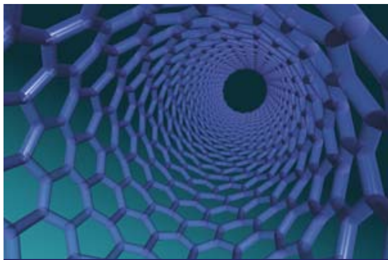
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Dr. Beck directs Gradient's nanotechnology, toxicology, and risk assessment practices.

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In This Issue

Recent Government Briefs

USEPA Draft Nanomaterial Research Strategy (NRS)

United States Environmental Protection Agency, Office of Research and Development

http://es.epa.gov/ncer/nano/publications/nano_strategy_012408.pdf

This strategy was developed as a guide to US EPA's Office of Research and Development (ORD) for evaluating and assessing the extent to which nanomaterials and products potentially affect the environment and human health. In this paper, ORD has identified four key nanomaterials research themes: sources of nanomaterials, research to develop risk assessment and test methods, case studies, and preventing and mitigating risks. From these themes, the authors raise key nanomaterials science questions. For each science question, the document addresses the topic background and relevance, describes the proposed research activities, and discusses the anticipated outcomes. Example questions include topics such as nanomaterials fate and transport, detection of nanomaterials in the environment, types of nanomaterial exposures, and how risk assessment needs to be modified to account for nanomaterial properties. The expected end result from the NRS will be guided research products that can be further used to address risk assessment and management needs for nanomaterials.

National Nanotechnology Initiative: Strategy for Nanotechnology-Related Environmental, Health, and Safety Research

http://www.nano.gov/NNI_EHS_Research_Strategy.pdf

In this report, the Nanotechnology Environmental Health and Implications Working Group lays out a framework to guide and address a nanotechnology research program that supports risk-based decision-making. The framework describes the strategy for addressing priority research on the environment, health, and safety aspects of nanomaterials. The authors analyze current research in five major research categories: instrumentation and analytical methods, nanomaterials and human health, nanomaterials and the environment, human and environmental exposure assessment, and risk management methods. They identify research needs, such as developing methods of detecting nanomaterials in biological matrices, understanding how chemical and physical modifications of nanomaterials affect their properties, developing methods for standardizing assessment of nanoparticle size and geometry, developing reference materials to characterize nanomaterials, and developing materials to characterize nanomaterial composition and heterogeneity. The discussion of data gaps and needs is accompanied by a recommended implementation strategy and an adaptive management process. This strategy, which specifies roles for each government agency, is the next step in the multi-level federal effort.

Reports, Reviews, White Papers, and Books

Warheit, D.B. 2008. "How meaningful are the results of nanotoxicity studies in the absence of aqequate material characterization?" *Toxicological Sciences* 101(2): .183-185.

This comment paper stresses the critical importance of adequately characterizing nanomaterials before exploring their effects in toxicological studies. This is a necessary step for nanotoxicological research particularly since physico-chemical properties (*e.g.*, size, surface area, surface chemistry, crystal structure) can vary widely between different types of nanomaterials, such that there may be large differences in their reactivity and biological effects. Nanoparticles can be of the same parent material but differ in structure; this can result in widely varying cytotoxic effects. In addition, studies (*e.g., in vitro* experiments) will often administer nanomaterials in a state not representative of how occupational or environmental exposures may actually occur. Studies that do use nanoparticles in other-than-dry states may induce agglomeration of nanoparticles, which can affect toxic potential as well. Despite the emerging body of findings that indicate the influence of various nanomaterial physical-characteristics on toxic potential, many recently published studies have estimated nanoparticle size and other characteristics only using unverified manufacturer data or other limited measurements that provide few robust data on the particles in question. Due to the potential for such unrepresentative exposures and effects, Dr. Warheit concludes that nanoparticle studies should adequately characterize particle size and distribution, crystal structure, aggregation status in relevant media, surface coatings, surface reactivity, method of synthesis, and purity before toxicity studies are conducted.

Application of the Toxics Release Inventory to Nanomaterials

Linda K. Breggin, Esq., and Read D. Porter, Esq., of the Project on Emerging Nanotechnologies, Woodrow Wilson Center for International Scholars
http://www.nanotechproject.org/process/assets/files/6088/brief2_eli_2_5_08.pdf

This research brief examines whether nanomaterials production and use falls under public chemical release inventories and community right-to-know laws. Upon an initial review of the statutory authority of the Toxics Release Inventory (TRI), the authors conclude that nanomaterials could fall under TRI jurisdiction. In order to trigger TRI requirements, nanomaterials would have to be identified as toxic by the US EPA; currently no nanomaterials have received this designation. Another key issue to the application of TRI reporting to nanomaterials is exactly how nanomaterials should be classified, since some nanomaterials are benign in their bulk form and potentially hazardous in their nanoscale form. In addition, reporting thresholds would need to be modified and new industrial classifications would need to be added to accommodate the unique nature in which nanomaterials are manufactured/used. The authors urge additional research and dialog with stakeholders to determine if applicability of TRI to nanomaterials is a policy priority.

