

NNI-ChI CBAN ESH Working Group

Recommended Topics for R&D Activities: Toxicological Hazard Assessment of Nanomaterials

The conventional wisdom regarding pulmonary toxicity of nanoparticles, based on studies in rats, is that such particles are more toxic (e.g., inflammogenic, tumorigenic) than an equivalent mass of fine-sized particles of identical composition (Oberdörster, 2000a; Donaldson et al., 2002). This concept is generally based on a limited number of systematic studies of three poorly soluble particle-types: namely, titanium dioxide, carbon black, and diesel soot particles (Oberdörster and Yu 1990; Oberdörster et al. 1992; Driscoll et al. 1996; Dasenbrock et al., 1996; Heinrich et al. 1995; Nikula et al., 1995). Data for titanium dioxide particles indicate that in the lungs of rats (on a per-mass basis), inhaled ultrafine P-25 particles are more inflammogenic, fibrogenic, and tumorigenic than chemically similar, fine TiO₂ particles (Lee et al. 1985; Oberdörster et al. 1992; Heinrich et al. 1995). However, on a surface-area basis, the potency of ultrafine particles to produce adverse pulmonary effects is considered to be similar to that of larger particles of similar composition (Oberdörster et al. 1992; Driscoll 1996a).

Ultrafine particles may deposit in all regions of the respiratory tract, including approximately 20-50% deposition in the lower respiratory tract in humans, which is approximately 2-5 times greater than that of larger respirable particles (ICRP 1994). Studies in rodents and in cells have shown that ultrafine particles also escape detection and clearance by macrophages to a greater extent than fine particles (Oberdorster, 2001; Donaldson et al., 2002; Renwick, et al. 2001), and may to a greater degree, translocate from alveolar regions to epithelial and interstitial sites (Ferin et al. 1992; Oberdörster et al. 1994; Kreyling et al. 2002) or to the blood circulation, where they may migrate to other organs (Nemmar et al. 2001, 2002; Oberdörster et al. 2002). Ultrafine particles can produce greater inflammatory effects via free radical generation and lipid peroxidation (Donaldson et al. 1998, 2004; Lundborg et al. 2001; Dick et al. 2003), as well as through their ability to produce cytoskeletal dysfunction in macrophages (Wottrich et al. 2004) and damage to mitochondria (Li et al. 2003).

Exceptions to the conventional wisdom, however exist. For instance, not all nanoparticles are more toxic than fine-sized particles of identical chemistry (Oberdörster et al., 2000b; Warheit, 2004, Frampton et al., in press). Moreover, significant differences in lung responses among species have been observed, with the rat being most sensitive, i.e., having enhanced pulmonary responses (e.g., inflammation) when compared to mice or hamsters with comparable internal lung burdens of either fine or ultrafine P-25 titanium dioxide particles (Bermudez et al. 2002, 2004). In addition, other contributing factors—such as particle shape, surface charge, and coatings—may contribute greatly to particle toxicity (Warheit et al. 2003a; 2003b; Rehn et al., 2003; Schins et al., 2002); and other factors such as surface reactivity may greatly influence the cytotoxicity of ultrafine particles (Sayes et al. 2004)

Clearly, much research and development is needed to provide satisfactory toxicology information for hazard assessment. In addition, there is a need to assess the extent to which existing toxicology studies of anthropogenically derived, ultrafine particles are suitable as

models for predictions of the potential hazard of new nanomaterials. The following list summarizes priority areas for research focus.

