TOXICOLOGICAL SCIENCES **101(2)**, 183–185 (2008)
doi:10.1093/toxsci/kfm279

TOXICOLOGICAL HIGHLIGHT

How Meaningful are the Results of Nanotoxicity Studies in the Absence of Adequate Material Characterization?

David B. Warheit[1]

DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, Delaware

Received November 6, 2007; accepted November 6, 2007

For the very few people who may not have an understanding of nanotechnology, here is a quick overview. Nanotechnology is an emerging multidisciplinary technology that involves the synthesis of molecules in the nanoscale (i.e., 10^{-9} m) size range. The origin of the term "nanotechnology" is derived from the Greek word "nano," meaning "dwarf." From a chemistry and material science perspective, the development of new products using nanomaterials is exciting because, for a given particle-type, as one moves down the nanoscale (i.e., as the particle size is decreased within the nanoscale range), fundamental physical and chemical properties appear to change—often yielding completely new and different physical/chemical properties.

For example, titanium dioxide particle-types, lose their white color and become colorless at decreasing size ranges < 50 nm. Other particle-types, known for electrical insulating properties, may become conductive at the nanoscale; or insoluble substances can become more soluble below 100 nm. Accordingly, these alterations in physical properties have generated great interest in this new technology (Colvin, 2003).

Given the excitement associated with all of the nanotechnology applications, evaluating the potential hazards related to exposures to nanoscale materials and its products has become an emerging area in toxicology and health risk assessment. The development of a safety database for nanoscale particles is evolving as new particles, materials, and exposure methodologies are being developed (i.e., implications research). Nanoparticle-types (often defined as < 100 nm in one dimension) may have different health impacts when compared to fine-sized (bulk) particle-types of similar chemical composition. In this regard, data from some pulmonary toxicity studies in rats demonstrate that exposures to ultrafine/nanoparticles produce enhanced toxicity responses when compared with larger-sized particles 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 the development of nanoparticle-related lung toxicity. In particular, some reports indicate that inhaled ultrafine/nanoparticles, following deposition in the alveolar regions of the lung, largely escape alveolar macrophage surveillance and transmigrate to the pulmonary interstitium or the systemic circulation following deposition in the alveolar regions of the lung (Donaldson *et al.*, 2001; Oberdorster, 2000). Alternatively, other recent studies indicate that the toxicity of some nanoparticulates may be related, in large part, to the surface reactivity of the particles, indicating that the particle surface–cellular interactions may take precedence over the core particle or particle size/surface area *per se* in influencing the development of inflammatory and cytotoxic responses in the lung (Warheit *et al.*, 2007a,b).

Particle surface and interfaces are important components of nanoscale materials. As the particle size is reduced, the proportion of atoms found at the surface is enhanced relative to the proportion inside its volume. This results in nanoscale particles, which are likely to be more reactive, thus generating more effective catalysts from an applications standpoint. However, from a health implications perspective, reactive groups on a particle surface are likely to modify the biological (potentially toxicological) effects. Therefore, changes in surface chemistry forming the "shell" on a (core) nanoparticle-type may be important and relevant for health effects. In addition, 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 note that surface coatings, functioning to reduce aggregation and to facilitate particle dispersion, enhance the efficacy of the particle-type in its *designed application*, but may also accelerate translocation of the nanoparticle from the respiratory tract to the systemic circulation and thereby significantly increase nanoparticle distribution throughout the body (Borm *et al.*, 2006; Oberdorster *et al.*, 2005). To capture this concept of the importance of nanoparticle core-shell dynamics, it

[1] For correspondence via E-mail: david.b.warheit@usa.dupont.com.

© The Author 2007. Published by Oxford University Press on behalf of the Society of Toxicology. All rights reserved.
For Permissions, please email: journals.permissions@oxfordjournals.org

Downloaded from http://toxsci.oxfordjournals.org/ by guest on August 31, 2014

should be noted that from a toxicological perspective, two different nanoparticle-types containing titanium dioxide as their "core" may not be biologically equivalent. 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 physicochemical differences are manifested in different pulmonary inflammatory and cytotoxic effects ranging from benign to more moderate health impacts (Warheit *et al*., 2007b).

In their publication in this issue, Murdock *et al*. (2007) have focused on the importance of developing adequate physicochemical characterization of nanomaterials prior to undertaking experiments for *in vitro* toxicity assessments. These authors have correctly suggested that for *in vitro* toxicity studies, particle size, size distribution, particle morphology, particle composition, surface area, surface chemistry, and particle reactivity in solution are important factors which need to be accurately characterized as prerequisites for implementing nanoparticle toxicity studies. This point cannot be overstated, because in a variety of recently published nanotoxicity studies, estimates of particle size and other characteristics have been reported using only the (1) manufacturer's data (without investigator confirmation), (2) via electron microscopy, or (3) via surface area Brunauer Emmett Teller (Brunauer *et al*.) measurements and these limited characterizations provide little information about the particle-type being studied.

