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## Recent Government Briefs

### **Request for Information on Carbon Nanotubes (CNTs) Including Single-Walled Carbon Nanotubes (SWCNTs) and Multi-Walled Carbon Nanotubes (MWCNTs)**

<http://www.gradientcorp.com/nano/documents/NIOSH-CNT.pdf>

On March 31, 2009, the National Institute for Occupational Safety and Health (NIOSH) published in the Federal Register a request for the submission of information, reports, and data regarding single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). These data are being requested from the public in order to compile information on the potential health risks of occupational exposure to carbon nanotubes. NIOSH is requesting information on published and unpublished results of toxicity bioassays, information on possible health effects observed in workers, information on workplaces and products in which CNTs can be found, description of work tasks and scenarios with a potential for exposure, workplace exposure data, and information on control measures that are being used in workplaces where potential exposures occur. Since the results of some *in vitro* and *in vivo* SWCNT and MWCNT studies have shown some adverse effects (e.g., cytotoxicity) and there is no estimate of the number of workers potentially exposed to carbon nanotubes, NIOSH seeks to obtain additional information to evaluate the possible health risks of occupational exposures to carbon nanotubes.

## Reports, Reviews, White Papers, and Books

### **Nanotoxicology: Characterizing the Literature, 2000-2007**

By AD Ostrowski, T Martin, J Conti, I Hurt, NH Harthorn  
<http://www.springerlink.com/content/1841012pq621170u/>

The authors of this article used several scientific literature-based search engines (e.g., Medline, Chemical Abstracts Service) to characterize the prevalence and distribution of nanotoxicology research in the current scientific literature. Using a search strategy based on previous studies, the authors examined the relative distribution of published nanotoxicology research across the areas of human health and the environment. They identified endpoints in human health and the environment that are most emphasized, exposure pathways that are frequently researched, and which stages of the nanomaterial life-cycle have been addressed in toxicological research. The authors identified 900 nanotoxicology articles in 58 peer-reviewed journals, a 600 percent increase since 2000. Most studies, across all specified nanomaterial types, relied on *in vitro* bioassays. There were few studies on semiconductors, none of which examined environmental and ecological effects. Also, most studies did not specify a relevant exposure pathway for the nanomaterial in question – when an exposure pathway was specified, inhalation was the most researched pathway. Mammalian tissues and organisms were the most commonly used cell/tissue type. Most studies focused on acute toxicity and mortality rather than chronic effects. Last, the authors found no evidence of research on the toxicological effects and environmental fate of consumer products containing nanomaterials; most studies focused on basic nanomaterials.

### **The Impact of Toxicity Testing Costs on Nanomaterial Regulation**

By JY Choi, G Ramachandran, M Kandlikar  
<http://pubs.acs.org/doi/abs/10.1021/es802388s>

This paper analyzed the impact of testing costs of nanomaterials, the ability to gather information on nanoparticle toxicity, and whether the emerging nanotechnology industry can afford these costs. The authors constructed a data set for all users of nanomaterials, and estimated the cost of nanomaterial-specific research and development. Next, they developed cost estimates of hazard assessment-related studies (e.g., *in vitro* and *in vivo* studies)

for currently used nanomaterials, and compared the amount to the sum spent on actual research and development. Based on this methodology, total costs for testing all existing nanoparticles would range from \$249 million to \$1.2 billion; these costs vary due to different assumptions on the degree of testing required for each nanoparticle. They also noted that it would require three to five decades to thoroughly test all existing nanomaterials. This analysis assumed existence of "testing tiers" (criteria based on amounts released to the environment) similar to that used in the European Union under REACH legislation. The authors concluded that in order for testing tiers to work, a nanomaterial classification scheme is required, which in turn requires additional information on exposures and toxicity.

