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A 21st Century Paradigm for Evaluating the Health Hazards of Nanoscale Materials?

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Over the past 5 years we have seen an increase in the attention focused on the assessment of the potential health risk posed by nanoscale materials. The diversity of these materials with respect to size, composition, and surface properties, and the rapid pace of their development and commercialization, poses significant challenges to traditional toxicity testing paradigms. At the same time the potential use of new high throughput "predictive "toxicity" strategies, such as that envisioned in the recent NRC report "Toxicity Testing in the 21st Century," have emerged as possible solutions to deal with the issue of how to assess the safety of the thousands of chemicals to which humans are potentially exposed. In this forum article we discuss how in some respects, the emergence of diverse engineered nanomaterials offers a tailor-made test case for the application of a new paradigm for assessing human heath risks. However, although this approach may have merit in the study of some specific nanomaterials, this approach does not consider the complexity involved in utilizing *in vitro* cell culture toxicology methods to evaluate the potential hazard of the wide array of current and future engineered nanomaterials.

Key Words: **nanoparticles; risk assessment.**

Concern over the potential environmental and human health impacts of nanoscale materials (nanomaterials) came to public attention ~6 years ago (Service, 2003) and through subsequent publications on the pulmonary toxicity of carbon nanotubes (Lam *et al.*, 2004; Warheit *et al.*, 2004). Since that time, increasing use and visibility of nanomaterials in commerce and concerns over the potential safety risks of a diverse array of commercially important nanomaterials has led to a corresponding rapid increase in the number of publications on their potential to cause adverse effects on human health and the environment.

About the same time that attention began to be focused on nanomaterials, in 2004, the National Toxicology Program released its Vision for Toxicity Testing in the 21st Century (http://ntp.niehs.nih.gov/ntp/main_pages/NTPVision.pdf), with the stated intent to move toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations. Similar themes were expressed in the National Research Council (NRC) report "Toxicity Testing in the 21st Century," released in 2007 (Andersen and Krewski, 2009; NRC, 2007). A report outlining the U.S. Federal Government response to the NRC document was published in 2008 and NIEHS, EPA, and the National Institutes of Health Chemical Genomics Center (NCGC) recently signed an agreement to collaborate on the development and evaluation of high throughput testing methodologies as a way to; (1) prioritize substances for further in-depth toxicological evaluation, (2) identify mechanisms of action for further investigation, and (3) develop predictive models for *in vivo* biological response for the toxicity of thousands of chemicals in commerce with inadequate or nonexistent toxicological data (Collins *et al.*, 2008). These events have catalyzed a healthy debate among toxicologists, regulators, and the public at large over the incorporation of new predictive approaches to address the human health risks for thousands of agents of public health concern.

In this forum article, we will discuss the convergence of these two issues. In some respects the emergence of diverse engineered nanomaterials offers a tailor-made test case for the application of a new paradigm for assessing human heath risks. But the question is, whether a 21st century toxicity testing paradigm is suitable for this 21st century technology?

Over the past 5 years there has been a continuing debate on the most appropriate strategies to use for evaluating the human and environmental health risks of nanomaterials (Bucher *et al.*, 2004; Maynard *et al.*, 2006; Oberdorster *et al.*, 2005a; Service, 2008; Stern and McNeil, 2008). Nanomaterials are loosely defined as any physical substance that has at least one dimension in the nanoscale range (1 to approximately 100 nm).

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Examples of nanomaterials include carbon fullerenes, nanotubes (single and multiwalled), metal oxides (titanium dioxide, zinc oxide, cerium oxide, iron oxide), quantum dots, dendrimers, and nanoscale metals (silver, gold, copper).

