The field of nanotechnology currently is undergoing a dramatic expansion in material science research and development. Most of the research efforts have been focused on applications; the implications (i.e., health and environmental effects) research has lagged behind. The success of nanotechnology will require assurances that the products being developed are safe from an environmental, health, and safety (EHS) standpoint. In this regard, it has been previously reported in pulmonary toxicity studies that lung exposures to ultrafine or nanoparticles (defined herein as particle size ≤100 nm in one dimension) produce enhanced adverse inflammatory responses when compared to larger particles of similar composition. Surface properties (particularly particle surface area) and free radical generation, resulting from the interactions of particles with cells may play important roles in nanoparticle toxicity. This brief review identifies some of the key factors for studying EHS risks and hazard effects related to nanoparticle exposures. Health and environmental risk evaluations are products of hazard and exposure assessments. The key factors for discussion herein include the importance of particle characterization studies; development of a nanomaterial risk framework; as well as corresponding hypothesis-driven, mechanistically-oriented investigations, concomitant with base set hazard studies which clearly demonstrate that particle size is only a single (and perhaps minor) factor in influencing the safety of nanomaterials.

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1. Introduction

Nanotechnology is an emerging multidisciplinary science that involves applications based upon the synthesis of molecules in the nanoscale (i.e., 10⁻⁹ m) size range. The concept of “nanotechnology” is
derived, in part, from the Greek word “nano”, meaning “dwarf”. From a material sciences standpoint, the generation of new products using engineered nanomaterials is exciting because as one moves down the nanoscale, i.e., reducing the particle size range below ~100 nm), the properties of particles are known to change; and implementation of these properties can be exploited to provide products with enhanced applications. For example, gold particles can change colors to red or blue as the particle size is decreased within the nanoscale range. Moreover, pigment-grade titanium dioxide particles (generally in the 300–400 nm size range), lose their white color and become colorless (i.e., transparent) at decreasing particle size ranges approaching 50 nm. This feature may be useful in the production of cosmetics (e.g., sunscreens) as well as other applications. Some particle-types which have been utilized for electrical insulating properties may become conductive at the nanoscale level; or insoluble substances can become more soluble below 100 nm. Consequently, alterations in physical properties serve to enhance versatility and efficacy in product development, resulting in more effective industrial and medical applications, concomitant with the production of more versatile and efficacious products (Colvin, 2003).

Nanoparticles (sometimes referred to as ultrafine particles) generally have been defined as particle-types in the size range <100 nanometers, in at least one dimension. A nanometer (nm) is roughly the width of 10 hydrogen atoms. For particle size comparisons to biological and cellular endpoints, a red blood cell (erythrocyte) is approximately 7 micrometers (µm) in diameter or 7000 nm. The size of some bacteria are often measured in the range of 1 µm (or 1000 nm) and some viruses are known to be in the 60–100 nm size range. The terms “ultrafine” and “nano” have frequently been used interchangeably, with the latter being viewed as a more contemporary nomenclature. This has led to some confusion, as some investigators have referred to the term “ultrafine” to pertain to particles generated via combustion sources, while engineered nanoparticle-types are often intentionally manufactured to specific particle size ranges for particular applications.

Given the alterations that are encountered in physical and chemical properties as particle sizes are decreased within the nanoscale range (and therefore take on different properties versus the “larger” fine-sized particle-types), it is not unreasonable to assume that the biological effects associated with exposures to nanoparticles may also differ from their bulk counterparts. As a consequence, the assessments of potential health risks related to exposures to engineered nanomaterials is an emerging area in toxicology, exposure assessment and health risk evaluations. The development of toxicity data sets as well as methodologies for facilitating exposure assessments for various nanoparticle-types are emerging as new particles and materials are being developed.