### **DEFRA-funded review of completed and near completed environment, health, and safety research on nanomaterials and nanotechnology.**

[http://www.safenano.org/Uploads/EMERGNANO\\_CB0409\\_Full.pdf](http://www.safenano.org/Uploads/EMERGNANO_CB0409_Full.pdf)

Recently, the UK Department for Environment, Food, and Rural Affairs (DEFRA) funded a study, conducted by several universities and private groups, that reviewed active research into the environment, health, and safety risks of nanotechnology. Their review noted important progress in the areas of characterization, exposure, human toxicology, and ecotoxicology of nanomaterials research. It summarized the findings of 260 studies and analyses that examined the environmental and health aspects of engineered nanoparticles. The authors identified and categorized all relevant studies since 2004, evaluated each study for overall contribution to pre-defined research areas (these eighteen areas include subjects such as characterization, exposure measurement, environmental fate, and cellular toxicity), undertook a risk management appraisal, and made an assessment of remaining gaps and future priorities. The study noted imbalance in all eighteen research areas. For example, it cited that the largest number of nanomaterial studies focused on toxicology and exposure, with a substantially lower number of studies addressing environmental effects and characterization of nanomaterials. The authors also stated that the few available characterization- or standardization-based studies are not conclusive enough to provide new approaches in setting characterization/measurement guidelines. Last, they pointed out that the review indicates that silver nanoparticles, titanium dioxide nanoparticles, and carbon nanotubes may have an adverse effect on human health or the environment, and recommended further investigation into these materials.

## **Upcoming Meetings and Conferences**

### **International Conference on the Environmental Implications and Applications of Nanotechnology**

<http://www.umass.edu/tej/conferences/nanoconference/>  
June 9-11, 2009, Amherst, Massachusetts

Hosted by the University of Massachusetts Environmental Institute and the US EPA Office of Superfund Remediation and Technology Innovation, this conference will bring together researchers and practitioners to address the environmental implications and applications of nanotechnology. The three-day program will include presentations, poster sessions, and an exhibitor hall. Topics to be covered at the conference include materials characterization, green nanotechnology, regulatory issues, environmental fate and transport, toxicology, and pollution control.

### **The Nanotechnology Health & Safety Forum**

<http://www.nhsf2009.org/index.asp>  
June 8-9, Seattle, Washington

Sponsored by Battelle, the University of Washington, the University of Oregon, Oregon State University, and several private companies, the Nanotechnology Health & Safety Forum (NHSF) will focus on occupational health, environmental health, and safety standards of nanotechnology. The agenda will be broken down into five units that will cover the different aspects of nanotechnology health and safety, with topics to include international nanotechnology standards, international regulatory dialogue, nanomaterial state-of-the-science, management of nanomaterial risk, and current activities of new nanomanufacturers. Keynote speakers and panelists include individuals from academia, the National Nanotechnology Coordination Office, former legislators, nanotechnology interest groups, and the National Institute for Occupational Safety and Health (NIOSH). NHSF coincides with the committee meeting of the American National Standards Institute (ANSI)/International Organization for Standardization (ISO) Technical Committee on Nanotechnologies, also to be held in Seattle, June 8-12. NHSF is a separate event, but will cover similar topics, specifically environmental/occupational standards.

### **Joint FAO/WHO Expert Meeting on the Application of Nanotechnologies in the Food and Agriculture Sector: Potential Food Safety Applications**

[http://www.fao.org/ag/agn/agns/expert\\_consultations/Nanotech\\_EC\\_Scope\\_and\\_Objectives.pdf](http://www.fao.org/ag/agn/agns/expert_consultations/Nanotech_EC_Scope_and_Objectives.pdf)  
June 1-5, Rome, Italy

There are a number of applications of nanotechnology that can affect agriculture, food processing, food packaging, and consumption of food products. The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) are convening this expert meeting to identify issues, review current literature, and develop guidance for nanomaterials in the food industry. The scope of the meeting will cover application of nanotechnologies used in primary food production, packaging, and distribution.

Nanotechnologies directly applied in the environment will be included if evidence of impacts on food safety are demonstrated during the course of the meeting. The expert meeting will inventory applications and identify health implications of nanotechnology used in the agricultural and processing sector, identify data gaps and tools/metrics to measure impacts, evaluate current risk assessment methodologies for assessing the safety of nanomaterials used in the food chain, and identify priority areas for which scientific advice regarding nanomaterials should be requested from FAO and WHO. The expert meeting will also provide an analysis of efforts that have been made to promote communication among nanotechnology stakeholders. This expert meeting will *not* cover occupational health matters, although these topics may be noted for further consideration.

