tissue injury caused by other components of polluted atmosphere, such as oxidant gases for injuries necessary to cause irreversible degenerative changes. The exact level of injury needed to cause irreversible degenerative changes in human lungs continues to be of considerable investigative interest [12].

Particles < 0.5 microns (500 nm) are cleared from lung with minimal efficiency by lung phagocytes or simply ignored and travel to the pulmonary interstitial space. Lack of clearance can be one source of persistence of the particles and continued injury. Relative solubility is a second source of persistence for poorly soluble particles [14, 15]. However, diffuse interstitial pulmonary fibrosis $\geq$ intensity and inflammation than DSWCNT, the most persistent of nanoparticles, is reversible anatomically, biochemically and physiologically [11].

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