Dating back to the mid 1990s, the few pulmonary toxicity studies that have been conducted with ultrafine particles in the rat model have demonstrated that lung exposures to ultrafine or nanoparticles produce greater adverse inflammatory and fibrotic responses when compared with larger-sized particles of similar or identical composition at equivalent doses/mass concentrations. Virtually all of these particle studies have been conducted using rats (a known sensitive species) and under (high dose) particle overload conditions. Contributing to these effects is the high size-specific deposition of nanoparticles when inhaled as monodispersed rather than aggregated particles. Some evidence suggests that inhaled ultrafine or nanoparticles which deposit in the lung will, to a greater degree, escape normal alveolar macrophage clearances efforts and gain access to other anatomical compartments of the respiratory system, including the pulmonary interstitium and the systemic vasculature. Results from the limited toxicological database have fostered the perception that all nanoparticles are likely to be more toxic than fine-sized particulates (Oberdorster, 2000; Donaldson, Stone, Clouter, Renwick, & MacNee, 2001).

This brief review is designed to summarize and identify some of the key factors and issues which are likely to influence environmental, health and safety risks related to exposures to nanomaterials. The paper is not designed to be a comprehensive treatise on nanotechnology, but will focus on some important and contemporary aspects associated with assessments of health effects. These include the following: 1) importance of particle characterization studies; 2) development of a Nano Risk Framework and corresponding base set hazard studies; 3) one example of mechanistic pulmonary toxicity bioassay studies with nanoquartz particles; 4) studies on the development of in vitro screening assays for pulmonary toxicity to particle-types; and 5) safe handling issues for nanomaterials in the laboratory.

2. Importance of conducting physicochemical characterization studies on nanoparticle-types

As discussed in the Introduction section, the development of a safety database for nanoscale particles is evolving as new particles, materials and exposure methodologies are being developed. Data from some pulmonary toxicity studies in rats demonstrate that exposures to ultrafine/nanoparticles (defined as <100 nm in one dimension) may produce enhanced toxicity when compared to fine-sized (bulk) particle-types of similar chemical composition (Donaldson et al., 2001; Oberdorster, 2000). Particle surface area and particle number determinations have been postulated to play significant roles in influencing the development of nanoparticle-related lung toxicity. The assumptions made from these studies were that the only differences (i.e., variables) between the ultrafine and fine-sized particle-types were the particle sizes; however, a closer analysis of these studies indicates that a number of other physicochemical characteristics including crystal structure, aggregation potential, and surface coatings were different in the various particle-types that were being compared. Moreover, findings of other recent studies with nanoquartz and ultrafine titanium dioxide particle-types demonstrate that the toxicity of some nanoparticle-types may be related, in large part, to the surface reactivity of the particles, in influencing the development of inflammatory and cytotoxic responses in the lung (Warheit, Webb, Colvin, Reed, & Sayes, 2007a; Warheit, Webb, Reed, Frielics, & Sayes, 2007b).

Surfaces and interfaces of particles are particularly important components of nanoscale materials. As the particle size is decreased, the proportion of atoms found at the surface is magnified relative to the proportion inside its volume. This results in nanoscale particle-types which are likely to become more reactive; thus generating more effective catalysts in a variety of applications. However, when considering the potential health implications, reactive groups on the surface or surface coatings which tend to passivate. As a consequence, modifications in surface chemistry forming the “shell” on a (core) nanoparticle-type may be important and relevant for health effects following exposures (see Fig. 1). Moreover, surface coatings can be utilized to alter surface properties of nanoparticles to prevent aggregation or agglomeration with different particle-types, and/or can serve to “passivate” the particle-type to mitigate the effects of ultraviolet radiation induced reactive oxidants. It is interesting to