## Hot-off-the-Presses Peer-Reviewed Research Articles of Note

### **1. Rothen-Rutishauser, B., et al. 2009. Direct combination of nanoparticle fabrication and exposure to lung cell cultures in a closed setup as a method to simulate accidental nanoparticle exposure of humans. *Environ Sci Technol* 43(7): 2634-2640.**

**Abstract:** <http://pubs.acs.org/doi/abs/10.1021/es8029347>

#### **Synopsis:**

- Given the vast number of commercially available engineered nanoparticles that have varying chemical-physical properties and potential toxicities, it is well-recognized that pre-screening is an essential step in identifying and prioritizing nanoparticles for *in vivo* toxicity testing. Together with detailed physical-chemical characterization, *in vitro* assays are a commonly used pre-screening tool for engineered nanoparticles. However, *in vitro* assays are considered to have a number of significant limitations, including the fact that particles are generally added to cell cultures as suspensions in liquid, thus potentially altering the particles' properties and poorly mimicking the physiological condition of lung epithelial cells. This study was conducted to address this limitation, directly combining the generation of cerium oxide nanoparticles with exposures to cell cultures at the air-liquid interface within a glovebox. Cerium oxide nanoparticles are a commercially important nanoparticle due to their applications in polishing and computer chip manufacturing.
- Within a closed glovebox apparatus, cerium oxide nanoparticles were generated by flame spray pyrolysis, with simultaneous exposure of cultured A549 lung cells. To allow for air-liquid interface exposures, cell culture plates were opened for increasing lengths of time, resulting in the following number/mass deposition at the cell surface:  $0.95 \times 10^{12}$  particles/cm<sup>2</sup>  $\approx$  0.012 mg of ceria/cm<sup>2</sup> for 10 min,  $1.43 \times 10^{12}$  particles/cm<sup>2</sup>  $\approx$

0.019 mg of ceria/cm<sup>2</sup> for 20 min, and  $1.9 \times 10^{12}$  particles/cm<sup>2</sup>  $\approx$  0.024 mg of ceria/cm<sup>2</sup> for 30 min.. Following particle exposures, cell cultures were incubated for an additional 24-hours at the air-liquid interface. A suite of cellular analyses were then conducted, including cytotoxicity *via* release of lactate dehydrogenase (LDH), transepithelial electrical resistance (TEER), structure of tight junctions (TJ), oxidative DNA damage, volume density of lamellar bodies, and cell morphology using laser scanning microscopy (LSM) as well as transmission electron microscopy (TEM). Particle samples were also collected for measurement of hydrodynamic particle size distribution, shape, and agglomerate morphology.

- Based on the hydrodynamic particle size measurements, cerium oxide nanoparticles were found to follow a log-normal distribution, with a mean hydrodynamic diameter of 19 nm and a geometric standard deviation of 1.49. Based on particles collected in TEM grids within the glovebox apparatus, homogenous distributions of cerium oxide nanoparticles were observed, with few aggregates. Cellular analyses revealed little evidence of cytotoxicity or impairment of cell viability, but dose-dependent responses were observed for the tightness of the lung cell monolayer, the mean total lamellar body volume, and the generation of oxidative DNA damage.

Overall, while some dose-dependent cellular responses were observed, neither a cytotoxic reaction nor a remarkable change in the cytoskeleton or cellular ultrastructure was observed in the epithelial cells, and the authors concluded that "these results show that the cells were not damaged by the exposure in the glovebox."