The problem is that most of the reported studies with nanoparticles have been conducted under *in vitro*/cell culture conditions (i.e., in the wet phase) wherein the physicochemical characteristics of the particles, including particle size, are likely to change from the "just received" (i.e., dry phase) nanopowder contained in a vial. This represents a misconception regarding the nature of the particle-type being studied because the characterization likely was conducted under dry state conditions and clearly has limited relevance for characterizing the nanoparticle–cell interactions under cell culture conditions. Therefore, in the Murdock *et al*. study, these investigators have focused on characterizing a wide range of nanomaterials including metals, metal oxides, and carbon-based structures using dynamic light scattering (DLS) concomitant with transmission electron microscopy, for particles dispersed under wet conditions in cell culture media, with and without serum. Some basic cell viability and morphology studies were correlated with DLS particle size characteristic experiments to assess toxicity from observed agglomeration alterations under the various experimental conditions. Murdock and coworkers concluded that many metals and metal oxide nanomaterials tend to agglomerate in solution. Moreover, other variables, such as the addition of serum in the culture media, can affect toxicity measurements, likely due to influences affecting agglomeration and/or surface chemistry of nanoparticles. These factors represent important considerations that have not been previously recognized.

Although measurements of particle size characteristics in cell culture media were a main focus of their methodology-based study, Murdock and colleagues also described other techniques for assessing nanoparticle physicochemical characteristics as well as the impacts of preparing nanoparticles for toxicological study. X-ray photoelectron spectroscopy techniques were utilized to detect surface chemical composition of silver nanoparticles. In addition, it was reported that sonication of nanoparticles reduces agglomeration and has a minimal effect on particle surface charge. Sonication has been utilized to facilitate particle dispersion and solution mixture, prior to the implementation of cell culture experiments.

Consistent with the message of the studies by Murdock *et al*., many scientific organizations or task forces have strongly recommended that toxicologists adequately characterize physicochemical properties of the nanoparticle-types that are being evaluated for hazard testing. However, too often this recommendation becomes a "laundry list" of physicochemical characteristics and does not have adequate prioritization. As a consequence, in order to adequately describe the physical characteristics of the nanoparticle-type being evaluated, I would recommend that, at a minimum, toxicologists 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 postsynthetic modifications (e.g., neutralization of ultrafine TiO_2 particle-types); and
- Purity of sample.

This represents a focused approach concomitant with a minimum, standardized assessment of physicochemical properties that should be investigated prior to the development of toxicity testing with nanoparticles.

Perhaps the most significant impact of the Murdock *et al.* publication is to raise the issue of the importance of adequately characterizing the nanomaterial preparation prior to the initiation of toxicological experimentation. Indeed, in the absence of a careful and complete description of the nanoparticle-type being evaluated (as well as the experimental conditions being employed), the results of nanotoxicity experiments will have limited value or significance. Moreover, the results of reported studies will not be comparable with other studies conducted with similar nanomaterial-types. In essence, the hazard database for nanomaterials would be cheated. Given the paucity of nanotoxicity health effects and ecological data currently available, this would continue to be an unfortunate oversight.

Downloaded from http://toxsci.oxfordjournals.org/ by guest on August 31, 2014

REFERENCES

Borm, P. J. A., Robbins, D., Haubold, S., Kuhlbusch, T., Fissan, H., Donaldson, K., Schins, R. P. F., Stone, V., Kreyling, W., Lademann, J., *et al.* (2006). The potential risks of nanomaterials: A review carried out for ECETOC. *Part. Fibre Toxicol.* **2006**(3), 11.

Brunauer, S., Emmett, P. H., and Teller, E. (1938). Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* **60,** 309–319.

Colvin, V. L. (2003). The potential environmental impact of engineered nanomaterials. *Nat. Biotechnol.* **21,** 1166–1170.

Donaldson, K., Stone, V., Clouter, A., Renwick, L., and MacNee, W. (2001). Ultrafine particles. *Occup. Environ. Med.* **58,** 211–216.

Murdock, R. C., Braydich-Stolle, L., Schrand, A. M., Schlager, J. J., and Hussain, S. M. (2007). Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. *Toxicol. Sci.*

Oberdorster, G. (2000). Toxicology of ultrafine particles: In vivo studies. *Philos. Trans. R. Soc. Lond. A* **358,** 2719–2740.

Oberdorster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J., Ausman, K., Carter, J., Karn, B., Kreyling, W., Lai, D., *et al.* (2005). Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. *Part. Fibre Toxicol.* **2,** 8.

Warheit, D. B., Webb, T. R., Colvin, V. L., Reed, K. L., and Sayes, C. M. (2007a). Pulmonary bioassay studies with nanoscale and fine quartz particles in rats: Toxicity is not dependent upon particle size but on surface characteristics. *Toxicol. Sci.* **95,** 270–280.

Warheit, D. B., Webb, T. R., Reed, K. L., Frerichs, S., and Sayes, C. M. (2007b). Pulmonary toxicity study in rats with three forms of ultrafine-TiO2 particles: Differential responses related to surface properties. *Toxicology* **230,** 90–104.

Downloaded from http://toxsci.oxfordjournals.org/ by guest on August 31, 2014