![Fig. 1. Schematic of a TiO₂ particle (core) with surface treatments (shell) containing amorphous silica and alumina (as the outer coatings). (copied from Warheit et al. Toxicol Sci. 88:514–524, 2005).](image-url)
consider that surface coatings, functioning to reduce aggregation concomitant with facilitating particle dispersion, are likely to augment the efficacy of the particle-type in its designed application, but may also facilitate nanoparticle translocation from the respiratory tract to the systemic circulation and thereby significantly enhance nanoparticle distribution and exposures to sites throughout the body (i.e., potential double-edged sword?) (Oberdorster et al., 2005; Borm et al., 2006). To summarize this concept regarding the importance of nanoparticle core-shell dynamics for biological effects, it should be noted that from a toxicological perspective, two different nanoparticle core-shell dynamics for biological effects, it should be noted that from a toxicological perspective, two different nanoparticle types containing titanium dioxide as their “core” may not have the same or even similar hazard potentials. There can be differences in crystal structures (anatase vs. rutile), surface reactivity, aggregation status, particle size distribution, surface area as well as surface coatings – including passivation and neutralization. These differences in physicochemical particle characteristics despite a similar “core”, may result in comparative differences in the potencies of pulmonary inflammatory and cytotoxic endpoints, ranging from benign to more moderate health impacts (Warheit et al., 2008).

Many scientific organizations or task forces have strongly recommended that investigators conduct thorough characterizations of physicochemical properties of the nanoparticle-types that are being assessed for toxicity testing. However, too often this recommendation becomes an extensive laundry list of material characteristics that does not have adequate prioritization. As a consequence, in order to adequately describe the physical characteristics of the nanoparticle-type being evaluated, we have previously recommended that, at a minimum, experimentalists should characterize the following (prioritized) physicochemical properties prior to conducting hazard studies with nanoparticle-types:

- Particle size and size distribution (wet state) and surface area (dry state) in the relevant media being utilized – depending upon the route of exposure;
- Crystal structure/crystallinity;
- Aggregation status in the relevant media;
- Composition/surface coatings;
- Surface reactivity;
- Method of nanomaterial synthesis and/or preparation including post-synthetic modifications (e.g., neutralization of ultrafine TiO$_2$ particle-types);
- Purity of sample; (Warheit, 2008).

This recommended physicochemical characterization of nanoparticle-types should be implemented prior to the initiation of toxicological experimentation. The point cannot be overemphasized that in the absence of an adequate description of the physicochemical characteristics of the nanoparticle-type being studied (as well as the experimental conditions being employed), the results of toxicity experiments with nanoscale materials will have limited value or significance. Moreover, the findings of previously reported studies may not be comparable to past or future studies conducted with similar nanomaterial-types, because the material characteristics of the particle-types under investigation have not been specified.

3. Evaluating the risks associated with nanomaterial exposures: the nanorisk framework

A Nano Risk Framework (2007) has been developed and promulgated jointly by two organizations, namely Environmental Defense and the DuPont Company with the intent of facilitating a systematic process for recognizing environmental health and safety (EHS) risks associated with exposures to newly developed products containing engineered nanoscale materials. First and foremost, determination of health or environmental risks requires an understanding of both hazard and exposure assessments. Moreover, in many circumstances, the exposure assessment or potential cannot be quantified, due either to limitations of measurement (methodologies) in the environmental setting or because of technological limitations in measuring nanoparticle exposures in the workplace. Therefore, exposure assessments often have to be estimated based upon reasonable and informed considerations of the product lifecycle. In addition, the EHS framework could include a base set of hazard studies which would provide a reasonable and pragmatic assessment of the toxicity of the nanoparticle-type for human health and environmental considerations.

The Nano Risk Framework consists of six basic steps corresponding to stages of development and is an interactive process. This framework can be downloaded at the following website address: www.nanoriskframework.com. Briefly, the six steps are outlined below:

Table 1

| Protocol for nano and fine quartz bioassay study |

<table>
<thead>
<tr>
<th>Exposure Groups</th>
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<tr>
<td>PBS (control)</td>
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<tr>
<td>Nanoquartz Particles</td>
<td></td>
</tr>
<tr>
<td>Fine Quartz Particles</td>
<td></td>
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<tr>
<td>Carbonyl Iron Particles (negative control)</td>
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Fig. 2. Cytocentrifuge preparation of lung cells recovered by bronchoalveolar lavage 3 months after intratracheal installation exposure to carbonyl iron. Some phagocytic macrophages (dark staining cells) can be observed 3 months after exposure.

