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REVIEW

Nanotechnology Safety Concerns Revisited

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Nanotechnology is an emerging science involving manipulation of matter at the nanometer scale. Due to concerns over nanomaterial risks, there has been a dramatic increase in focused safety research. The present review provides a summary of these published findings, identifying areas of agreement and discordance with regard to: (1) the potential for nanomaterial exposure, (2) the relative hazard nanomaterials pose to humans and the environment, and (3) the present deficits in our understanding of risk. Special attention is paid to study design and methodologies, offering valuable insight into the complexities encountered with nanomaterial safety assessment. Recent data highlight the impact of surface characteristics on nanomaterial biocompatibility and point to the inadequacy of the current size-dependent mechanistic paradigms, with nanoscale materials lacking unique or characteristic toxicity profiles. The available data support the ability of the lung, gastrointestinal tract, and skin to act as a significant barrier to the systemic exposure of many nanomaterials. Furthermore, the acute systemic toxicity of many nanomaterials appear to be low. By contrast, the potential pulmonary toxicity of certain nanomaterials, such as carbon nanotubes, is significant, requiring a better understanding of exposure to further evaluate their risk. While these findings arrive at an overall picture of material-specific rather than nanogeneralized risk, any conclusions should clearly be tempered by the fact that nanomaterial safety data are limited. Until such time as the exposures, hazards, and environmental life cycle of nanomaterials have been more clearly defined, cautious development and implementation of nanotechnology is the most prudent course.

Key Words: **nanotechnology; nanomaterials; nanoparticles; ultrafines; particle toxicology; safety evaluation.**

Nanotechnology is an emerging science involving manipulation of matter at the nanometer scale. The recent rise in the interest surrounding nanotechnology stems from its potential to revolutionize such diverse fields as engineering and medicine; for example, carbon nanotubes (CNTs) are being used in low-weight, high-strength building materials (Miyagawa *et al*., 2005), while targeted nanoparticle drug formulations have been shown to greatly improve the toxicity profile of anticancer therapeutics in animal models (Kukowska-Latallo *et al*., 2005). Along with the excitement over the prospects of nanotechnology, there have been increasing concerns regarding the risks this science may pose. As an example, a recent story in the international news described respiratory distress associated with the use of the German bathroom cleaner called Magic Nano (Weiss, 2006). The story was billed by some groups as an example of how nanotechnology is dangerous, and safeguards to protect the public and the environment from this emerging technology are not in place. Subsequent reports uncovered the fact that Magic Nano did not contain any nanoscale components, and the respiratory ailments associated with its use were likely due to the ethanol–water aerosol released when the cleaner was sprayed (Wolinsky, 2006). The Magic Nano story is a clear example of the need for accurate reporting and vetting of facts when it comes to nanotechnology. Most importantly, there is also a need for good science and public dissemination of findings to counter the negative perceptions that all too easily could result in the demise of this promising technology that is still in its infancy. The goal of this review is to objectively examine the current knowledgebase regarding the health risks posed by engineered nanoparticles, in order to put nanotechnology safety concerns in perspective.

Nanoparticles, the building blocks of nanotechnology, have been broadly defined as having at least one dimension at 100 nm or less. For biomedical application, this definition has been expanded to include particles greater than 100 nm, such as liposomes, in order to encompass particle sizes that take advantage of anatomical considerations, such as vascular gaps surrounding tumors (Garnett and Kallinteri, 2006). For the purposes of this review, nanoparticles can be categorized as either engineered or incidental depending on origin (Fig. 1). Engineered nanoparticles, such as the quantum dots and dendrimers (Fig. 1), are particles generated to exploit the

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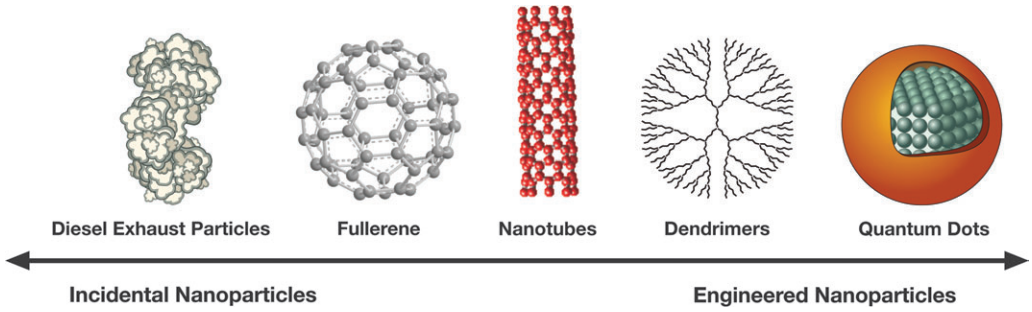

FIG. 1. Examples of incidental and engineered nanoparticles.

size-related properties inherent in the nanoscale (e.g., conductivity, spectral properties, biodistribution). Currently, there are many consumer products on the market that claim to have an engineered nanotechnology component. These products range from light-weight tennis rackets to stain-resistant clothing (www.nanotechproject.org/consumerproducts). Alternatively, incidental nanoparticles, such as diesel exhaust particles (Fig. 1), are defined as particles either from unintended anthropogenic sources (e.g., combustion derived) or of natural origin (e.g., particles generated in forest fires). Some particles, such as fullerenes and CNTs (Fig. 1), are both engineered materials and incidental components of air pollution.

The interaction of nanoparticles with humans and the environment is not a recent event. It is estimated that the average person consumes 10^{12} submicron-sized particles per day in a normal diet as a result of food additives consisting primarily of titanium dioxide (TiO_2) and aluminosilicates (Lomer *et al*., 2002). Incidental nanoparticles are also found in such common sources as wood smoke, and automobile and furnace exhaust (Barregard *et al*., 2006; Chang *et al*., 2004; Fang *et al*., 2005). Levels of incidental nanoparticles in the outdoor environment near heavy traffic areas can range from 5000 to 3,000,000 particles/cm^3! (Utell and Frampton, 2000). Correspondingly, the study of the hazardous effects of these particles, particulate toxicology, has been underway for many decades. Particle toxicology encompasses the study of various types of incidental nanoparticles, termed ultrafine particles, as well as toxic nanoscale mineral fibers (such as asbestos). Research into the health effects of these incidental nanoparticles has consisted of epidemiological studies and experimental studies in animal models. The mechanistic paradigms of particle toxicology have been used as the foundation for the emerging field of nanoparticle toxicology, coined "nanotoxicology" (Donaldson *et al*., 2004).

NANOPARTICLE RISK

Fundamentally, risk assessment involves an estimation of the potential for exposure and characterization of hazard. Potential routes of nanoparticle exposure include inhalation, dermal, oral, and in the case of biomedical applications, parenteral (Fig. 2). Toxicity resulting from nanoparticle exposure could occur at the various portals of entry, such as the lungs and skin,

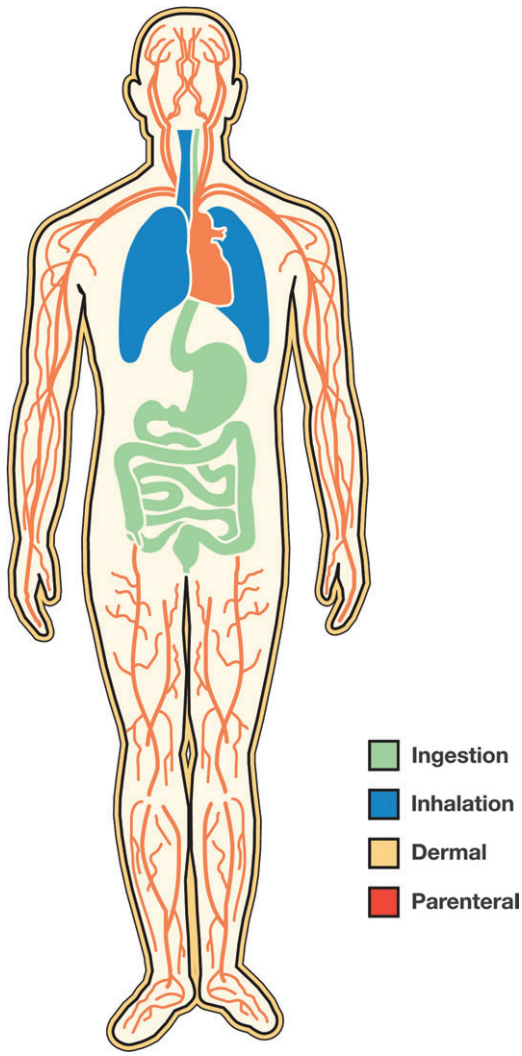

FIG. 2. Potential routes of nanoparticle exposure.

