FORUM SERIES

Research Strategies for Safety Evaluation of Nanomaterials, Part I: Evaluating the Human Health Implications of Exposure to Nanoscale Materials

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Nanotechnology has the potential to dramatically improve the effectiveness of a number of existing consumer and industrial products and could have a substantial impact on the development of new products ranging from disease diagnosis and treatment to environmental remediation. The broad range of possible nanotechnology applications could lead to substantive changes in industrial productivity, economic growth, and international trade. A continuing evaluation of the human health implications of exposure to nanoscale materials will be essential before the commercial benefits of these materials can be fully realized. The purpose of this article is to review the human health implications of exposure to nanoscale materials in the context of a toxicological risk evaluation, the current scope of U.S. Federal research on nanoscale materials, and selected toxicological studies associated with nanoscale materials to note emerging research in this area.

Key Words: nanomaterials; nanoscale materials; nanotechnology; risk assessment; toxicology.

Definitions Associated with Nanoscale Materials

Any discussion on the human health implications of exposure to nanoscale materials will be impacted by the definition of the size range of these materials. One of the more widely used definitions for nanotechnology was adopted by the National Nanotechnology Initiative (NNI), a U.S. Federal research and development program established to coordinate the multiagency efforts in nanoscale science, engineering, and technology. This definition equates the term nanotechnology with a process or product only if each of the following is involved: (1) research and technology development at the atomic, molecular, or macromolecular levels, in the length scale of approximately 1–100 nanometer range; (2) creating and using structures, devices, and systems that have novel properties and functions because of their size; and (3) the ability to control or manipulate on the atomic scale. Additional definitions vary in terms of specifying the size dimensions of nanoscale materials, with some including sizes over 100 nm (www.lanl.gov/mst/nano/definition.html), and others identifying nanoscale materials as “those which have structured components with at least one dimension less than 100 nm” (Royal Society, 2004, Page 7). Some refer to nanoscale materials as those intentionally produced in the laboratory or industrial settings. These “engineered” nanoscale materials would exclude the broad range of naturally occurring particulates (from forest fires, biological particulates, etc.) and unintentional anthropogenic particulate by-products (from diesel engines, power plants, etc.) in the 1–100 nm size range.

Engineered nanoscale materials can be further subdivided on the basis of how they are produced: they can be made by either “top down” or “bottom up” techniques (Royal Society, 2004). Top-down processing involves etching or milling of a larger single sample of material to obtain the nanoscale material in the desired configuration, while bottom-up approaches assemble smaller subunits to obtain the larger nanoscale material through processes such as chemical synthesis. Many top-down applications such as the lithographic processes used to manufacture computer chips have been used for years, while other bottom-up approaches such as production of carbon nanotubes are relatively new. The specific technique used to produce a nanoscale material could influence the human health risk associated with that material.

Due to their size, nanoscale materials often exhibit unique physical/chemical properties and can impart enhancements to engineered materials, including better magnetic properties, improved electrical and optical activity, and increased structural integrity. In addition, these materials are often much more reactive than their bulk material counterparts as a result of their
larger surface area. Consequently, the use of nanotechnology can create more reactive chemical intermediates in industrial processes, and have a dual benefit as powerful reaction catalysts to initiate chemical reactions with less energy input. These properties allow nanoscale materials the ability to improve the performance of a wide range of products and services including sports equipment, textiles and fabrics, energy generation and distribution, drugs and medical devices, and food processing. As a result of the unique physical-chemical properties of nanoscale materials, research has been initiated at private and public organizations to develop approaches for evaluating the human health implications from exposure to these materials.

The Scope of Current U.S. Federal Research

The scope of U.S. Federally funded research on nanoscale materials has been increasing. All major aspects of federal funding of nanotechnology noted here can be found at www.nano.gov, with some more specific details on health and environmental research found at www.nano.gov/html/facts/EHS.htm. Agencies participating in the NNI funded an estimated $106 million in research on the health and environmental aspects, including applications and implications, of nanoscale materials in 2004 (Subcommittee on Nanoscale Science, Engineering and Technology, 2005). This funding included research directed to: (1) increase the fundamental understanding of nanoscale material interactions at the molecular and cellular level addressed in in vitro experiments and models; (2) increase fundamental understanding of nanoscale materials’ interactions with the environment; (3) increase understanding of the fate, transport, and transformation of nanoscale materials in the environment; and (4) identify potential exposure, possible toxicity, and needs for personal protective equipment when working with nanoscale materials. This research also included studies that aimed to exploit nanotechnology to benefit human health and the environment, and yet some of these findings will also assist in the assessment of the potential risks associated with nanoscale materials. Finally, some of these funds have been earmarked for evaluating societal implications and for education-related activities.