A Nano Risk Framework (2007) has been developed and promulgated jointly by two organizations, namely Environmental Defense and the DuPont Company with the intent of facilitating a systematic process for recognizing environmental health and safety (EHS) risks associated with exposures to newly developed products containing engineered nanoscale materials.
described below. Step 2C is designed to identify and characterize the potential for human or environmental exposures to the nanomaterial — including exposures related to the intended use of the product as well as by accidental releases during the lifecycle of the product. The lifecycle aspect of the framework strongly encourages the user to consider how the nanomaterial's physicochemical properties, hazards, and/or exposures may be altered during the material's lifecycle — e.g., following the normal lifespan of the product, possibly followed by disposal.

With regard to the implementation of the hazard component of the Framework with a new material, the toxicity results of a base set of hazard tests on a newly developed, well-characterized, ultrafine rutile TiO$_2$ (uf-TiO$_2$) particle-type have previously been reported (Warheit et al., 2008).

**Fig. 3.** (top) Pulmonary inflammation in particulate-exposed rats and controls as evidenced by % neutrophils (PMN) in BAL fluids at 24 h, 1 week, 1 month and 3 months postexposure (pe). Instillation exposures resulted in transient inflammatory responses carbonyl iron exposed groups at 24 h pe. However, exposures to various Quartz and Nanoquartz particle-types at 1 and 5 mg/kg produced a sustained lung inflammatory response. *p<0.05. (bottom) Cytocentrifuge preparation demonstrating pulmonary inflammation recovered from bronchoalveolar lavage (BAL) fluids in rats following exposures to Min-U-Sil quartz particles. The micrograph demonstrates the sustained inflammatory responses 3 months postexposure in the lungs of rats exposed to quartz particles.
et al., 2007c). These consisted of acute pulmonary, as well as oral toxicity studies, skin irritation and sensitization studies, ocular irritation studies, genotoxicity studies, and screening aquatic toxicity studies. Pulmonary instillation bioassay studies were implemented with a protocol similar to the nanoquartz studies described in the next section. The acute dermal irritation tests were conducted in rabbits according to OECD 404 guidelines. The local lymph node assay in mice was utilized to assess dermal sensitization (OECD 429 Guideline). The acute oral toxicity test was conducted with rats (OECD 425 guideline). In vitro mutagenicity assays were implemented using the Bacterial Reverse Mutation (Ames) test (OECD 471) and the in vitro mammalian chromosome aberration test in Chinese Hamster Ovary cells (OECD 473). In addition, acute aquatic toxicity assays were carried out using rainbow trout, daphnia, as well as with green algae.

The results of base set toxicity tests demonstrated that exposures to ultrafine titanium dioxide particles in the lungs of rats produced low inflammatory potential and lung tissue hazards. Results of acute oral toxicity studies in rats, dermal irritation studies in rabbits and skin sensitization assays in mice demonstrated that uf-TiO₂ had low oral toxicity, was not a skin irritant or dermal sensitizer. Genotoxicity tests demonstrated that uf-TiO₂ was negative in both the Bacterial Reverse Mutation Test and in an In Vitro Mammalian Chromosome Aberration Test in Chinese Hamster Ovary Cells. The results from aquatic toxicity screening assays demonstrated that uf-TiO₂ exhibited low concern for aquatic hazard using the water flea, Daphnia magna; exhibited low concern for aquatic hazard using the rainbow trout, Oncorhynchus mykiss; and exhibited medium concern for green algae Pseudokircheriella subcapitata. In summary, most of the tests demonstrated low hazard potential in mammals or aquatic species following acute exposures to ultrafine rutile TiO₂ particles (Warheit et al., 2007c). These findings provide confidence for EHS considerations in developing this new ultrafine TiO₂ product; and concomitant with occupational exposure measurements and lifecycle considerations, the Nano Risk Framework methodology provides reasonable assurances that the commercialization of this product has a very low health and environmental risk potential.