#### **Implications:**

- By directly combining NP production with cell culture exposures, this study demonstrated a more realistic and reproducible *in vitro* exposure system for investigating the potential toxicity of engineered nanoparticles. A key advantage of this exposure system compared to more conventional suspension experiments involves its capability to better simulate the degree of NP agglomeration, size, and surface coating that could arise during an accidental exposure situation at a manufacturing or handling site. In addition, it allows for the precise determination of exposure doses.
- By relying on air-liquid interface exposures, this exposure system offers a more sensitive technique for screening nanoparticle toxicity, given that previous work has demonstrated that air-liquid interface exposures can elicit responses at doses several orders of magnitude lower than those for exposures to particles in suspensions.
- While some dose-dependent cellular responses were observed, including decreased epithelial tightness, additional research is needed to elucidate the mechanisms underlying these responses. In addition, additional testing of other nanoparticle types in this exposure system is needed to understand the biological significance of the observed responses for the cerium oxide nanoparticles. Similar responses (e.g., oxidative stress responses and altered gene

expression) have been observed *in vitro* assays of other nanoparticle types, but the findings from these studies cannot be compared to those of the present study due to the differences in the exposure systems and uncertainties in the exposure doses of suspension experiments. Furthermore, like any *in vitro* assay, validation with *in vivo* toxicity testing is also needed.

**2. Phenrat, T., et al. 2009. Partial oxidation (“aging”) and surface modification decrease the toxicity of nanosized zerovalent iron. *Environ Sci Technol.* 43 (1), 195-200. Abstract:**

<http://pubs.acs.org/doi/abs/10.1021/es801955n>

**Synopsis:**

- A consistent body of evidence has shown that the high surface to volume ratio, and small particle size of certain engineered nanomaterials (ENMs) such as metal oxides and zerovalent iron make them powerful materials for remediating contaminants such as PCBs, PAHs and arsenic. However, the same properties also influence biological interactions between ENMs and cellular targets. Increased chemical activity of ENMs relative to their macro-size counterparts has been attributed to increased generation of reactive oxygen species (ROS), resulting in inflammation, oxidative stress, and cytotoxicity to the cells. For example, surface free radicals on insoluble ENMs retained in the lungs can cause oxidative stress as well as recruit an excessive number of host defense cells into the lungs, which can cause inflammation and fibrosis.
- In addition to size, the redox activity (ability to generate electrons), which is dependent on the oxidation state of the element, is also known to influence the ability of ENMs such as zerovalent iron to generate ROS. In this work, nano zerovalent iron (nZVI) was used to assess the effect of physical properties and oxidation state on toxicity to mammalian cells (microglia and neurons). Oxidative stress was assessed using mouse microglia, and neurotoxicity was assessed using isolated mouse N27 neurons. These cell types were selected based on their previous use in determining oxidative stress-induced brain damage associated with interaction to particulate matter.
- To enhance long-term stability of ENMs as individual nano-sized particles rather than as aggregates, and to increase particle mobility, surface modification techniques using polymer coatings are being increasingly used in recent years. Two surface modified (SM-nZVI) particles coated with a sodium polyaspartate polymer were used in this study, in addition to fresh (not “aged” or oxidized), “aged” or oxidized nZVI and magnetite (iron oxide). One of the SM-nZVI particles was commercially available, while the other was laboratory generated using a similar procedure as the commercially available SM-nZVI. Since the physical properties of the commercially available SM-nZVI was unknown due to proprietary reasons, both the materials were used to account for any possible differences between the two SM-nZVI particles.
- Cells were exposed to suspensions of nZVI and iron oxide in both reduced serum (1%) exposure media and Hank’s balanced salt solution (HBSS), at exposure times ranging from 1-24 hours. Oxidative stress and neurotoxicity was determined by analyzing ROS and intracellular adenosine triphosphate (ATP) levels in microglia and neurons, respectively, exposed to nZVI particles. Particle size and sedimentation rate were also measured to determine the effect of agglomeration and sedimentation on cellular responses.
- Particle size measurements (taken during the exposure period) in salt solution and cell culture media clearly indicated that surface modification of nZVI improves particle dispersion in aqueous media, resulting in less agglomeration. Aged nZVI agglomerated faster than fresh nZVI, while polymer coated nZVI agglomerated the slowest due to electrostatic stabilization provided by the polymer. The particle agglomeration rate and thereby particle size increased with time initially. Subsequently, larger particles fell out of solution due to sedimentation, resulting in an apparent decrease in the particle hydrodynamic radius. Zeta potential measurements indicated surface charge of all nZVI particles was mildly positive and varied based on the Fe<sup>0</sup>/iron oxide content in the particles.
- Based on measurement of hydrogen peroxide (an indicator of oxidative stress), generation of intracellular ROS occurred in microglia exposed to fresh nZVI and aged nZVI but not in microglia exposed to SM-nZVI. The cellular response to aged nZVI was delayed, compared to the response to fresh nZVI. Both SM-nZVI behaved identically in the study. Decreases in intracellular ATP, an indicator of “apoptosis” or cell death, was also observed immediately in response to fresh nZVI, while it was negligible for aged nZVI, magnetite and SM-nZVI. Response of N27 neurons to the nZVI particles and magnetite was determined by measuring intracellular ATP levels. Neurotoxicity of the test particles decreased in the order: fresh nZVI > SM-nZVI > aged nZVI = magnetite. Traces of both fresh nZVI and SM-nZVI were observed within the nuclei of the neurons as observed using scanning and transmission electron microscopy.