Upcoming Meetings and Conferences

2nd International Conference on Nanotoxicology

September 7-10, 2008, Zurich, Switzerland
http://www.nanotox2008.ch

Hosted by the University of Zurich and the Swiss Federal Institute of Technology, this conference aims to address the hazards of and exposure to nanomaterials. Participants, who will include the public and stakeholders, will have the chance to attend panels on the biological effects of nanomaterials and learn about the development of new methods and standardization processes. The conference program will cover topics such as exposure to and uptake of nanomaterials, the biological effects and mechanisms of nanomaterials, and the properties of special materials associated with nanotechnology.

Risk, Uncertainty and Decision Analysis for Nanomaterials: Environmental Risks and Benefits and Emerging Consumer Products

April 27-30, 2008, Lisbon, Portugal
http://www.risk-trace.com/portugal2008/

Presented by the North Atlantic Treaty Organization (NATO), the Society for Risk Analysis (SRA), and other trade and government groups, this conference aims to bring together those interested in minimizing exposure to and liability from nanomaterials. Its goal is to identify issues in emerging nanotechnology applications which may have environmental and health implications. The workshop will examine the decision analyses used to weigh benefits and risks of nanotechnology, discuss future research options of emerging nano-enabled products, and ascertain nanomaterial management strategies for developing countries.

The Second Saint-Petersburg International Conference on NanoBiotechnologies

June 16-18, 2008, St. Petersburg, Russia
http://www.spbcas.ru/nanobio/

Hosted by several governmental and academic groups in Russia, Nanobio'08 is intended to showcase the latest R&D breakthroughs in nanotechnology, as well as discuss problems and define future trends. Biologists, engineers, physicists, and computer scientists will have the chance to bridge the gap between materials science and life science in an international forum. Topics discussed will include nanomaterials design, current and future applications of nanomaterials, and human health and environmental risk assessment implications of nanotechnology. The program will consist of oral, poster and position presentations.

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

EJ; Huang, Q; Weber, WJ. 2008. "Ecological uptake and depuration of carbon nanotubes by Lumbriculus variegatus." *Lumbriculus variegatus." Environ. Health Perspect.* 116(4):496-500. Abstract:
http://www.ehponline.org/docs/2008/10883/abstract.html

Synopsis:

- Although the cellular uptake and ecotoxicological effects of carbon nanotubes have been documented, no quantitative studies have been performed to investigate the bioaccumulation potential of carbon nanotubes. This study determined the bioaccumulation of single-walled carbon nanotubes (SWNTs), multi-walled carbon nanotubes (MWNTs), and pyrene [(a three ringed polycyclic aromatic hydrocarbon (PAH) for comparison with the nanotubes]. Both update and depuration experiments were conducted using a freshwater oligochaete (Lumbriculus variegates), which is a standard test organism for determining a substance's bioaccumulation potential.

- Carbon-14 (C^{14}) labeled SWNTs and MWNTs were synthesized using a novel chemical vapor deposition method, and characterized using transmission electron microscopy (TEM) and thermal gravimetric analysis (TGA) for impurities. TEM micrographs and TGA analyses confirmed the purity of the nanotubes, showing carbon percentages after purification of 92.0±0.4% for SWNTs and 99±1% for MWNTs. Diameters typically ranged from 1 to 2 nanometers for SWNTs and from 30 to 70 nanometers for MWNTs.
- Uptake experiments were conducted according to US EPA Method 823; worms were exposed to sediments separately spiked with C^{14} SWNTs (0.03 or 0.003 mg/kg dry sediment), C^{14} MWNTs (0.37 or 0.037 mg/kg dry sediment), and C^{14} Pyrene (0.054 mg/kg dry sediment). Biota-sediment accumulation factors (BSAFs)—the ratio of a substance's concentration in an organism normalized by the organism's lipid fraction to the substance's concentration in the sediment normalized by the sediment's organic carbon fraction—were determined after particular exposure intervals. In depuration experiments, worms were exposed to either nanotubes or pyrene for 14 or 28 days, and were then transferred to clean water or clean sediment for BSAF determination at one day intervals for up to three days.
- No increased mortality (compared to controls) of the worms was observed for the experimental conditions of this study. Worms exposed to sediments spiked with SWNTs, MWNTs, and pyrene for 28 days had BSAFs of 0.28±0.03, 0.40±0.1, and 3.6±0.2, respectively.
- When organic carbon content of sediment was decreased by a factor of eight, BSAF values decreased from 0.51±0.09 to 0.035±0.015 after 14 days of exposure. BSAF values were not found to significantly change with changes over several orders of magnitude in the concentrations of SWNTs and MWNTs used to spike the sediments.
- After about three days of depuration in clean water, the worms had purged >80% of carbon nanotubes that remained following the initial 6 hours of depuration, while only 13% of pyrene was excreted over this time period. Depuration rates of MWNTs in clean sediment and water were significantly higher than in the clean water alone; after two days of depuration in clean sediment and water, carbon nanotube concentrations in the worms were below background levels.