4. Pulmonary bioassay (toxicity) studies of fine and nanoscale α-quartz particle-types

In earlier studies, we had demonstrated that surface characteristics of nanoparticles, such as surface reactivity may be as or more important in influence pulmonary toxicity when compared to particle size (Warheit et al., 2007b). To test this hypothesis, three different forms of ultrafine or nano titanium dioxide particles were evaluated. Measurements of particle size ranges indicated that the particle-types had similar median particle size characteristics as measured in the wet phase— ranging from ~120–140 nm (with “tails” reaching below 100 nm). However, the three ultrafine TiO₂ particle-types demonstrated very different core and surface characteristics — including differences in surface reactivity, surface coatings, crystal structures, and pH values. These comparative alterations in particle surface characteristics correlated with significant hazard effects; despite the fact that all of the particle-types would be characterized as “ultrafine TiO₂ particles” — owing to their similar core compositions (Warheit et al., 2007b).

In subsequent studies, we tested the particle size hypothesis with a “cytotoxic” particle-type, namely quartz particles. It was postulated that if Min-U-Sil (primary particle size ~500 nm) quartz particles are highly cytotoxic, it seemed likely that nanoscale quartz particles (average particle sizes 12 and 50 nm, respectively) would be substantially more toxic to the lungs of exposed rats (Warheit et al., 2007a).

Accordingly, the aims of this study were to compare the pulmonary toxicities of cytotoxic quartz particle-types of various size ranges including 1) synthetic 50 nm nanoquartz I particles; 2) synthetic 12 nm nanoquartz II particles; 3) synthetic ~300 nm fine quartz particles; and to compare the pulmonary hazard effects to a known cytotoxic positive control particle-type — namely (mined) Min-U-Sil quartz particles (~500 nm); A second objective was to assess the physicochemical characteristics — including the particle surface reactivities among the samples (as evaluated by erythrocyte hemolysis studies) as they relate to toxicity. Accordingly, groups of rats were intratracheally instilled either with doses of 1 or 5 mg/kg of carbonyl iron particles (as a negative control) or with the various α quartz particle-types contained in phosphate buffered saline solutions. The pulmonary effects were evaluated using a dose response/time course experimental protocol. Pulmonary endpoints included bronchoalveolar lavage fluid biomarkers for inflammation and cytotoxicity, lung tissue cell proliferation indices, and lower respiratory tract histopathological

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**Table 2**

<table>
<thead>
<tr>
<th>Summary of selected α-quartz endpoints</th>
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<tr>
<td><strong>Particle size</strong></td>
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<tr>
<td>Cytotoxicity</td>
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<tr>
<td>Hemolytic potential</td>
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<tr>
<td>Lung inflammation</td>
</tr>
<tr>
<td>Particle size</td>
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<td>Surface area</td>
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**Fig. 4.** Cytocentrifuge preparation demonstrating pulmonary inflammation recovered from bronchoalveolar lavage (BAL) fluids in rats following exposures to nano-sized quartz particles at 24 h postexposure. The micrograph demonstrates the inflammatory responses in the lungs of rats exposed to nanoscale quartz particles. These pulmonary inflammatory responses become sustained and progressive.

**Fig. 5.** Lung tissue response in a rat exposed to carbonyl iron particles. The lung displays normal architecture 3 months after instillation of particles.
evaluation of lung tissue at 24 h, 1 week, 1 month and 3 months post intratracheal instillation exposures (see Table 1).