or at distant sites. For prediction of systemic toxicity following nonparenteral exposures, systemic dose is another important parameter to consider. The systemic dose is dependent upon both the barrier function and clearance mechanisms at the portals of entry. Studies addressing the systemic translocation of nanoparticles from sites of deposition are beginning to unravel the dynamics of nanoparticle–organism interaction, and provide the means to relate exposure and hazard data (Elder and Oberdorster, 2006).

NANOPARTICLE EXPOSURE

Exposure to nanomaterials could occur during their development, manufacture, use, or following disposal. Presently, nanomaterial exposure studies are severely lacking. Considering nanomaterial-containing consumer products are presently on the market, this absence of exposure data represents a significant research need (Thomas *et al*., 2006). Though the nanoscale components of these nanomaterial-containing consumer products are often bound to the material, and not in a free form, there is still potential for release of these materials during their wear. The following discussion of nanomaterial exposure highlights the limited exposure studies that have been conducted, as well as studies on the ability of the external surfaces of the body to limit systemic exposure and mechanisms of nanomaterial translocation.

Inhalation Exposure

Inhalation is thought to be an important route of nanoparticle exposure, since nanoparticles can travel great distances in air by Brownian diffusion and are respirable, depositing within the alveolar regions of the lung (Bailey, 1994). In the case of engineered nanoparticles, very few airborne exposure studies have been conducted. Often a confounding factor in airborne nanoparticle exposure studies has been the high level of background incidental nanoparticles. For example, an occupational exposure study in an engine machining plant designed to measure the levels of airborne metalworking fluid mist, found that background incidental nanoparticles from direct-fire, natural gas furnace exhaust often exceeded those produced by the machining processes (Peters *et al*., 2006b). In studies that were able to account for background incidental nanoparticules, airborne nanoparticle exposures have been found to be quite low. A study by Maynard *et al.* (2004), using an enclosed system to eliminate background, found the airborne mass concentration of CNT in a simulated production environment to be below 53 $\mu g/m^3$. The majority of the particulates in the 53 $\mu g/m^3$ estimation were CNT agglomerates greater than 1 μm in diameter, with many of these particulates not of respirable size. To put this level of CNT exposure in perspective, the level of background incidental particles resulting from entering and leaving the monitoring enclosure was as high as 500 $\mu g/m^3$. Low nanoparticle exposures have also been observed in a carbon black production facility (Kuhlbusch *et al*., 2004). Airborne concentrations of nanoscale carbon black were undetectable during production hours, while microscale carbon black particles were observed. Nanoscale particulates that were present originated from forklift and gas heater emissions. These low-nanoscale CNT and carbon black exposures in a "worst-case" occupational setting are thought to be due to the tendency for airborne nanoparticles to agglomerate and dissipate as a result of sedimentation from the air (Kuhlbusch *et al*., 2004; Maynard *et al*., 2004).

The tendency for airborne nanoparticles to form larger particles, as a result of either particle agglomeration or condensation of vapors onto existing particles, has been noted for combustion-generated incidental nanoparticles from furnace and vehicle sources (Chang *et al*., 2004; Sioutas *et al*., 2005). Free-particle half-life has been shown to directly correlate with particle size and inversely correlate with particle concentration (Preining, 1998). This short airborne lifetime of free, non-agglomerated nanoparticles limits their inhalation exposure, since the agglomerated nanoparticle would be expected to deposit in the lungs as a larger particle in the upper airways, and with increasing size, agglomerated nanoparticles have an increased tendency to settle from the air. While the settling of the resulting larger nanoparticle agglomerates diminishes inhalation exposures, it may increase the potential for dermal exposure, and hand-to-mouth or object-to-mouth oral exposure, as discussed below. This natural tendency of nanoparticles in both air and liquid to agglomerate would be expected to limit dermal, oral, and inhalation exposure to free nanoscale particles. Nonetheless, regardless of this aggregation tendency, the various portals of entry would still be expected to encounter nanostructured aggregated particles of high surface areas, and these aggregated particles could still undergo disaggregation at these sites or within subcellular compartments. Additionally, surface coatings on engineered nanoparticles that limit particle–particle interaction and protein binding, such as the polyethylene glycol–coated surfaces of nanoparticles intended for biomedical application, would be expected to diminish this aggregation tendency.

Systemic Translocation from Lung

Clearance mechanisms in the lungs include the mucocilliary escalator and phagocytosis by alveolar macrophages. Several studies in rodents have also demonstrated that nanoparticles deposited in the lungs can translocate to the pulmonary interstitium (Oberdörster *et al*., 1992; Oberdörster, 2000). In these studies it is important to take dose into account, as the fraction of the dose interstitialized has been shown to increase with increasing dose (Oberdörster *et al*., 1992). Thus, the interstitialization of nanoparticles observed at high doses may not be relevant to normal exposure conditions. However, it is expected that, compared with rodents, humans would tend to interstitialize a greater proportion of an inhaled nanoparticle

dose in an equivalent exposure scenario (Nikula *et al*., 1997). This movement of particles from the lung toward the circulation and secondary organs is of concern, since studies support a direct role for inhaled nanoparticles in systemic disease, such as cardiovascular disease. For example, CNTs have been shown to induce platelet aggregation *in vitro* and enhance thrombosis *in vivo* (Radomski *et al*., 2005).

The results of studies examining the translocation of nanoparticles from the lungs to secondary organs are mixed, with some studies demonstrating translocation and others not. For instance, Kreyling *et al*. (2002) demonstrated that < 1% of the administered dose of 80- and 15 nm iridium particles translocated from the lungs of rats to liver, spleen, heart, and brain, and the extent of this translocation was greater for the smaller-sized particles. This study utilized tracheal instillation and was able to demonstrate that gastrointestinal (GI) absorption was not a likely route of the systemic exposure. A study examining the translocation of inhaled ^{13}C nanoparticles (20–29 nm) in rats also observed translocation and hepatic distribution (Oberdörster *et al*., 2002). However, in this study, it was difficult to control for the possible ingestion and GI absorption of nanoparticles, and the authors concluded that a significant portion of the observed systemically distributed nanoparticles was likely accounted for by this phenomenon. In contrast to findings supporting the systemic absorption of nanoparticles deposited in the respiratory tract, a study examining the translocation of technetium-99m–labeled carbon nanoparticles (60 nm) in humans failed to demonstrate distribution to secondary organs (Brown *et al*., 2002). Overall, the available data suggest that the respiratory tract represents a formidable barrier to the systemic exposure of some nanoparticles.