Although this may seem like a limited number of potential agents of concern, differences in primary size, aspect ratio, shape, coatings, and surface functionalization can lead to thousands of possible variants. Moreover, production information and human exposure data are lacking for many of these substances and, in many cases, the specific nanomaterial within a "class" used for a specific application is not clearly defined. As such, one of the greatest challenges facing the toxicology community is the prioritization of nanomaterials to evaluate and the depth of toxicological evaluation that should be conducted. Clearly, not all can be evaluated in *in vivo* studies. It has been estimated that the costs to conduct traditional *in vivo* studies on even those nanomaterials currently in commerce could total a billion dollars and take 30–50 years to complete (Choi *et al*., in press). Moreover, because limited information is available on production volumes, the potential for human exposure or levels that might occur in the environment, an incorrect decision to evaluate a specific nanomaterial could potentially prove to be not only costly but irrelevant in the context of human risk.

From a pragmatic point of view, we first need to ask why would the requirements for safety testing for nanomaterials be any different from chemicals, drugs or other entities that we have encountered before. In essence there are several reasons why they need to be considered differently. Firstly, it has been known for some time that decreasing the size of a material below barrier cutoffs for portals of entry can lead to new unintended routes of exposures (Hillyer and Albrecht, 2001). Secondly, that once internalized, surface properties can have profound impacts on the kinetics and bio-distribution of materials of similar size and shape, leading to differences in target organ dosimetry (Bullard-Dillard *et al*., 1996). Thirdly, the new properties that make nanomaterials attractive for commercial applications could result in new biological interactions leading to unanticipated toxicity (Linse *et al*., 2007; Lynch *et al*., 2006). Finally, for some nanomaterials, "dose" can scale with a size-dependent property such as surface area. Thus, assessments of relative risk based on mass-based dose may lead to erroneous assertions of relative risk (Oberdorster *et al*., 2007).

Given the diversity of nanomaterial size, structure, and composition, it is clear that a one-by-one approach to toxicity testing is not a tenable strategy for dealing with all of the existing and new emerging nanomaterial-enabled products in commerce. The NRC paradigm lays out a scheme for toxicity testing and risk assessment that is based on chemical characterization, toxicity pathways assessment, targeting testing and dose-response modeling as core elements of a testing strategy. In considering whether this approach could be applied to assessing the risks of nanomaterials, it is likely that most of the elements of this strategic approach would not differ when employed for chemicals versus nanomaterials.

However, there is one critically important element that we believe makes the NRC proposal far less attractive for nanomaterials than for chemicals or other simpler substances. Clearly chemical characterization and the interrelated aspects of dosimetry for nanomaterials is an area that requires significantly more attention than would be paid to most chemicals. Nanomaterials possess molecular, supramolecular and physical attributes that may influence their biological effects. As such, the suite of analytical procedures used for assessing the identity of nanomaterials is considerably more complex than for simple chemical characterizations. In addition, assessment of what constitutes "purity" for a nanomaterial is also more complicated. For a defined molecule, a statement such as "99% pure" is often used to support the assertion that the effects are attributable to the specific agent tested. But how would we assess and communicate "purity" for a nanomaterial that may be composed of multiple entities, have a distribution for its primary particle size, may aggregate, and may also have a surface coating? In addition how would we assess deviation from that stated purity? Using size as an example, would we consider a nanomaterial with 95% of the particles between 50 and 70 nm the same as of one with 95% of the particles between 30 and 90 nm even if the reported average size were the same? With regards to the test systems, changes to nanomaterials within an *in vitro* and *in vivo* testing environment should be expected. It must be recognized that the specific composition of an *in vitro* and *in vivo* test system will likely play a huge role in how a nanomaterial interacts with a cell, or other biological target. This adds a significant complication to the prediction of *in vivo* health effects from *in vitro* findings and extrapolation to humans. Agglomeration and aggregation is now recognized as a major issue in both the *in vitro* and *in vivo* evaluations of effects of nanomaterials. Depending on the experimental conditions used, the pH, and specific protein content of the environment, methods used to "solubilize" nanomaterials, etc., what was "tested" may often bear little resemblance to the material as it exists in the real world or in a different test system.