Intratracheal instillation exposures to the various fine and nano-sized α-quartz particle-types resulted in different degrees of pulmonary inflammation and cytotoxicity (See Figs. 2–4), and these findings were not always associated with particle size characteristics but correlated better with surface reactivity endpoints, as evidenced by red blood cell hemolytic potential (see Table 2). Although lung tissues in carbonyl iron-exposed (negative particle control) rats appeared normal (Fig. 5), histopathological observations of lung tissues in all of the quartz samples demonstrated common inflammatory-related pulmonary effects—dose-dependent lung inflammatory “foamy” macrophage accumulation responses along with the early development of lung tissue thickening and the initiation of corresponding pulmonary fibrosis (Fig. 6). The various α-quartz related pulmonary effects were very similar in form but with different degrees of potency. The range of particle-related lung toxicities and tissue effects in order of greatest hazard potency were: nanoscale quartz II<Min-U-Sil quartz<fine quartz<nanoscale quartz<carbonyl iron particles. The conclusions derived from this study demonstrated that pulmonary hazard effects following α-quartz particle exposures were not directly correlated (i.e., enhanced) with reduced particle size (the size range for the most potent quartz particle-types were 12 nm (Nanoquartz II) and 500 nm (Min-U-Sil), respectively compared to quartz particle sizes of 50 nm and 300 nm), but were more consistent with surface reactivity effects (Warheit et al., 2007a). The results measured in this intratracheal instillation model for Min-U-Sil quartz and carbonyl iron particles were similar to previously demonstrated studies using inhalation models in the rats and therefore, the nanoquartz results could be bridged to the Min-U-Sil quartz results (see Fig. 7).

4.1. Bridging Studies

Pulmonary bridging studies can serve to provide relevant and accurate screening hazard data when assessing the safety of compounds in commercial development or when making modifications to existing products, such as particle size alterations on particulates. The strength of the bridging strategy is dependent upon having good inhalation toxicity data for comparisons to data developed from instillation studies. The materials for which there are inhalation data can then be used as reference particle-types for comparisons to the pulmonary bioassay (i.e., intratracheal instillation route of exposure) results. Thus, in describing the basic bridging concept, the effects of the instilled materials serve as a reference (known) particle-type and then are “bridged” or benchmarked to the inhalation toxicity data for that particle-type, concomitant with the new materials being tested. The results of bridging studies in rats are then useful as preliminary pulmonary toxicity screening (i.e. hazard) data, because consistency in the response of the inhaled and/or instilled control material serves to validate the responses with the newly tested particle-type. In this case, the results from pulmonary bioassay instillation studies with Min-U-Sil were similar in nature to previously conducted short-term inhalation studies with Min-U-Sil quartz particles. Therefore the nanoquartz studies can be bridged to the inhalation and instillation pulmonary bioassay studies with Min-U-Sil quartz particles.

5. Attempts to substitute in vivo screening studies for in vivo pulmonary toxicity studies with fine and nanoscale particle-types

In earlier attempts by other investigators to develop in vitro models as predictive screens for pulmonary toxicity to particles, published reports have indicated very little correlation when comparing pulmonary toxicity effects following in vivo intratracheal instillation of particles relative to in vitro cell culture exposures. Recently, we assessed the capacity of in vitro screening studies to predict in vivo pulmonary toxicity of several fine or nano particle-types in rats. In the

Table 3

<table>
<thead>
<tr>
<th>Hazard/risk management tool for nanomaterials</th>
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<tr>
<td>• Describe established and reasonably anticipated activities by lifecycle stage; materials sourcing, manufacturing, distribution, use/reuse/maintenance, recycle/waste management.</td>
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<tr>
<td>• Identify and characterize the nanomaterial’s physical and chemical properties, including property changes, throughout the full lifecycle.</td>
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<tr>
<td>• Establish a hazards profile of the nanomaterial that characterizes the material’s potential health, environmental, and safety hazards over the entire lifecycle.</td>
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<tr>
<td>• Identify and characterize the potential for human and environmental exposures across the full lifecycle.</td>
</tr>
<tr>
<td>• For each lifecycle stage, appropriate to the phase of development analyze the properties, hazard and exposure profiles to understand the nature, likelihood and magnitude of adverse effects on human health and the environment.</td>
</tr>
<tr>
<td>• Determine how best to pursue practices, conduct process and safely produce, use and dispose or recycle the nanomaterial and/or product.</td>
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Fig. 6. Lung tissue response in a rat exposed to nanoscale quartz particles. Two common features of quartz-induced pulmonary response are apparent: 1) the development of foamy alveolar macrophages filling alveolar airspaces; and 2) the early tissue thickening response leading to the progressive development of pulmonary fibrosis.