Neuronal Translocation

The ability of inhaled nanoparticles to undergo neuronal translocation from the nasal epithelium to the olfactory bulb is supported by several studies in rats (Elder *et al*., 2006; Oberdörster *et al*., 2004). Rat inhalation studies using 30 nm manganese oxide (MnO_2) or 35 nm ^{13}C-carbon particles, found elevated levels of Mn or ^{13}C, in the olfactory bulb (Elder *et al*., 2006; Oberdörster *et al*., 2004). The fact that these cited studies measured the molecular constituents of the nanoparticles and not the nanoparticles themselves leaves open the possibility that distribution of the disassociated particles could have occurred instead of neuronal translocation of the intact nanoparticle. While these studies did attempt to account for the possibility of particle disassociation, studies examining the neuronal translocation of micron-sized MnO_2 particles (Fechter *et al*., 2002) far larger than the narrow (< 200 nm) diameter of the rat olfactory neurons (Plattig, 1989) suggest that a disassociation mechanism is also possible and requires consideration. A rat inhalation study utilizing 1.3-μm MnO_2 particles found a significant increase in cortical Mn levels and a trend toward increased olfactory bulb Mn levels, which failed to reach significance (Fechter *et al*., 2002). Though the increase in olfactory bulb Mn levels found in this study was low and highly variable, and could conceivably have resulted from neuronal translocation of a nanoscale fraction within the larger, micron-sized population, there remains the possibility that particle disassociation was involved. Indeed, in the nanoscale MnO_2 study of Elder *et al*. (2006) discussed above, Mn levels were also elevated in deeper regions of the central nervous system (CNS), including the frontal cortex, striatum, and cerebellum, and this increase was at least partially attributed to circulatory transport of ionic Mn.

Due to physiological and anatomical considerations, the nasal sensory nerve uptake of nanoparticles proposed in the rat model may not be predictive of human exposures: rats are obligatory nose breathers; 50% of their nasal mucosa is olfactory (vs. 5% in humans); and the weight of their olfactory bulb is approximately 177-fold greater than that of humans, when normalized to body weight (85 ng/0.2 kg rats vs. 168 ng/70 kg human) (Oberdörster *et al*., 2005). In support of the relevance of the neuronal translocation pathway in man, non-human primate studies have also demonstrated olfactory bulb uptake of intranasally instilled 50-nm gold nanoparticles (de Lorenzo, 1970). Furthermore, the ability of nanosized particles to migrate via neuronal translocation in humans is supported by the known movements of viruses within nerves (Snyder *et al*., 2006). Importantly, neuronal translocation via nasal sensory neurons is a potential route of nanoparticle CNS exposure in humans and, considering the predicted high deposition of nanosized particles in the nasopharyngeal region of the human respiratory tract (Bailey, 1994), it is reasonable to be concerned about the possible adverse effects that might arise from such exposure. Indeed, a recent *in vitro* study supports the ability of manganese nanoparticles to induce adverse effects in neuronal cells, including loss of cell viability, induction of oxidative stress, and dopamine depletion (Hussain *et al*., 2006). Although CNS uptake of inhaled nanoparticles has yet to be proved in humans, the neuronal and circulatory translocation of inhaled incidental nanoparticles has been postulated to be involved in the etiology of neurodegenerative CNS diseases associated with airborne pollutants (Peters *et al*., 2006a). Further research is required to provide direct evidence that the neuronal translocation mechanism of CNS uptake is operational in humans under normal nanoparticle exposure conditions, and that such translocation results in deleterious effects.

Dermal Exposure

The interaction of nanoparticles with skin has received significant attention recently because of the increasing use of nanoscale particles in stain-resistant clothing, cosmetics, and sunscreens. The dermal route of exposure is also important because of the tendency of agglomerated airborne nanoparticles to settle on surfaces and the difficulties in preventing dermal contact with these settled particles. Several studies have been

conducted examining the ability of nanoscale TiO_2, used as a ultraviolet (UV)–absorbing component in sunscreens, to penetrate the epidermis in human volunteers, and animal and *in vitro* models (Gamer *et al*., 2006; Lademann *et al*., 1999; Mavon *et al*., 2007; Pflucker *et al*., 2001; Schulz *et al*., 2002). The primary TiO_2 particles used in these studies ranged in size from 10 to 60 nm, with larger sized aggregates present. In all of these studies, nanoparticles did not appear to penetrate past the stratum corneum of the epidermis, though in some cases, accumulation in the hair follicles was observed (for a recent review see Nohynek *et al*., 2007). Studies have found 20 nm, negatively charged polystyrene spheres to behave similarly to nanoscale TiO_2 with regard to their follicular localization and lack of stratum corneum penetration (Alvarez-Roman *et al*., 2004).

In contrast to the studies of nanoscale TiO_2 and polystyrene, a study examining the dermal penetration of quantum dots has shown limited dermal penetration (Ryman-Rasmussen *et al*., 2006). This study by Ryman-Rasmussen *et al*. (2006) utilized scanning florescent confocal microscopy to examine the influence of size, shape, and surface charge on quantum dot penetration in an *in vitro*, flow-through diffusion porcine skin model. A small fraction of the applied dose for some quantum dot species was shown to penetrate the stratum corneum, with an even smaller fraction escaping the epidermis and accumulating within the dermis. This penetration was dependent upon size, shape, and surface charge of the quantum dots, with smaller, spherical particles demonstrating greater penetration than larger, ellipsoidal particles. None of the quantum dot species were shown to pass through the entire skin thickness to the opposing perfusate side of the diffusion cell. This limited dermal penetration has also been shown recently for < 10-nm maghemite and iron nanoparticles using an *in vitro* human skin model (Baroli *et al*., 2007), suggesting that "smaller" nanoparticles may have this ability. Though studies have yet to examine the effect of skin condition on nanoparticle absorption, damaged skin would be expected to provide less of a barrier, and this issue may be important in certain situations, such as the application of nanoparticle-containing sunscreens to sun-damaged skin. In support of this idea, the flexing of skin *in vitro* has been shown to increase dermal penetration of micron-sized dextran particles and derivatized fullerenes (Rouse *et al*., 2007; Tinkle *et al*., 2003). Together, the available data suggest that healthy, intact skin is a significant barrier to certain nanomaterials.

GI Exposure

Recent studies have addressed the issue of GI absorption of nanoparticles following oral exposure. Like dermal exposure, oral exposure could be a significant occupational and environmental route, resulting from ingestion of contaminated food and water, the swallowing of inhaled particles, or hand-to-mouth transfer of particles. Alternatively, nanoparticle drug formulations may be used to increase the oral bioavailability of poorly soluble or labile drugs, or target GI lymphatic tissues (Florence and Hussain, 2001). While many nanoparticles have been shown to undergo limited GI absorption, mainly to lymphatics, most studies have demonstrated low systemic exposure following oral administration. For example, studies of the oral absorption of ^{14}C-radiolabeled fullerenes and ^{192}Ir nanoparticles in rats observed minimal systemic absorption (Kreyling *et al*., 2002; Yamago *et al*., 1995). The available data suggest that the absorption of nanoparticles from the GI tract is governed by both the size (Hillyer and Albrecht, 2001) and surface characteristics (Jani *et al*., 1989) of the particle, with the smaller, hydrophobic, neutral particles having increased absorption (Hussain *et al*., 2001). Absorption of ^{125}I-radiolabeled polystyrene nanoparticles in rats, for example, was found to be size dependent (50 nm > 100 nm > 300–3000 nm) and was mainly confined to the Peyer's patches of the gut associated lymphoid tissue (Jani *et al*., 1990), which appears to be the predominant absorption pathway for nanoparticles from the gut.

NANOPARTICLE HAZARD

Data regarding the hazard of nanomaterials have come mainly from epidemiological studies of pollution-derived incidental nanoparticles and limited experimental studies of both incidental and engineered nanomaterials, using both animal models and *in vitro* systems. Epidemiological studies support an association between particulate air pollutants and pulmonary, cardiovascular, and CNS disease (Calderon-Garciduenas *et al*., 2002; Delfino *et al*., 2005; Penttinen *et al*., 2001; Peters *et al*., 1997, 2006b). However, it is worth noting that in many epidemiological studies, the nanoparticle component of the particulate pollution was never specifically measured, and it was not possible to separate nanoparticle-related effects from the effects of larger particulates that were omnipresent. The nanoscale component of particulate air pollution has been implicated in the generation of these adverse effects, since nanoscale particles make up the greatest particle number concentrations and surface area of particulate air pollution, and nanoscale particles have greater lung deposition and potential for systemic translocation. The size fractions of particulate air pollution have also been shown to have different inflammatory, oxidative, and cytotoxic potencies in experimental systems (Jalava *et al*., 2007; Li *et al*., 2003).