The Nanoscale Science and Engineering and Technology (NSET) Subcommittee’s Nanotechnology Environment and Health Implications (NEHI) Working Group has been formed in part to identify the nanotechnology environmental and health research that will be needed to support regulatory decision-making by agencies that have such authorities. The members of the NEHI Working Group include agencies such as the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the National Institute for Occupational Safety and Health (NIOSH), which support nanotechnology research and/or have responsibilities for protecting human health and the environment.

Many Federal agencies have initiated focused efforts to study the potential risks of exposure to nanoscale materials, and others have been continuing to conduct research that is expected to facilitate risk evaluation. The National Science Foundation (NSF) funds basic research on environmental effects of nanoscale materials with a focus on the production and dispersion of nanoparticles in air, water, soil, and biological systems. A list of 100 environment-related grants funded by NSF is publicly available (www.nsf.gov/funding/pgm_list.jsp?type=xcut). NSF also supports three Nanoscale Science and Engineering Centers (NSECs) that address health and environmental concerns (www.nsf.gov/crssprgm/nano/info/centers.jsp): the Center for Biological and Environmental Nanotechnology (CBEN) at Rice University (funded in 2001 for five years at $11.8 million) is focused on dry-wet interface and the biological effects of nanostructures released in the environment (http://cben.rice.edu/index.cfm); the center at the University of Pennsylvania (2004–present) is focused on the individual cell response to nanostructures; and the center at Northeastern University (2004–present) has research related to nanomanufacturing safety (www.azonano.com/news_old.asp?newsID=335). NSF also supports exploratory research at the University of Rochester on size-dependent neural translocation of nanoparticles towards brain tissue. Finally, NSF-supported studies on reverse engineering cellular pathways from human cells exposed to nanoscale materials are being conducted at the Texas Medical Center in Houston.

The National Toxicology Program (NTP) on nanotechnology funds research on the potential toxicity of nanoscale materials. The NTP program was initiated in 2004, and the materials that will be evaluated at the beginning of the program include titanium dioxide, several types of quantum dots, and fullerenes (www.niehs.nih.gov/oc/factsheets/nano.htm). The first studies to be initiated will characterize the distribution and uptake by the skin of titanium dioxide, fullerenes, and quantum dots. The NTP is also considering conducting inhalation studies of fullerenes, and is collaborating with NIOSH to develop methods for controlled inhalation exposure for carbon nanotubes. NIOSH provides research, information, education, and training in the field of occupational safety and health. In 2004, NIOSH initiated several research projects focused on nanotechnology, including a five-year program to assess the toxicity of ultrafine and nanoparticles, funded at approximately $1.7 million in FY2004, increasing to approximately $2.3 million in FY2005 (www.cdc.gov/niosh/topics/nanotech/). In an action related to environmental health and safety research, NIOSH released its Frequently Asked Questions regarding nanotechnology and occupational safety and health research in 2005 (www.cdc.gov/niosh/topics/nanotech/faq.html).

The Department of Defense has at least one major project focused on evaluating the potential toxicity of nanoparticles. To better understand and predict the effects of nanoscale materials on biological systems, the Air Force Multi-disciplinary University Research Initiative (MURI) recently announced funding
a new research effort involving the University of Rochester, Washington University, and the University of Minnesota (www.nano.gov/html/news/Oberdorster_Article.htm). The new research is intended to identify the relationship between the physicochemical characteristics and toxicological properties of nanoscale materials. The ultimate goal is to develop a computational model that will predict toxic, salutary, and biocompatible effects based on nanostructural features. The project is to receive $5.5 million over five years.

Within their broader portfolios of nanotechnology-related research, both the EPA and the Department of Energy (DOE) have activities that address human and environmental health issues. The EPA is funding research at universities to examine the toxicity of manufactured nanoscale materials such as quantum dots, carbon nanotubes, and titanium dioxide (http://es.epa.gov/nceer/rfa/2004/2004_manufactured_nano.html). In addition, current and past studies on ultrafine particulates at EPA can help further the understanding of the potential effects of nanoparticles on human health. DOE is supporting research at the Molecular Foundry at Lawrence Berkeley National Lab in California aimed at understanding the transport and transformation of nanoparticles in the environment, and exposure and risk analysis (http://foundry.lbl.gov/).