Fig. 7. Schematic demonstrating the strategy for conducting pulmonary bioassay bridging studies. Bridging studies can have utility in providing an inexpensive preliminary safety screen when evaluating the hazards of new developmental compounds. The basic idea for the bridging concept is that the effects of the instilled materials serve as control (known) materials and then are “bridged” on the one hand to the inhalation toxicity data for that particle-type being studied.
in vivo portion of the study, rats were exposed by intratracheal instillation to two different doses of the following particle-types: carbonyl iron, crystalline silica, amorphous silica, nano zinc oxide, or fine zinc oxide. Following exposures, lung inflammation and cytotoxicity biomarkers were measured at 24 h, 1 week, 1 and 3 mno post-exposure. For the in vitro component of the study, three different culture conditions were employed, using the same test particle-types are discussed with the in vivo studies. Cultures of rat lung epithelial cells, primary alveolar macrophages from lavaged rats, as well as alveolar macrophage/L2 lung epithelial cell co-cultures were incubated with the same particle-types as identified above at 1 h, 4 h, 24 h, or 48 h. Similar to the in vivo investigations, the culture fluids were evaluated for cytotoxicity endpoints and inflammatory cytokines at several different time periods. Data from the in vivo pulmonary toxicity studies demonstrated that instilled carbonyl iron particles produced little toxicity. Crystalline quartz silica particle exposures produced sustained inflammation and cytotoxic effects. Exposures to amorphous silica particles produced transient inflammatory responses which were reversible at 1 week postexposure. Finally, intratracheal instillation of nano or fine-sized zinc oxide particles produced potent but transient inflammation/cytotoxic effects which were resolved by 1 month post-instillation exposure. Results of in vitro pulmonary cytotoxicity studies demonstrated a variety of inconsistent responses to the different particle-types. The potency of the in vitro responses to crystalline silica particles were dependent, in large part, upon different cell types, cell culture conditions, different time course incubation periods (i.e., 1, 4, 24 or 48 h); and cytotoxicity endpoints were significantly elevated only at particle overload concentrations. With regard to the LDH cytotoxicity biomarkers, L2 lung epithelial cells were the most sensitive cell-types (vs. macrophages or co-cultures) and exposures to nano or fine-sized zinc oxide for 4 or 24 h produced greater toxicity when compared to cells incubated with crystalline or amorphous silica particles. Macrophages were essentially resistant to these effects and epithelial/macrophage co-cultures generally reflected the results at 4 and 24 h incubation, but not at 48 h incubation. Based upon the results of these studies, it was concluded that in vitro cellular systems will need to be further developed, standardized and validated (relative to in vivo effects) in order to provide useful predictive screening data on the relative pulmonary toxicities of inhaled particles (see Sayes, Reed, & Warheit, 2007 for details).