Hazard Assessment

The properties that make nanoparticles unique and drive the current interest in their industrial and biomedical application are the same properties that raise safety concerns. Nanoparticles have increased surface area for reactivity, and the potential for unique biodistribution governed by size (e.g., lung deposition) or protein interaction (e.g., opsonization). Nanoparticle properties (Fig. 3) that must be taken into account when assessing hazard include size, shape, agglomeration state,

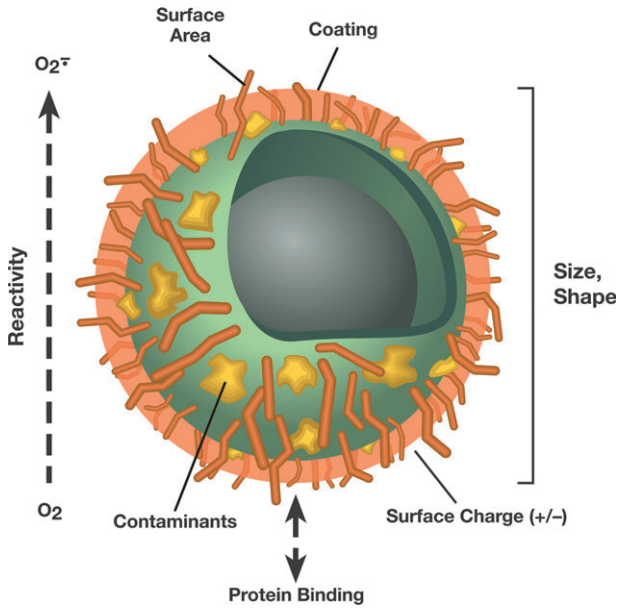

FIG. 3. Physicochemical properties of nanoparticles that may influence biocompatibility.

solubility, and surface properties (e.g., surface area, surface charge) (Patri *et al*., 2007). It is fundamental to properly characterize these physicochemical properties, so that variables affecting biocompatibility can be identified and comparisons to other hazard studies can be made.

Due to their unique properties (e.g., large surface area for absorption of reagents, spectral properties, catalytic activity), nanoparticles also have the potential to interfere with hazard assessment assays. This interference can confound interpretation of toxicological studies, leading to "false-positive" or "false-negative" results. CNTs, for example, have been shown to interfere with the MTT cytotoxicity assay (Worle-Knirsch *et al*., 2006) by absorbing the reduced formazan dye, resulting in an overestimation of cytotoxic potency. While it is likely that the majority of hazard assessment methods will be suitable for most nanomaterials, it is essential that assays first be validated. The use of multiple independent measures of hazard in order to confirm consensus behavior is also helpful.

When evaluating nanoparticle toxicology studies, it is important to note any experimental conditions that can influence interpretation of the data. Since nanoparticles have a tendency to aggregate, dispersive agents, such as surfactants, are often used to maintain the nanoscale or increase solubility. Studies that use dispersive agents may not be relevant to normal exposure conditions, since these dispersive agents, themselves, can have biological activity. Recently, an often-quoted study relating to the ability of fullerenes to induce oxidative stress in the brain of fish (Oberdörster, 2004) was called into question because of the possibility that residual tetrahydrofuran (THF) used to solubilize the nanoparticle resulted in the observed toxic mechanism (Andrievsky *et al*., 2005). Follow-up studies demonstrated that the majority of the oxidative stress could be attributed to residual THF (Zhu *et al*., 2006). Other experimental conditions that can affect assay outcome are light exposure, as some nanomaterials are photoreactive (e.g., fullerenes; Rancan *et al*., 2002), and serum proteins in cell culture media that can modulate agglomeration state (Tirado-Miranda *et al*., 2003).

In many cases, the toxicities associated with nanoparticles have been attributed to contaminants that adsorb to the particle surface during their creation or transport, and not the nanomaterial itself. For instance, transition metal and redox active organic contaminants are thought to be responsible for the oxidative stress generated by particulate air pollutants. One study found oxidative stress generated in macrophages treated with nanoscale pollution particulates correlated with the organic contaminant content (Li *et al*., 2003), and a subsequent study concluded that quinone and polyaromatic hydrocarbon contaminants were responsible for the mitochondrial dysfunction observed in macrophages treated with incidental diesel exhaust nanoparticles (Xia *et al*., 2004). Since engineered nanomaterials are formed by controlled production processes, toxic contaminants, if present, could be removed from the final product. For example, nonpurified, iron-rich CNTs have been shown to be more potent in inducing oxidative stress in macrophages than purified CNTs with reduced iron content (Kagan *et al*., 2006). Alternatively, coatings can be applied to engineered nanoparticles to make them more biocompatible. Surface coatings have been shown to dramatically influence the toxicity of nanoparticles. For instance, studies by Derfus *et al.* (2004) demonstrated that ZnS "capping" of CdSe quantum dots results in decreased toxicity to rat primary hepatocytes. Another study examining the effect of surface characteristics on quantum dot toxicity in human epidermal keratinocytes, found neutral, polyethylene glycol-coated quantum dots to be nontoxic, while amine surfaces were cytotoxic, and carboxylic surfaces were both cytotoxic and inflammatory (Ryman-Rasmussen *et al*., 2007).

Mechanistic Paradigms

Inflammation and oxidative stress have been identified as possible mechanisms underlying the etiology of nanoparticle-associated toxicities (Dick *et al*., 2003; Donaldson and Stone, 2003; Xiao *et al*., 2003). Nevertheless, it cannot be said that the ability to induce inflammation and oxidative stress are typical properties of nanomaterials in general. For instance, a screen of nanomaterials in a murine macrophage cell line (RAW 264.7) concluded that nanoscale TiO_2, carbon black, and carboxylated polystyrene did not induce oxidative stress, while cationic polystyrene and incidental pollution-derived nanoparticles did (Xia *et al*., 2006). Upregulation of inflammatory biomarkers was observed only in cells treated with the pollution-derived nanoparticles, and not with the cationic polystyrene particles. While the current inflammation and oxidative stress paradigms

provide a starting point for toxicological investigation of nanomaterials, it is still an attempt to generalize the mechanistic toxicology of an ever-increasingly diverse group of materials. The toxicity of cationic dendrimers, for example, appears to be related not to oxidative stress generation, but to disruption of cell membrane integrity through interaction of the positively charged dendrimer terminal groups and the anionic lipids composing the cell membrane (Mecke *et al*., 2004). Likewise, the toxicity of cationic liposomes and polymers, under investigation as nonviral gene vectors, appears to be dependent upon charge considerations (Lv *et al*., 2006).

Pulmonary Toxicity of Nanoparticles

In several animal studies, shifts in the pulmonary toxicity dose–response relationships for nano- versus micron-sized particles have been observed. For example, a 3-month inhalation study found nanoscale TiO_2 to be more inflammatory than submicron-sized TiO_2 (Oberdörster *et al*., 1994). When normalized to surface area, the dose–response curves for the nano- and submicron-sized TiO_2 particles in the 3-month study were similar, suggesting that the pulmonary inflammation was mediated by surface effects (Oberdörster *et al*., 1994). The increased inflammogenic effect of nano- compared with submicron-sized particles has also been noted for carbon black particles instilled in rats, and, like TiO_2, the inflammation correlated with the surface area dosimetric (Donaldson *et al*., 2002). These shifts in dose–response could potentially require adjustment in threshold limit values for some nanoscale material.