Important issues to consider during the first phase of research:

- Investigate basic issues regarding nanoparticle toxicity.
 - Determine the major factors that cause pulmonary nanoparticle toxicity. Consider solubility, particle size (including specific diameters/lengths), surface coatings, shape, surface charge, composition (e.g., gas or liquid phase), and aggregation/disaggregation.
 - Investigate whether anthropogenic ultrafine/nanoparticles behave similarly to natural or engineered nanoparticles.
 - Determine the best metric for assessing particle toxicity—for example, mass, surface area, or particle number.
 - Assess the health impacts of inhaled particles that translocate into the brain.
 - Determine the major issues related to potential nanoparticle dermal toxicity, skin penetration, and phototoxicity. How should nanoparticulates be tested for dermal toxicity? (The various methodologies for assessing dermal effects have not yet been validated or standardized.) Determine whether a difference exists between macro/microscale particles and nanoparticles with respect to skin irritation, skin penetration, and skin sensitization.
 - Determine the representative nanomaterials for testing. Suggested examples include nanotubes (single and double walled); fullerenes (mainly carbon plus its derivatives, as well as others such as MnS); nanowires/whiskers; metal oxides (e.g., TiO₂, ZnO); quantum dots; and nanoclays.
 - Develop a reasonable testing strategy for assessing the toxicity of nanoparticulates. Should a tiered testing approach be implemented? What are the short-, intermediate-, and long-term endpoints that should be of principal concern?
 - Determine what new methods or techniques need to be developed prior to evaluating representative samples of nanoparticles. Methods must address issues of purity (including presence or absence of other materials from production), surface characterization, and presence or absence of adsorbed contaminants or surface coatings.
 - Assess the absorption, distribution, kinetics, and clearance of representative nanoparticulates. Because the sparse available literature suggests that nanoscale particles are more likely to escape (via absorption) the GI tract and transmigrate to other organs, oral toxicity (which likely depends upon the inherent toxicity of each specific chemical/particle type) needs to be included.

- Develop a Preliminary Hazard Assessment tool for nanotechnology, recognizing that *health risk* is a combination of *hazard* and *exposure*.
 - Address preliminary issues.
 - Determine the presumed exposure: for example, powder vs aqueous suspension.
 - Consider basic life-cycle issues. For example, is nanoscale iron in remediation still a possible inhalation exposure?
 - Determine the availability of information on the micro- or macroscale chemical/product (e.g., toxicology information or an occupational exposure limit).

- Using literature review and other approaches, evaluate the three likely routes of exposure: inhalation, dermal, and oral. Determine which exposure route predominates and which should be of primary concern.
- Consider the following issues regarding inhalation hazard for a research chemical.
 - Explore the use of pulmonary screen bioassay methodology (intratracheal instillation exposure in rats). The in vitro approach is not recommended at this time.
 - As the product becomes more successful—or if already in widespread use—recommend short-term to subchronic inhalation studies.
 - Consider the issue of translocation of inhaled nanoparticles to the brain (may be particle type specific). At present, the ramifications are unknown.
- Develop methodology and assess toxicity of nanomaterials to aquatic organisms.

Important issues to consider during a second phase of research:

- Assess the biopersistence of particle-types as well as particle surface coatings
- Evaluate the susceptibility of different subpopulations to nanoparticles
- Evaluate acute vs. chronic effects

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Recommended Topics for R&D Activities: Measurement/Detection of Nanomaterials

Measurement Methods

- Survey existing methods used in industry for the measurement of occupational (within-plant-boundary) and environmental (outside-plant-boundary) emissions of nanomaterials.
- Determine what new measurement parameters are critical to the measurement and detection of engineered nanoparticle emissions with respect to occupational and environmental emissions.
- Determine if any bulk techniques (mass based) are applicable to the measurement of nanoparticles. (Mass-based techniques are susceptible to large biases resulting from even trace levels of contaminants, which may have several orders of magnitude more mass than the nanoparticles.)
- Facilitate measurements of individual nanoparticles via development of measurement schemes with nanoscale resolution.
 - Develop and validate analytical tools for the characterization of nanoparticles by electron-beam analysis methods.
 - Address the need for improved analytical spatial resolution (<500 nm) in X-ray micro/nanoanalysis.
 - Develop methods for the 3-D chemical characterization of nanoparticles at 1-nm resolution.
 - Develop and validate analytical tools for the characterization of nanoparticles by optical microscopy/spectroscopy.
 - Address the need for expanding nanoparticle chemical analysis through the development of novel techniques such as NSOM.
 - Determine the feasibility of nanoparticle characterization using super-resolution optical microscopy at <100-nm resolution.
 - Develop and validate measurement methods to characterize the surface properties of nanoparticles, for example, surface charge and surface speciation.
 - Develop and validate methods for measuring biological activity associated with nanoparticles.
 - Develop and validate methods for measuring the dissolution of nanoparticles in water and biological fluids.
 - Develop methods to measure the potential for nanoparticles to pass through cell membranes.