Scientists funded by the NIH are studying the effects of nanoscale materials in the body, in cell cultures, and in laboratory use for diagnostic and research tools. While many are looking for potentially beneficial effects—for example, whether these particles are useful in drug delivery—this research is creating a significant body of knowledge of nanoparticulate reactions with biological materials. The National Cancer Institute has just established the Nanotechnology Characterization Laboratory (NCL), which collaborates with FDA and the National Institute of Standards and Technology (NIST) to better characterize nanotechnologies for cancer therapies and diagnostics (http://ncl.cancer.gov/). This effort will examine the physical attributes of nanoparticles, including those critical parameters related to absorption, distribution, metabolism, excretion, and toxicity profiles of these materials.

Finally, researchers at NIST are developing measurement tools, tests, and analytical methods necessary to ensure the reliability and comparability of the results of environmental and health-related studies (www.nist.gov/public_affairs/nanotech.htm). Accurate measurement and characterization of nanoscale particles in the atmosphere, for example, are required to relate levels of exposure to biological responses (also known, in epidemiological studies, as concentration-response functions).

Current Studies Related to Toxicology of Nanoscale Materials

Several recent studies that evaluated the effects of nanoscale materials on biological systems have provided very useful data for understanding the human health implications from exposure to these materials and have helped to identify priorities for further research. While most of the existing studies on manufactured nanoscale materials have focused on a very limited range of materials, including carbon nanotubes, fullerenes, and quantum dots, the data derived from these studies provides useful information for evaluating the potential human health and environmental effects that could result from exposure to nanoscale materials. The increased reactivity of nanoscale materials that arises as a consequence of their larger surface area has created considerable interest in development of a better understanding of the effects of nanoscale materials on biological systems. Nanoscale materials have been evaluated in both in vitro and in vivo systems to explore effects from dermal and inhalation exposure. In a broad sense, these studies were designed to characterize the extent to which these materials interact with organ systems and cellular organelles, the biological effects of those interactions, and the potential health effects from acute and chronic exposure to nanoscale materials.

Fullerenes, a type of nanoscale material that could have utility in several areas, including in the development of novel drug delivery systems, have been shown to induce oxidative stress in juvenile largemouth bass (Oberdorster et al., 2004). Exposure to uncoated C60 fullerenes caused lipid peroxidation in the brain tissue of juvenile largemouth bass. While the specific mechanism(s) responsible for this effect was not clear, it was postulated to be associated with a selective transport mechanism from the olfactory nerve into the olfactory bulb. In another study, a time-dependent increase in nanoscale elemental 13C was observed in the olfactory bulb of rodents following inhalation exposure (Oberdorster et al., 2004). The increase was measured subsequently to exposure to 13C and was attributed to neuronal transport from the olfactory nerve to the olfactory bulb.

These data underscore the need to develop a more comprehensive understanding of the translocation specificity of fullerenes, and other lipophilic nanoscale materials, into lipid-rich tissues and cellular organelles and any subsequent effects on biological function. Additional studies are needed to determine the reversibility of the observed translocation, and to determine whether similar effects would be seen in humans. Another important consideration for evaluating the biological effects of nanoscale materials is the presence and type of surface coating on the material. Although uncoated material was used for the largemouth bass study, a significant amount of the material that will be used in commerce is likely to be coated with one of a variety of materials that have varying levels of biological availability and persistence. The characteristics of the underlying nanoscale material therefore must be understood in combination with the relevant surface coating or treatment.

Multi-walled carbon nanotubes (MWCNTs) were recently examined for effects that could result from dermal exposure in workers and consumers. Simple in vitro tests using human epidermal keratinocytes indicate that MWCNTs could induce a cytokine-mediated inflammatory response, and localize in cytoplasmic vacuoles, in mammalian systems (Monteiro-Riviere et al., 2005). Such results could be evaluated more...
comprehensively in vivo to elucidate the clinical significance of these in vitro effects.

Respiratory exposure to nanoscale materials such as carbon nanotubes has also received a considerable amount of interest. Because of the difficulties associated with generating aerosols of carbon nanotubes to facilitate an evaluation of inhaled material, and the high cost of homogeneous well-characterized material, several in vivo pulmonary studies have employed intratracheal instillation as the exposure methodology. Single-walled carbon nanotubes (SWCNTs) have been evaluated in rodents using this technique. SWCNTs instilled into the lungs of mice produced granulomas in the pulmonary interstitium of the lungs (Lam et al., 2004). Rats exposed to SWCNTs developed multifocal granulomas in the absence of any pulmonary inflammation or cellular proliferation (Warheit et al., 2004) which suggests that SWCNTs may act via a different mechanism of toxicity than other inhaled toxicants such as crystalline silica.