As a rule, nanoscale particles are not necessarily more toxic than larger particles of the same material, as several studies contradict this trend. A study by Warheit *et al*. (2006b) demonstrated that intratracheal instillation of rats with 1 or 5 mg/kg of 300-nm pigment-grade TiO_2 particles, 200 nm $\times$ 35 nm TiO_2 rods, or 10-nm TiO_2 dots all resulted in equivalent pulmonary inflammation and tissue injury at 24-h postexposure. It is important to note that the pigment-grade particles used in the study were of rutile-type crystalline structure, while the rods and dots were anatase, so the crystalline nature of the material was not identical. The lack of an effect of TiO_2 particle size on toxicity has also been observed in cell culture studies; cytotoxic and inflammatory responses of human dermal fibroblasts and lung epithelial cells to TiO_2 nanoparticles correlated with the ability to generate reactive oxygen species (ROS) and were independent of particle size and surface area (Sayes *et al*., 2006).

Recent studies suggest that surface properties, as opposed to size or surface area, are a more important determinant of particle biocompatibility (Warheit et al., 2006a, 2007). A study by Warheit *et al*. (2006a) examined the inflammatory potency of four different quartz particles in rats, ranging in size from 12 to 500 nm. Pulmonary inflammation in this study was determined by inflammatory cell and enzyme profiles in bronchoalveolar lavage fluid, the degree of lung cell proliferation, lung weight, and lung histopathology. Nanoparticle surface reactivity was measured by hemolytic potential and electron spin resonance. Inflammatory potency was best correlated not with particle size or surface area, but with surface reactivity as measured by hemolytic potential. In this study, the smallest, 12 nm, quartz particle was less inflammogenic on a mass basis than the largest, 500-nm particle. A previous study examining the inflammatory potency of micron-sized quartz particles in rats also found hemolytic potential to correlate well with *in vivo* inflammation (Clouter *et al*., 2001).

The majority of animal studies assessing nanoparticle pulmonary toxicity to date have utilized the rat and intratracheal instillation, which may not be the most predictive model for human toxicity. Rats have been shown to more sensitive to nanoparticle-induced pulmonary sequela than other animal models (Bermudez *et al*., 2004), and intratracheal instillation is nonphysiological, delivering nonrespirable aggregates and bypassing nasal deposition. Additionally, the doses utilized in many nanoparticle pulmonary toxicity studies were so high as to result in a condition termed "lung overload". Lung overload results when pulmonary clearance mechanisms are overwhelmed, resulting in increased deposited alveolar lung burdens (ILSI, 2000). In rats, this condition can initiate a cycle of lung inflammation and fibrosis, culminating in tumor generation that appears to be species specific: it has not been observed in mice and hamsters, and most likely is not relevant to humans. For example, while chronic inhalation studies with microscale TiO_2 particles in rats have found treatment-related increases in lung tumors (Lee *et al*., 1985), epidemiological studies addressing mortality risks in TiO_2 manufacturing workers have found no increase in risk of lung cancer or any other cause of mortality (Boffetta *et al*., 2004). This discrepancy has been attributed to lung overload conditions in the animal studies.

There are several reasons to believe that nanoparticles may cause lung overload at lower administered doses: nanoparticles have increased alveolar deposition relative to larger particles, and, once deposited, nanoparticles may not be cleared as effectively as larger particles, since particle surface area has been shown to correlate with diminished alveolar macrophage clearance capacity (Moss and Wong, 2006). In support of this concept, nanoscale TiO_2 particles have been shown to cause tumors in rats at much lower exposure concentrations than micron-sized TiO_2 particles (Heinrich *et al*., 1995; Lee *et al*., 1985). This size-dependence of TiO_2 particle tumorigenicity was most likely due to increased nanoparticle lung burden, as these rat tumors were associated with particle lung overload (Lee *et al*., 1985). This is an example of how adverse effects attributed to the nanoscale size of particles in some animal studies could be related to overload conditions, and may not be a consequence of size *per se*, but of increased deposited load for a given particle mass. It is important to clarify which animal studies resulted in lung overload conditions, and if these study findings are relevant to humans.

Pulmonary Toxicity of Nanotubes

Nanoscale fibers, such as asbestos mineral fibers, have gained notoriety for causing pulmonary disease, including fibrosis and cancer, raising concerns that new, engineered nanofibers might behave similarly (Donaldson and Tran, 2004). For this reason, CNTs have received special attention with regard to their potential health risks. From classical fiber toxicology, the primary predictors of a fiber's pulmonary toxicity are biopersistence and length (Donaldson and Tran, 2004). The fiber length is important since long fibers are not as easily cleared by alveolar macrophage, resulting in prolonged pulmonary residence. Indeed, the 60-day lung residence of intratracheally instilled multiwalled CNTs in rats has been shown to be size dependent, with longer fibers having increased residence (Muller *et al*., 2005). This prolonged residence of long CNTs within the lungs would tend to increase fiber–epithelium interactions, and these interactions may result in the development of inflammation through mechanisms such as the generation of oxidative stress and cell membrane damage (Kagan *et al*., 2006; Panessa-Warren *et al*., 2006). Apart from its influence on lung residence, CNT fiber length has also been shown to directly correlate with induction of subcutaneous inflammation *in vivo* (Sato *et al*., 2005).

Experimental studies in rodents have demonstrated that instillation of multiwalled and single-walled CNTs can cause pulmonary inflammation, granulomas, fibrosis, and even death (Table 1). The deaths observed in at least two of these studies were attributed to mechanical obstruction of the airways, and may not be meaningful due to the high doses administered (Carrero-Sanchez *et al*., 2006; Warheit *et al*., 2004). The observed epithelial granulomatous lesions were associated with agglomerated CNTs, and in some cases interstitial fibrosis was observed at the sites of the granulomatous lesions and at distant sites (Carrero-Sanchez *et al*., 2006; Lam *et al*., 2004; Muller *et al*., 2005; Shvedova *et al*., 2005; Warheit *et al*., 2004). The fibrosis distant to the granulomatous lesions was presumed to be in response to either single or less-aggregated particles (Shvedova *et al*., 2005). Though the induction of interstitial

TABLE 1
CNT Acute Pulmonary Toxicology Studies

Species	Nanoparticle[a]	Administration route/study duration	Dose	Adverse effects/lesions	Ref.
Mice	MWCNT	Intratracheal instillation/1, 2, 3, 7 and 30 days	1, 2.5 and 5 mg/kg	• Dose-dependent lethality[b] • Inflammation • Dose- and time-dependent fibrosis and granulomas	Carrero-Sanchez *et al*., 2006
Guinea pig	MWCNT	Intratracheal instillation/4 weeks	25 mg/animal	• No evidence of inflammation • No perturbation of lung function	Huczko *et al*., 2001
Guinea pig	MWCNT	Intratracheal instillation/90 days	15 mg/animal	• Nonspecific desquamative interstitial pneumonia–like reaction • Increased lung resistance	Huczko *et al*., 2005
Mice	SWCNT	Intratracheal instillation/7 and 90 days	0.1 and 0.5 mg/animal	• Deaths in high dose group • Progessive, dose-dependent multifocal epithelial granulomas • Interstitial inflammation • Peribronchial inflammation and necrosis	Lam *et al*., 2004
Rats	MWCNT	Intratracheal instillation/1 and 2 mo	0.5, 2 and 5 mg/animal	• Inflammation and dose-dependent fibrosis • Bronchiolar granulomatous lesions	Muller *et al*., 2005
Mouse	SWCNT	Pharyngeal aspiration/1, 3, 7, 28 and 60 days	40 μg/animal	• Transient inflammatory response • Dose-dependent epithelioid granulomas and interstitial fibrosis • Decreased bacterial clearance, and dose-dependent loss of pulmonary function	Shvedova *et al*., 2005
Rats	SWCNT	Intratracheal instillation/24 h, 1 week, 1 and 3 months	1 and 5 mg/kg	• Deaths in high dose group[b] • Transient inflammatory and cell injury responses • Nonprogressive, non-dose-dependent multifocal granulomas	Warheit *et al*., 2004

[a]MWCNT = multiwall carbon nanotube, SWCNT = single-wall carbon nanotube.
[b]Deaths were attributed to mechanical obstruction of upper airways by nanotube aggregates.

fibrosis and pulmonary granulomas in rodents has also been described for other manmade and natural fibers (Lemaire *et al*., 1989), the rapid onset and lack of persistent inflammation associated with the CNT-induced fibrosis was atypical (Shvedova *et al*., 2005). It is important to point out that in these studies, CNTs were administered by nonphysiological intratracheal inhalation or pharyngeal aspiration, and the nanotubes were at least partially in the form of aggregated, larger-diameter bundles (Thess *et al*., 1996). Considering that a large portion of these aggregated CNTs would not have been respirable, the results of these studies are not necessarily representative of physiological inhalation exposures.