6. Safe-handling of nanomaterials in the laboratory

Given the relative dearth of safety data on health risks related to exposures to nanoparticles, a prudent product stewardship tool/safe-handling methodology for nanotechnology would begin with the general concept that health risk is a function of both hazard assessment and exposure evaluations. Moreover, as a general principle, personnel should treat all new nanomaterials as potentially hazardous. Where there is scientific data available on the hazards of nanomaterials, these should be managed in a consistent manner associated with the defined risks. An effective risk management process will identify and characterize the nature, magnitude and probability of risks presented by the nanomaterial and its anticipated application. With regard to the hazard assessment tool, some of the initial questions that should be addressed are the following: 1) Are nanomaterials generated during the process and where are the potential exposures during the handling life cycle? 2) The nanomaterial should be well characterized, and the following questions addressed: e.g. solid or liquid state, how dusty is the material, what is the size distribution, is the material water soluble? 3) What is the presumed exposure? — the four major routes of occupational exposure are the respiratory tract (i.e., inhalation exposure), the skin, eyes, and the gastrointestinal tract (via oral or inhalation exposures). 4) Which route of exposure predominates? — this is a primary concern. A risk management assessment process evaluates the available options for managing the risks and recommends a course of action (see Table 3). Containment and control of potential exposures should follow prudent protection practices appropriate to the activity and consistent with the hierarchy of controls. Such measures should be taken to control exposures as low as is reasonably practical. The hierarchy of control measures include: Eliminate, Substitute, Enclose, Engineering control, Procedural control and Personal Protective Equipment (PPE). Selection of the controls will depend on the acceptable level of risk. Engineering controls are effective where airborne nanomaterials are generated and PPE, as a last option for control, such as High Efficiency Particulate Air (HEPA) filter respiratory protection and personal protective clothing can often prevent dermal and ocular exposures in many cases. In the absence of adequate toxicology data on the nanoparticle-type of interest, it is important to determine whether any hazard information is available on the micro or macroscale form of the chemical product (i.e., bulk form). This may provide some initial information regarding the potential toxicity of the nanoparticle-type, but must be followed up by relevant hazard studies with the nanomaterial. Adequate pulmonary, dermal, and oral toxicity tests/bioassays are available which can provide important hazard information on the nanoparticle-type of interest. Exposure assessment determinations are also important components for determining health risks. However, current methodologies were not designed for quantifying nanoparticulate exposures, and thus, may not be sufficiently sensitive, and should be validated. This is because most of the exposure data is in the form of mass/volume of air, i.e., mg/m³. However, to investigate exposures in terms of mass, it might require hours or days of sampling nanoparticles in the workplace — in order to acquire sufficient mass for making assessments. Therefore, the dose metrics for exposure measurements of nanoparticles is likely to change to particle number (# particles/cc) or surface area metrics (m²/g).

Similar to the examples discussed with health-based studies, current information on the environmental fate of nanoparticles is very limited. In future studies it will be important to determine the routes through which engineered nanoscale particles enter the environment, the modes of dispersion in the environment, assessing whether the nanomaterials are persistent and bioaccumulative, and/or undergo transformation in the environment. An estimate of nanoparticulate exposure potential in the environment will require information about releases, emissions, transport, distribution and transformation. The reader is directed to the following publications for additional guidance on the safe-handling of nanomaterials (Schulte, Geraci, Zumwalde, Hoover, & Kuempel, 2008; ASTM Subcommittee E56.03, 2007; NIOSH Approaches to Safe Nanotechnology, 2007; British Standards Institute. Nanotechnologies, 2007).

7. Summary

In conclusion, this brief review has been developed to identify some of the important issues which influence the assessment of environmental health and safety risks related to exposures to nanomaterials. We have focused on the important issue of particle characterization studies, which are critical prerequisites for studying health and environment impacts of nanomaterials. In addition, a thoughtful risk management model framework for identifying the potential risks related to exposures to nanomaterials has been developed. Health and environmental risks are products of both hazards and exposures. Too often hazard data from a toxicity study are confused with the concept of health risk. It should be noted that exposure is an integral part of this equation. Risk management should be an integral part of an occupational safety and health program, which is based on recognition of the nanomaterial hazards, evaluation of the exposure potentials, and application of control measures to reduce the risk. Finally, examples of mechanistic pulmonary toxicity studies with nanoparticles are described. Studies are ongoing to
develop in vitro models for predicting pulmonary effects to nanomaterials, although further development and validation are required.

References