**Discussion and Implications:**

- The toxic response to fresh nZVI was greater and occurred more rapidly in both microglia and neurons, when compared to “aged” nZVI or iron oxide. This study also demonstrated that higher elemental iron content resulted in increased toxicity to both microglia and neurons. Fresh nZVI contains a greater amount of elemental iron (as Fe<sup>0</sup>), compared to “aged” nZVI and iron oxide, and would therefore be more chemically reactive, and could subsequently generate higher levels of ROS. Exposure to SM-nZVI resulted in negligible oxidative stress. The reduced oxidative stress observed with SM-nZVI could be attributed to the effect of the polymer coating, which mitigated the redox activity of the iron. The lower ROS generation observed with exposure to SM-nZVI also indicates that agglomeration and sedimentation (settling rate) may also

affect toxicity of ENMs. Since fresh nZVI agglomerated faster than SM-nZVI, fresh nZVI would have had contact with the cells for much longer than SM-nZVI, which may have contributed to the increased oxidative stress observed with fresh nZVI.

- In contrast to generation of ROS, neurotoxicity of SM-nZVI was moderately greater compared to that of “aged” nZVI. The authors propose that, unlike “aged” nZVI, SM-nZVI particles may have a greater ability to interact with cells due to the biopolymer coating, which is known to facilitate such interactions. The ability of the biopolymer coating to facilitate interaction between the SM-nZVI and cells may hence contribute to relatively greater toxicity of SM-nZVI compared to “aged” nZVI, as well as the presence of SM-nZVI inside the neurons.
- Nanomaterials such as nano zerovalent iron (nZVI) are gaining prominence for remediation of a variety of organic and inorganic contaminants. The key to the successful development of this remediation technology is the ability of particles to travel long distances and reach contaminated areas. In the environment, natural colloids and ENMs interact among themselves to form larger particles (*i.e.*, they agglomerate), which inhibits their mobility. While surface modification and “aging” or oxidation of nZVI reduced its toxicity, modification also facilitated entry of SM-nZVI into the cells. Such observations may have long-term biological consequences, which can not be determined from short-term exposure studies. Understanding the effect of surface modification on physical properties of ENMs, and subsequent toxicity is critical to understanding their potential environmental impact.

## Guest Contributor

by Frank Leone

Nanotechnologies have been incorporated into hundreds of consumer products, including toys, baby products, fitness equipment, home and garden products, clothing, appliances, electronics, and computers that the US Consumer Product Safety Commission (CPSC) regulates. Such products include pacifiers, teddy bears, socks, pillows, and refrigerators, all containing nanosilver bactericide (See <http://www.nanotechproject.org/inventories/consumer/>). Although much attention has been focused on the potential regulation of nanotechnology by the much larger US Environmental Protection Agency (TSCA chemical and FIFRA pesticide regulations) and the US Food and Drug Administration (drug and cosmetic regulations), CPSC may find itself on the front line of nanoregulation issues.

Somewhat coincidentally, Congress last August adopted the Consumer Product Safety Improvement Act of 2008 (CPSIA or the Act), Pub. L. No. 110-314, which implements the most sweeping revision of United States consumer product safety laws since 1972. The Act expands the CPSC’s regulatory manufacturers, importers, and retailers of consumer products.