In two of the animal studies cited above, single-walled CNTs were compared to equal mass doses of nanoscale carbon black (Lam *et al*., 2004; Shvedova *et al*., 2005), and in both cases neither fibrotic nor granulomatous lesions were observed in the carbon black-treated animals. The lack of toxicity in the nanoscale carbon black-exposed animals in comparison with those treated with an equal mass of CNT, both carbon allotropes, highlights the importance of particle characteristics on toxicity. These effects appear to be primarily due to the surface properties and aspect ratio of CNTs, rather than their nanosize, *per se*. While CNTs clearly have the potential to cause adverse pulmonary effects, preliminary exposure assessments predict low inhalation exposure, suggesting reduced overall risk (see exposure section above). Additionally, many of the intended applications of CNTs involve composite materials that would further reduce exposure to free nanotubes.

Cutaneous Toxicity of Nanoparticles

As discussed above, a likely route of exposure to nanomaterials is by contact with the external surfaces of the body. Therefore, the potential hazards associated with cutaneous exposure to nanomaterials are of great concern. The limited *in vivo* studies that have been conducted to address the issue of cutaneous toxicity (Table 2), have identified only mild irritation as an adverse response to topical nanomaterial application. Nanoscale metal oxides, for example, are currently used in commercially available sunscreens, and have undergone extensive animal and clinical testing to fulfill regulatory requirements (SCCNFP, 2000, 2003). These studies found minimal irritancy potential, and no evidence of photo-irritation, sensitization, or photo-sensitization. The carbon-based nanomaterials, fullerene and CNT, similarly did not produce any notable irritant or allergic responses in clinical patch test or rabbit ocular toxicity studies (Huczko and Lange, 2001; Huczko *et al*., 1999). This lack of irritancy for the CNT is

TABLE 2
Cutaneous Toxicology Studies

Species	Nanoparticle[a]	Study design/duration	Dose	Adverse effects/lesions	Ref.
Human	CNT soot	Patch test/96 h	Unknown, aqueous suspension	• No irritation or signs of allergic response	Huczko and Lange, 2001
Rabbit	CNT soot	Ocular irritation (modified Draize test)/24, 48, and 72 h	Unknown, aqueous suspension	• No irritation or signs of allergic response	Huczko and Lange, 2001
Human	Fullerene soot	Patch test/96 h	Unknown, aqueous suspension	• No irritation or signs of allergic response	Huczko *et al*., 1999
Rabbit	Fullerene soot	Ocular irritation (modified Draize test)/24, 48 and 72 h	Unknown, aqueous suspension	• No irritation or signs of allergic response	Huczko *et al*., 1999
Rat	"Hat-stacked" carbon nanofibers	Subcutaneous implantation/ 1 and 4 weeks	Unknown	• Foreign body granuloma • No tissue necrosis • No severe inflammation	Yokoyama *et al*., 2005
Rat	CNT	Subcutaneous implantation/ 1 and 4 weeks	0.1 mg	• Foreign body granuloma • No tissue necrosis • No severe inflammation	Sato *et al*., 2005
Several studies—human, rabbits and guinea pigs	Nano-scale TiO_2	Skin and ocular irritation, sensitization, photo-irritation, photo-sensitization/ Various durations	Various doses	• TiO_2 considered non-irritant to mild irritant in all studies • No evidence of sensitization, photo-sensitization, or photo-irritation in any studies	SCCNFP, 2000
Several studies in humans	Nano-scale ZnO	Skin irritation, sensitization, photo-irritation, photo-sensitization/ various durations	Various doses	• ZnO considered non-irritant and non-photo-irritant • No evidence of sensitization or photo-sensitization in any studies	SCCNFP, 2003

[a]CNT = carbon nanotube.

surprising, since irritant contact dermatitis has resulted from exposure to structurally similar carbon fibers (Eedy, 1996). In contrast to these benign findings following topical application, many of these nanomaterials have been found to be cytotoxic and proinflammatory to dermal cell lines *in vitro* (Manna *et al*., 2005; Sayes *et al*., 2005, 2006; Shvedova *et al*, 2003). The discrepancy between the *in vitro* and *in vivo* findings may be due to limited dermal penetration *in vivo* (see exposure section above). In support of this posit, subcutaneous implantation of carbon nanotubes or "hat-stacked" carbon nanofibers in rats resulted in a more severe foreign body granuloma-like response (Sato *et al*., 2005; Yokoyama *et al*., 2005).

Systemic Toxicity of Nanoparticles

Few studies have examined the systemic toxicity of nanomaterials, and most of these have been cursory acute toxicity studies (Table 3), often without identification of target organs and often with extremely limited characterization of the test material. This lack of characterization is an important point, since without characterization, it is impossible to compare studies and recognize parameters that influence toxicity (Fig. 3). Nevertheless, some general trends can be gleaned from these preliminary investigations. In the majority of the studies cited, the LD_{50} values are in the high mg/kg–g/kg range regardless of administration route. This high mg/kg–g/kg range is defined as slightly toxic to moderately toxic for an oral LD_{50}, based on the Hodge and Sterner classification (scale: extremely toxic = < 1 mg/kg; highly toxic = 1–50 mg/kg; moderately toxic = 50–500 mg/kg; slightly toxic = 500–5000 mg/kg; practically nontoxic = 5000–15,000 mg/kg; relatively harmless = > 15,000 mg/kg) (Hodge and Sterner, 1949). In many of the studies, the LD_{50} could not even be estimated due to the lack of lethality observed at the doses administered.

It is interesting to note that many of the target organs that have been identified are members of the reticuloendothelial system (RES), including the liver and spleen. For example, acute toxicity studies of G3 cationic melamine dendrimers (Neerman *et al*., 2004) and G7 cationic polyamidoamine dendrimers (Roberts *et al*., 1996) in mice both determined that liver was most likely the primary target organ. This is not surprising, since it is has been shown that nanoparticles are commonly taken up by the RES, presumably following opsonization (Ogawara *et al*., 2001). Since nanoparticles used in biomedical applications are commonly coated in order to reduce opsonization and avoid the RES, the target organs for biomedical nanoparticles might be expected to shift away from the RES. Aside from the primary RES-related organs, the kidney also may be a common target organ of toxicity for nanoparticles. The kidney has been identified in preliminary pharmacokinetic studies as the primary clearance route for many nanoparticles, including CNTs, water-soluble polyalkylsulfonated fullerenes, and low-generation dendrimers (Chen *et al*., 1998; Lee *et al*., 2005; Wang *et al*., 2004). Some nanoparticles, such as dendrimers, have also been shown to distribute to kidney tissue (Nigavekar *et al*., 2004; Roberts *et al*., 1996). In one of the more comprehensive acute toxicology studies of nanoparticles in rats, the kidney was determined to be both the primary organ of clearance and the target organ of toxicity for polyalkylsulfonated fullerenes (Chen *et al*., 1998). The necropsy performed at study termination identified a treatment-related lysosome-overload nephrosis.