As a result, there is now a strong expectation that there should be an assessment of the appropriate physicochemical properties of a tested nanomaterial within the experimental test system for *in vitro* studies, as well as within an exposure system for *in vivo* studies (e.g., particle size distribution in the chamber for inhalation studies or particle size distribution in liquid based-dose formulations) (Warheit, 2008). Indeed there are international efforts geared to establishing characterization schemes for various nanomaterials. Although these concerns may simply be a result of the general toxicology community's unfamiliarity with engineered nanomaterials, they do highlight that chemical characterization is one of the key issues for assessing nanomaterial toxicity. The NRC strategy does address the need for "characterization" as way to predict the

environmental behavior of a chemical, and it is this part of the strategy that, for nanomaterials, requires a significant increase in effort. An emphasis on determining from the physicochemical characteristics, the likelihood that a nanomaterial might actually present itself to the environment in a nanoscale form may reduce considerably the number of entities we may have the highest concerns about.

A reason for this focus on characterization is due to the fact that the what constitutes ''dose'' for an nanomaterial, in terms of assessing dose-response relationships may not always be ''mass''(Oberdorster *et al*., 2007). For dose-response assessment, we routinely use mass-related dose metrics (ppm, mg/kg, mol/l) to compare the potencies of different chemicals. Because effects of nanomaterials may be related to both their physical as well as molecular/chemical properties, the dose metric of concern may be not only mass based but also related to some aspect of its physical structure. This is well appreciated in particle and fiber toxicology where the metric of concern may be the number of fibers of a given length or the surface area of a particle (Oberdorster *et al*., 2005b). Consequently, any changes to the physical structure through agglomeration or aggregation or changing particle size within the experimental test system may affect the ''dose'' of concern for the nanomaterial. As such, apparent adverse responses from such studies may either underpredict, or overpredict the hazard of the material actually encountered in real world human exposures. And in this context, real world exposures refers to not only the level of exposures in the environment and populations, as noted in the NRC strategy, but also to the physical/chemical aspects of the nanomaterial in the environment. If predictive strategies are to be useful for nanomaterials, a significant increase in our capabilities to measure and characterize nanomaterials in the environment and in biomonitoring studies is needed. This reinforces recommendations that we (Bucher *et al*., 2004) and others (Warheit, 2008) have made previously concerning the critical need to try to characterize and report as fully as possible the physical and chemical characteristics of nanomaterials within the experimental system used, and to develop approaches and technologies to measure them in the environment (Maynard *et al*., 2006). Doing so will ensure that informed conclusions can be made about which physicochemical parameter is driving any observed adverse response within a given test system and its relevance to exposures of concern.

A cornerstone of the NRC paradigm is the focus on evaluation of perturbations in ''toxicity'' pathways as exposure increases to the point where adaptation transitions to adversity. The application of this idea to toxicity testing is a key approach proposed in the NTP's vision for toxicity testing and is a key feature in the collaborative high throughput approach employed by NTP, EPA, and the NCGC (Collins *et al*., 2008). There have been many attempts to apply similar approaches to the study of nanomaterials. For example, Nel *et al*. have advocated this approach building on current knowledge on the induction of ROS and oxidative stress by ultrafine particles (Nel *et al*., 2006). In addition, Shaw *et al*. (2008) have used this approach to prioritize nanomaterials for further *in vivo* testing. From a practical point of view it is likely that, as with high throughput screening for chemicals, these approaches will ultimately be successful for a few classes of nanomaterials that are well behaved and compatible with the available test systems. It is also likely that by virtue of their physical attributes and unpredictable and/or artifactual behavior in *in vitro* systems, the majority of nanomaterials, may not be amenable to study in high throughput assays.

In summary, the NRC paradigm proposes a largely *in vitro* approach for the evaluation of the toxicity of substances of public health concern. As pointed out, this approach is in keeping with the efforts that the National Toxicology Program is taking to move toxicology to a more predictive science based on discovery of common mechanisms of toxic action. However, although this approach may have merit in the study of some specific nanomaterials, this approach does not consider the complexity involved in utilizing *in vitro* cell culture toxicology methods to evaluate the potential hazard of the wide array of current and future engineered nanomaterials.

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