These studies highlight the need for further experimentation to fully elucidate the mechanisms that are responsible for the behavior of carbon fullerenes and nanotubes. Specifically, to develop a comprehensive evaluation of the human health implications from exposure to nanoscale materials, more data are needed on the adsorption, distribution, metabolism, and excretion of these materials in biological systems. In addition, more data are needed to assess the extent to which the fundamental properties of these materials, such as particle size and size distribution, affect the distribution and elimination of these materials in the body. More studies are also needed to characterize the toxicity of a broader range of commercial nanoscale materials as a function of chemical composition, shape, surface characteristics, and method of production. Several of these properties, particularly surface coating, could have a substantial effect on the biological activity of these materials. While only a few studies have been noted here, other recently published review articles capture a broader range of studies that have been performed to evaluate the human health implications of exposure to nanoscale materials (Oberdorster et al., 2005; The Royal Society, 2004).

**Emerging Research Directions**

In fiscal year 2005, the NNI research and development budget for nanoscale materials is estimated at $1.08 billion (a 9% increase over the 2004 actual investment), and the fiscal year 2006 budget request is $1.05 billion across 11 agencies (Subcommittee on Nanoscale Science, Engineering and Technology, 2005). There are numerous commercial products that may benefit from incorporation of nanoscale materials in the near future. For example, one of the most promising uses for nanotechnology is the detection, diagnosis, and treatment of disease. Nanoscale materials could be used in diagnostic devices to detect diseases at earlier stages when treatment can be more effective and less costly. The fact that nanoscale materials are similar in size to biomolecules makes these materials ideally suited for use as markers for tracking biological molecules such as enzymes and receptor ligands. In addition, therapeutic devices and novel delivery systems based on nanoscale materials may target specific disease cells for treatment or elimination with fewer side effects. Nanoscale materials are already showing potential for use in the treatment of cancer and neurological and developmental disorders. Many Federal agencies have developed long-term programs to exploit the benefits of nanotechnology.

To balance the research leading to benefits from this technology, the total fiscal year 2006 NNI budget that will be devoted to research and development efforts whose primary purpose is to understand and address potential risks to health and the environment posed by this technology is estimated at $38.5 million (Subcommittee on Nanoscale Science, Engineering and Technology, 2005). These funds will be administered by nine agencies or departments including NSF, EPA, NIOSH, and NIH. Future activities will include the continuance of those already under way that are principally risk assessment research oriented (NIOSH, NTP, and some DOD initiatives, for instance), and other efforts such as those at the NSF nanotechnology center at Rice University and the National Cancer Institute, which also have risk-related components.

NIOSH will spend approximately $2.3 million in fiscal year 2005 for nanotechnology safety and health research coordination, including the following: generation and characterization of occupationally relevant airborne nanoparticles, pulmonary toxicity of carbon nanotube particles, role of carbon nanotubes in cardiopulmonary inflammation and related diseases, particle surface area as a dose metric, and characterization of ultrafine aerosols from diesel-powered equipment. NIOSH will have further deliverables that are aimed at establishing "recommended practices" for safe handling of nanoscale materials. In 2006, NIOSH will finalize the establishment of a Center of Excellence for Nanotechnology Research that will coordinate nanotechnology-related activities across the institute. EPA, NSF, NIOSH, and USDA plan an expanded joint extramural research program for 2005 and 2006 that addresses potential environmental, health, and safety implications of nanoscale materials, including development of standards for environmental and toxicological studies of nanoparticles and a metrology infrastructure supporting these standards.

Some of the Federal efforts noted previously have yielded results that advance the field and indicate directions for future research. For example, NIOSH researchers recently reported adverse lung effects in mice following exposure to SWCNTs using a dosing regime that can be correlated with the OSHA Permissible Exposure Limit (PEL) for graphite (Shvedova et al., 2005). The doses include a 20 μg/mouse dose that can be correlated with the dose deposited in a person being exposed to the graphite PEL for approximately 20 8-h workdays. This dosing regime is more relevant to risk assessment because the experimental exposures can be related to workplace exposures.
for other particulates that have PELs (as was done by Lam et al., 2004). The study also is the first one to use the pharyngeal aspiration method for dosing animals with carbon nanotubes (earlier SWCNT studies have used pulmonary instillation). While the advantages of pharyngeal aspiration have been reported previously (Rao et al., 2003), there remains considerable debate about the relevance of this dosing technique to human inhalation exposure. The reported advantages of pharyngeal aspiration over other approaches such as intratracheal instillation or inhalation dosing approaches include: 1) it causes minimal stress to test animals, allowing particulates to reach the lung in a physiologically relevant manner and resulting in good particle distribution in the lung; 2) it uses minimal amounts of test material; 3) it is repeatable and simple; and 4) resulting exposures are highly correlated with the administered dose. The findings of the Shvedova et al. study suggest that exposure to SWCNTs in mice leads to pulmonary inflammation and oxidative stress, followed by the development of multifocal granulomatous pneumonia and fibrosis. Finally, the study is also the first to demonstrate the effects of SWCNTs on bacterial clearance from the mouse lung.