Lysosomal disorders may be a common side effect of nanoparticle exposure, as some nanoparticles have properties of substances known to cause these conditions (Kovacs and Seglen, 1982; Schneider *et al*., 1997), including lysosomal localization (Bottini *et al*., 2006; Shukla *et al*., 2005), enzyme-inhibiting ability (Shcharbin *et al*., 2006; Ueng *et al*., 1997), and biopersistence. Correspondingly, there are many examples of particle-induced lysosomal dysfunction. Studies have implicated changes in lysosomal permeability, and the subsequent release of lysosomal enzymes, as one of the mechanisms involved in the induction of apoptosis in alveolar macrophages by silica microparticles (Thibodeau *et al*., 2004). Nanoscale neodymium oxide particles (Chen *et al*., 2005), quantum dots (Seleverstov *et al*., 2006), and fullerenes (Yamawaki and Iwai, 2006) have all been shown to induce autophagic activation *in vitro*. Though the lesions in the cited nano-copper (Chen *et al*., 2006) and cationic dendrimer studies (Roberts *et al*., 1996) in Table 3 were not identified as lysosomal pathologies, both copper (Myers *et al*., 1993) and polycationic drugs (Mingeot-Leclercq *et al*., 1988) are known to cause lysosomal disorders. Additionally, the histological features of the liver lesions in these studies (Table 3) are consistent with those associated with copper and cationic drug-induced lysosomal pathologies, i.e., steatosis (Cai *et al*., 2005) and vacuolization (Anderson and Borlak, 2006). A thorough understanding of these potential mechanisms of nanoparticle toxicity is essential for hazard assessment and identification of exposure biomarkers.

Carcinogenicity of Nanoparticles

Researchers have just begun examining the carcinogenic potential of engineered nanoparticles in various *in vitro* and *in vivo* systems. In a dermal carcinogenesis bioassay, topical treatment of 7,12-dimethylbenzanthracene preinitiated mouse skin with 200 μg fullerene in benzene, two times per week for 24 weeks, failed to generate tumors (Nelson *et al*., 1993). These results agree with several *in vitro* findings; fullerene was found to be nonmutagenic in the Ames assay and nongenotoxic in a Chinese hamster lung cell chromosomal aberration assay, at concentrations up to 5 mg/ml (Mori *et al*., 2006). Derivatized, water-soluble fullerenes have even been shown to possess antimutagenic properties (Babynin *et al*., 2002). In contrast to these data, fullerenes were found to be mutagenic *in vitro* when irradiated with visible light in the presence of liver microsomes (Sera *et al*., 1996). The mutagenicity of the

TABLE 3
Acute Systemic Toxicology Studies

Species	Nanoparticle	Route	LD_{50} (g/kg)	Adverse effects/lesions	Ref.
Mouse	Fullerene (C_{60})	ip	> 2.5[a]	• Fullerene was absorbed, localized in spleen and liver • No deaths observed • Liver-hypertrophy of the perisinusoidal cells	Moussa *et al*., 1996
Rat	Fullerene (C_{60})	po	> 2[a]	• No evidence of toxicity • No effect on body weight	Mori *et al*., 2006
Mouse	Multiwalled CNT	po, ip	> 0.005, > 0.005[a]	• No evidence of toxicity	Carrero-Sanchez *et al*., 2006
Mouse	G7, cationic PAMAM dendrimer	ip	> 0.045[b]	• **Liver**, vacuolization	Roberts *et al*., 1996
Mouse	G3, cationic melamine dendrimer	ip	0.040–0.160	• **Liver**, necrosis	Neerman *et al*., 2004
Rat	Polysulfonated fullerene (C_{60})	ip	0.6	• **Kidney**, lysosomal overload	Chen *et al*., 1998
Mouse	Polyhydroxylated fullerene (C_{60})	ip	1.2	• Dose-dependent increases in liver/body weight noted	Ueng *et al*., 1997
Mouse	20 nm Fe_3O_4	po, ip, iv	> 2.1, > 1.6, > 0.4[a]	• No deaths observed • No histopathological lesions	Xia *et al*., 2005
Mouse	58 nm Zn	po	> 5[c]	• **Kidney**, tubular dilation, casts • **Liver**, hydropic degeneration	Wang *et al*., 2006
Mouse	25 nm Cu	po	0.4	• **Kidney**, proximal tubular necrosis • **Liver**, steatosis • **Spleen**, atrophy	Chen *et al*., 2006
Mouse	25, 80, and 155 nm TiO_2	po	> 5[a]	• **Kidney**, glomerular swelling • **Liver**, hydropic degeneration, spotty necrosis	Wang *et al*., 2007
Rats	Nanoscale TiO_2 T805 (primary particle 21 nm)	po	> 2.2[a]	• No evidence of toxicity • No gross lesions • No effect on body weight	SCCNFP, 2000
Rats	Nano-scale TiO_2	Topical	> 2[a]	• Hypokinesia, ataxia, chromodacryorrhea observed 24-h postdose • No deaths • No gross lesions • No effect on body weight	SCCNFP, 2000
Rats	CdTe quantum dots, 6 nm	iv	> 2 μmol/kg[a]	• No deaths • No histopathological lesions • No changes in clinical chemistry	Zhang *et al*., 2007

[a]No deaths were observed up to the maximum dose administered.
[b]1/5 of the animals in the high-dose group died.
[c]2/20 of the animals in the 5 g/kg–dose group died; deaths attributed to intestinal obstruction.

irradiated fullerenes appeared to be mediated by lipid peroxides produced by fullerene-generated singlet oxygen. Photo-dependent cytotoxicity has also been observed for fullerene and fullerene derivatives (Rancan *et al.* 2002; Sakai *et al*., 1999; Yang *et al*., 2002), and the underlying mechanism is also thought to involve ROS-mediated oxidative stress (Kamat *et al*., 1998; Yamakoshi *et al*., 2003).

Similar to the data for fullerenes, carcinogenicity data for nanoscale TiO_2 are also conflicting: *in vitro* studies with nanoscale TiO_2 have demonstrated photo-genotoxicity (Nakagawa *et al*., 1997), while *in vivo* studies have shown protection against photo-induced carcinogenesis in mice (Bestak and Halliday, 1996). The genotoxicity of the nanoscale TiO_2 observed *in vitro* was dependent upon UV irradiation, and in the absence

of irradiation, TiO_2 was not genotoxic (Nakagawa *et al*., 1997). Photo-dependent cytotoxicity of TiO_2 has also been demonstrated in human skin fibroblasts and colon carcinomas (Wamer *et al*., 1997; Zhang and Sun, 2004). Since nanoscale TiO_2 particles, such as those used in commercial sunscreens, have been shown to generate ROS upon UV irradiation (Brezova *et al*., 2005), oxidative stress is a plausible mechanism underlying this reported UV-dependent genotoxicity and cytotoxicity. It should be noted, however, that these findings of photo-toxicity and photo-genotoxicity were not supported by numerous industrial regulatory studies (SCCNFP, 2000). In any event, since coating of nanoscale TiO_2 particles has been shown to stabilize the particles and prevent photocatalysis (Allen *et al*., 2005), the proper coating of commercial TiO_2 may eliminate any concerns over dermal carcinogenesis from topical application. Overall, while some *in vitro* studies support the carcinogenic potential of irradiated TiO_2 and fullerenes, the lack of carcinogenesis observed *in vivo* is more relevant and should be given more weight.