The Act does not specifically address nanoproducts, but it will affect potential CPSC regulation of such products.

Congress drafted the CPSIA “in anger” resulting from a number of high-profile product safety recalls, most notably recalls of Chinese-manufactured jewelry and painted toys that contained excessive, and in some cases dangerous, amounts of lead. The Act addresses toys and children’s products, and, over a short time period, (1) lowers permissible lead levels in paint; (2) imposes maximum permissible limits for lead in product substrates and components; (3) bans certain uses of six phthalates (plasticizers); and (4) incorporates an ASTM (American Society for Testing and Materials) toy standard as a CPSC rule. Furthermore, the CPSIA added new requirements governing children’s products, including for testing and certification of compliance with regulations, use of tracking labels, and warnings in connection with advertisements.

The CPSIA also imposes additional new requirements affecting all consumer products (not just children’s products), including greater CPSC recall authority, mandatory recall notice standards, broadened reporting requirements, adoption of a class-wide product hazard list, creation of a publicly accessible Consumer Product Safety Database identifying harmful products, weakened protections for preventing public disclosure of confidential business information, State Attorney General enforcement of standards through injunctive relief, increased civil and criminal penalties for violations, a requirement for a GAO (Government Accountability Office) study of formaldehyde, and limitations on preemptive effects of consumer protection statutes.

As an initial matter, although the CPSIA did not impose any specific regulations on nanoproducts, Congress in passing the Act made it clear that it can and will impose substance-specific limitations and bans when it believes that such actions are appropriate. Congressional action need not comply with notice and comment requirements and is subject to limited judicial review; Congress therefore can take implement aggressive actions without the strong scientific evidence required of agency action.

The initial Senate version of the Act (S. 2663, sec. 3(d)) provided \$1 million for CPSC research on nanoproducts, but this provision was dropped from the final statute. CPSC may seek to use its limited scientific research facilities to address nanoproducts, but more likely the agency will continue to participate in the National Nanotechnology Initiative and benefit from toxicity and exposure research carried out by better funded government agencies. The CPSIA requires CPSC to convene a Chronic Hazard Advisory Panel (CHAP) regarding phthalates and consider the CHAP’s findings in evaluating additional regulation. It has been suggested that CPSC also convene a CHAP at least regarding children’s nanoproducts, but the agency is unlikely to do so anytime soon.

The CPSIA streamlined the agency process for adopting products safety standards, regulations, labeling requirements, and product bans. CPSC currently appears to be fully occupied with implementing specific CPSIA provisions, along with its usual focus on children’s hazards (*e.g.* choking), and fire, electrical and mechanical hazards. But the agency may increase its involvement in voluntary standards organizations with the goal of eventually

adopting standards for nanoproducts. Once such standards are adopted, manufacturers will have to certify compliance with the standards and violation of such standards would subject a product to recall.

CPSA Section 15(b) requires manufacturers, importers, distributors and retailers to notify CPSC immediately if they obtain information reasonably supporting the conclusion that a consumer product distributed in commerce (1) failed to comply with any CPSC rule or with a voluntary consumer product safety standard upon which CPSC has relied as a consumer product safety rule; (2) contained a defect which could create a substantial product hazard; or (3) created an unreasonable risk of serious injury or death. 15 U.S.C. § 2064(b). These provisions currently apply to all consumer products, including those containing nanomaterials.

At about the same time Congress was enacting the CPSIA, the Woodrow Wilson Center Project on Emerging Nanotechnologies issued its report on the CPSC and Nanotechnology (<http://www.nanotechproject.org/publications/archive/pen14/>), which concluded that CPSC lacked the budget, staff, and authority to regulate nanoproducts properly. CPSIA may add have addressed some of these concerns, but CPSC still lacks explicit authority to require manufacturers to identify the presence of nanomaterials in their products and to promulgate standards for new products where there exists a lack of information adequate to determine the products' safety. New legislation regarding CPSC regulation of nanoproducts is unlikely in the near future. Nevertheless, as more nanoproducts enter the market, manufacturers must be aware of CPSC's current authority over such products.

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