- Develop methods that have sufficient particle number sensitivity to be useful and determine uncertainties. (*Note:* Microscopic methods are often limited by time and the number of particles analyzed, leading to high statistical uncertainty.)
- Develop and verify appropriate sample collection schemes that are compatible with single-particle analysis methods.
 - Develop and verify methods for the representative sampling of nanoparticles in soil.
 - Develop and verify methods for the representative sampling of nanoparticles in water.
 - Develop and verify methods for the representative sampling of nanoparticles in air.
- Develop methods that can be used and evaluated with environmental; occupational; and animal, epidemiological, and other health-related studies.

Detection Methods

- Determine conditions under which existing detection schemes are applicable to engineered nanomaterials.
- Develop automated microscopic methods for rapid analysis/screening of a large number of particles.
- Determine the limits of detection required to address occupational and environmental emissions issues.
- Develop and validate methods for detection of nanoparticles in the air and water to address real-time monitoring issues. Monitoring techniques include those inside the plant and at the plant boundary.
- Develop and validate methods for detection of nanoparticles for long-term monitoring issues. Monitoring techniques include those inside the plant and at the plant boundary.
 - Develop and validate methods for separating nanoparticles from soil.
 - Develop and validate methods for separating nanoparticles from water.
 - Develop and validate methods for separating nanoparticles from air.

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Recommended Topics for R&D Activities: Worker Protection and Industrial Hygiene for Nanomaterials

- Survey existing knowledge (published and unpublished) on protective equipment, monitoring, and analysis currently used in nanoresearch and manufacturing.
- Develop and evaluate exposure monitoring methodologies.
 - Develop NIOSH/OSHA-validated filter air-sampling techniques.
 - Determine/create reliable, validated methodologies for determining the physical characteristics of nanoparticles released to the environment (work areas or external), and determine if (and how) these characteristics change over time.
 - Investigate the response of particles (or their aggregates) to changes in humidity, electrical fields, and variations in temperature.
 - Create and validate detection methodologies and equipment.
 - Develop and validate methods for analysis of environmental samples.
 - Develop methodologies to determine nanospecific safe exposure limits.
- Address issues related to PPE.
 - Determine whether the required level of respiratory protective equipment is appropriate.
 - Determine the sufficiency of HEPA filter respirator cartridges.
 - Evaluate the potential need to develop specific filters for various classes of nanoparticles.
 - Assess the adequacy of standard qualitative and quantitative fit test procedures.
 - Determine whether nanoparticles combined with organics or halogens require special protection or if standard chemical cartridges would be sufficient.
 - Address issues related to dermal exposure.
 - Determine whether some or all nanoparticles penetrate the skin.
 - Assess the effects of nanoparticles on the surface of the skin.
 - Evaluate the ability of commercially available disposable protective clothing to offer adequate dermal protection.
 - Determine the appropriate type of glove to be worn, specifically addressing the issue of whether a single type of glove material is appropriate for all nanoparticles.
 - Establish validation techniques for PPE penetration tests.
- Determine the adequacy of the currently commercially available air pollution control techniques.
- Consider the development of methods for evaluating the solubility of nanoparticles. (Appropriate techniques may be similar to those used for fibers.)
- Consider methods for relevant in vitro assessment of aggregation/agglomeration.
- Assess the effectiveness of communication of hazards from MSDSs.