Environmental Hazard of Nanoparticles

Over the course of a nanomaterial's life cycle, from the initial production to final disposal, there are ample opportunities for interaction with the environment. Specific to nanomaterials are the concerns that, because of their chemistry, size, and possible nonbiodegradable composition, they will rapidly distribute throughout the environment and bioaccumulate, with unknown consequences. On the contrary, experimental data suggest that many nanomaterials have extremely low mobility because of their rapid absorption to surfaces (Lecoanet *et al*., 2004). While some nanomaterials, such as metal oxides and carbon allotropes, may be biopersistent, many nanomaterials have been specifically developed to be biodegradable and are ideally suited for biological concepts, such as drug delivery. For example, drug delivery approaches have utilized poly(-D,L-lactide-*co*-glycolide acid) and chitosan as biodegradable nanoparticle drug carriers (Cui *et al*., 2006; Mitra *et al*., 2001).

The possibility that nanoparticles' interactions with the environment will have deleterious effects has received considerable attention. Preliminary investigations seeking to address nanoparticle environmental concerns have primarily been limited to fullerenes. Fullerenes were reported to be toxic to bacteria (Lyon *et al*., 2005) and aquatic species (Oberdörster, 2004), leading to speculation that nanomaterials may disrupt ecosystems (Feder, 2004). These results were later criticized due to the possibility that residual polar organic solvents used to disperse the fullerene influenced the results (Andrievsky *et al*., 2005). Indeed, other studies examining fullerene antibacterial properties and the toxicity of fullerenes to the aquatic organism *Daphnia magna* found greater toxicity for fullerenes solubilized with organic solvents (Lovern and Klaper, 2006; Lyon *et al*., 2006). The *D. magna* toxicity studies found a greater than 10-fold difference between the LC_{50} values of sonication-dispersed and THF-dispersed fullerenes (460 ppb vs. 7.9 ppm, respectively) (Lovern and Klaper, 2006), while the antibacterial study in *Bacillus subtilis* found a sixfold greater minimum inhibitory concentration for sonication-dispersed fullerenes. By contrast, fullerenes in their nondispersed, heavily aggregated state have been shown to be free of any bactericidal properties (Lyon *et al*., 2005). As discussed in a previous section, clearly it is important to take experimental conditions into account when evaluating the potential hazard associated with a nanomaterial. It is also important to not attribute the properties of one nanomaterial to all; for each instance of a bactericidal nanoparticle, nanoparticles have been identified that do not possess this property (e.g., hydroxylated fullerenes) (Lyon *et al*., 2005). Again, because nanomaterials differ greatly in their physicochemical properties, generalities with regard to their biocompatibility do not appear to be valid. The other side of the nanoparticle environmental issue is the potential direct benefit nanomaterials pose to the environment through pollution detection (Kim *et al*., 2006) and reduction (Dong *et al*., 2006; Sayle *et al*., 2005), and bioremediation (Tungittiplakorn *et al*., 2005). Further research is required to better characterize the fate of nanomaterials in the environment.

SUMMARY

No Two Nanomaterials are Exactly Alike

Though there is admittedly limited toxicological data available at the present, no unique or characteristic toxicities of nanoscale materials have been identified. Furthermore, traditional methodologies for toxicity characterization appear to be adequate for nanoparticle safety assessment. With the possible exception of nanofibers, nanomaterials appear to behave more like the microscale or bulk material than like other, unrelated nanomaterials. Thus, it is likely that a nanoscale particle of a toxic material will be toxic, and, likewise, that the nanoscale particle of a nontoxic material will also be nontoxic. This in no way means that the hazard associated with the use of a novel nanoparticle can be fully estimated from its bulk counterpart, as changes in dose–response have been observed for some nanoscale materials. This uncertainty warrants the use of common-sense techniques, such as containment, ventilation, and the use of personal protective equipment, to minimize exposure until such time as safety can be established.

Everything is Relative

It is important to put the known hazards of engineered nanoparticles in perspective, by comparing the toxic potency of these nanomaterials with that of other "model" toxic agents. In other words, relatively speaking, how toxic are the most toxic nanomaterials that have been evaluated? Consider the case of

cancer therapeutics: the chemotherapeutic agents used to treat neoplastic disease are some of the most toxic drugs known (taxol, rat iv $LD_{50} = 8$ mg/kg; Kim *et al*., 2001). Nanoparticles, which have great potential as drug carriers to affect biodistribution of these chemotherapeutics and thereby decrease drug-related side effects, are sure to be less toxic than the drugs they carry. Compared with toxic agents currently used in domestic applications, such as insecticides used to treat the family dog (pyrethrins, dog iv $LD_{50} = 7$ mg/kg; Soloway, 1976) or over-the-counter fungicides to treat dandruff (ketoconazole, man oral LDlo = 45 mg/kg; Lewis *et al*., 1984), the acute systemic toxicity studies identified in this review (Table 3) found nanoparticles to be significantly less toxic in the majority of cases. However, it can not be stressed enough that sparse safety data is available, and what is available consists primarily of acute studies, with chronic toxicology studies notably absent. An additional caveat is the noticeable lack of test material characterization that confounds study comparisons and conclusions regarding how hazard relates to nano-properties.

Some of the nanoparticle safety data discussed in this review, particularly that for CNT pulmonary toxicity, would suggest that researchers proceed with caution. This does not mean, however, that these nanomaterials should be abandoned outright, as risk is a combination of both the hazard and potential for exposure. There are many examples of dangerous materials that society has learned to work with safely, including ignitable fuels, such as gasoline, and radioactive isotopes used in nuclear energy and medicine. The insecticides and fungicides mentioned above are examples of hazardous substances that, with proper use, have low associated risk because the potential for exposure is low. At this time, the limited occupational and experimental studies available suggest that the potential for exposure to some engineered nanoparticles may be low, and supports the ability of the lung, GI tract, and skin to act as a significant barrier to the systemic translocation of many nanomaterials as well. However, further research is urgently needed to better clarify nanomaterial exposure and translocation pathways. On the other side of the risk equation is the fact that nanoparticle hazard appears to be modifiable. Several of the studies discussed in this review have highlighted the fact that by changing nanoparticle properties, such as surface characteristics, the biocompatibility of the particle can be dramatically altered. This "tunability" is unique, and holds the promise that with continued efforts to identify the factors involved in nanoparticle biocompatibility, safe engineered nanomaterials can be manufactured.

Addressing Unknowns

Under the auspices of the National Nanotechnology Initiative (NNI), many efforts are underway to address the uncertainties regarding nanotechnology safety (NNI report, *Environmental, Health, and Safety Research Needs for Engineered Nanoscale Materials*, September 2006). Examples include efforts by the National Institute of Standards and Technology (NIST) to standardize the methodology and instrumentation necessary for the characterization of nanomaterials, in order to aid in the implementation, interpretation, and interlaboratory comparison of safety studies. The National Cancer Institute has developed the Nanotechnology Characterization Laboratory to aid in the clinical translation of biomedical nanotechnology applications by identifying physicochemical properties that govern biocompatibility, and partnering with NIST and the U.S. Food and Drug Administration to help guide regulatory policy. Directly related to environmental and occupational concerns, the U.S. Environmental Protection Agency, the National Institute of Occupational Health, the National Institute of Environmental Health and Safety, and the National Toxicology Program are all actively involved in supporting research to define exposures, elucidate the hazards posed by nanomaterials throughout their environmental and occupational life cycle, and identify methods to ameliorate those hazards. These government agencies provide the checks and balances necessary to safeguard the public.

There is No Life Without Risk

There are many unanswered questions when it comes to nanotechnology safety concerns: What properties govern the biocompatibility of nanomaterials? What is the fate of nanoparticles in the environment? What are realistic exposures? In other words, is nanotechnology safe? Although nanoparticle safety studies have not raised any red flags, because of the unknowns, it is impossible to say that nanotechnology is free of risk. However, the same could be said of any new material in the research pipeline, nano or otherwise, from the latest small-molecule drug to the next generation polymer. In the future, when weighing the established risks against the established benefits of nanotechnology, it may be important to consider the risks that we already take for granted in our everyday lives, like the gasoline in our automobiles or the flea collar on our dogs.

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