New collaborations with the private sector are also forming to further toxicology testing and evaluations of nanomaterials. For example, the ILSI Health and Environmental Sciences Institute (HESI) has formed a consortium with representatives from government research laboratories, academic institutions, and multinational corporations to develop risk assessments for nanoscale materials. Initially, the focus of the consortium will be to evaluate the toxicity of nanoscale materials relative to their bulk counterparts and to explore the fate of nanoscale materials in the body on the basis of size.

In addition, the International Life Sciences Institute’s Risk Science Institute (ILSI RSI) formed a Nanomaterial Toxicity Screening Working Group to develop a screening strategy for the identification of hazards associated with engineered nanomaterials. The multidisciplinary working group is developing a report entitled “Principles for Characterizing the Potential Human Health Effects from Exposure to Nanomaterials: Elements of a Screening Strategy.” The report will present the elements of a screening strategy for evaluating the toxicity of engineered nanoparticles.

Other studies (Shimada et al., 2005) are emerging that provide an indication of the mechanisms involved in the translocation of nanoscale particles (14 nm diameter ultrafine carbon black) from the lung. Morphological alterations in endothelial cells of alveolar capillaries and other changes noted in electron microscopy images from this study suggest that pro-inflammatory chemokines may be responsible for morphological changes seen and may be involved in the movement of nanoscale particles through the airway-capillary barrier. Such studies may lead to better predictive capabilities for determining which tissues in the body, remote from the site of entry of nanoparticles, should be examined more closely for possible effects. While much of the nanoparticle effects research currently focuses on pulmonary effects, future research aimed at additional endpoints may be warranted, as has been the case for air pollutants. Linkages between particulate air pollutants and cardiovascular disease have been proposed (American Heart Association, 2004), as well as possible linkages between such pollutants and neurological and reproductive effects.

In addition to hazard concerns, a better understanding of exposures to nanoscale materials is necessary to gauge potential risks. Data have been generated by NIOSH that provide more detail on the type of airborne carbonaceous material that workers could be exposed to in facilities that handle unrefined carbon nanotube material. A 2004 NIOSH study (Maynard et al., 2004) indicated that an overall airborne concentration of nanotubes in facilities where SWCNT material was removed from production vessels and handled prior to processing was below 53 μg/m³, and that agitation of unprocessed SWCNT material in a laboratory setting resulted in a bimodal distribution of particles in the 10 to 1000 nm range. However, the study did not identify if any of the material fractions from either the laboratory or production facilities was associated more specifically with nanotubes, catalyst particles, or compact carbonaceous particles. Another study (Maynard, 2005) indicated that the airborne material from the laboratory agitation studies of unprocessed SWCNTs in the sub-100 nm mode is likely to be predominantly composed of nanoropes, but that compact non-tubular carbonaceous particles dominate the mode above 100 nm. Some nanoscale materials influence chemical reaction rates and may result in altered exposure scenarios to nanoscale materials and/or their by-products. For example, the use of nanoscale metal oxides is being explored as a treatment for halogenated organics and other chemical contaminants at hazardous waste sites. While nanoscale iron has been shown to be more efficient in the remediation of trichloroethene (TCE) than iron filings (Liu et al., 2005), a recent study on degradation of lindane by nanoscale zero-valent iron found biphenyl and trace amounts of benzene as by-products (Elliott et al., 2005). Beyond such assessments of altered exposure scenarios and by-product analyses, a more complete life-cycle evaluation of a nanoscale product from raw material acquisition through production to disposal could provide additional insights related to possible human health and environmental effects. It may also allow an evaluation of the inputs and outputs of materials and energy related to the development of products that contain the nanoscale material.

While sufficient information may not be available for nanoscale materials to allow a full life-cycle analysis at this time, toxicological and other information may soon be available to develop approaches for generating priorities and developing data to facilitate the assessment of nanoscale materials.

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