Implications:

- This study presents a novel synthesis method for production of C^{14} labeled carbon nanotubes and their use in quantitative determination of bioaccumulation potential of carbon nanotubes. The approach used in this study thus provides a reliable method for future studies to quantify various ecological effects of carbon nanotubes with varying properties.
- With observed BSAF values that were almost an order of magnitude less than those for pyrene, study findings indicate that the bioaccumulation potential of carbon nanotubes to likely be significantly reduced compared to that of PAHs.
- Depuration experiments provide evidence that nanotubes detected in the worms were associated with sediments remaining in the organism guts rather than absorbed into cellular tissues, as was observed for pyrene. These findings thus suggest that carbon nanotubes may not be readily absorbed into cellular tissues. However, ecological effects due to interactions between carbon nanotubes and other contaminants need to be studied given the strong sorptive properties of carbon nanotubes and their potential influence on the bioaccumulation and fate of other pollutants.

Tin-Tin-Win-Shwe; Mitsushima, D; Yamamoto, S; Fukushima, A; Funabashi, T; Kobayashi, T; Fujimaki, H. 2008. "Changes in neurotransmitter levels and proinflammatory cytokine mRNA expressions in the mice olfactory bulb following nanoparticle exposure." *Toxicology and Applied Pharmacology* 226: 192-198.

Abstract: http://tinyurl.com/6gtrzx

Synopsis:

- This study is one of the first to explore the effects of nanoparticles and possible synergism with other compounds in the brain. The authors investigated the *in vivo* effects of nasal exposure to nanoparticle carbon black (CB) and lipoteichoic acid (LTA), a cell wall component found in some bacteria. They measured changes in neurotransmitter levels and proinflammatory cytokine expressions in the olfactory bulb of mice, looking for possible potentiating effects caused by these two compounds.
- 14-nm CB particles, suspended in a saline solution, were intranasally instilled into male BALB/c mice. LTA, obtained from *Staphlococcus* bacteria, was injected intraperitoneally. There were four groups in the study: the CB-control and LTA-control group (VS), the CB-control and LTA-treated group (VL), the CB-treated and LTA-control group (CS), and the CB- and LTA-treated group (CL).
- CB (or control solution) was administered at hour zero of the experiment. LTA (or control solution) was administered six hours into the experiment. A microdialysis probe was used to continuously collect extracellular fluid from the olfactory bulb over a seven-hour period. The experiment was terminated 11 hours after CB was administered. Olfactory extracellular fluid from each group was measured for concentrations of glutamate and glycine, which are amino acids created by cells to form necessary

proteins. At the end of this period, olfactory bulbs were collected to measure proinflammatory cytokine (IL-1β, TNF-α, CCL2, and CCL3) mRNA expression.

- Significant differences in glutamate and glycine production were observed among the four treatment groups. For the group treated with CB *and* LTA, there was a significant increase of glutamate and glycine production (compared to the groups receiving only one or none of the treatments). For this same group, increases over time were also significant. For the group treated with CB *without* LTA, glutamate and glycine concentrations were significantly higher than the group not treated with CB. For this same group, however, glutamate and glycine levels did not significantly increase over time.
- After olfactory bulb collection, IL-1β mRNA expression was increased in the CL group over other groups. In CB-treated groups, the CL group showed a significant increase of IL-1β over the CS group. TNF-α was increased only in the CS group when compared to the VS group; TNF-α was not affected by LTA injection. No significant differences for CCL2 and CCL3 were observed in any of the groups.

Implications:

- This was one of the first studies to show the *in vivo* effect of nanoparticles on the extracellular amino acid neurotransmitters and proinflammatory cytokines. The results suggest that exposure could affect neurotransmitter levels, which are pivotal in neural development, learning, and memory.
- Glutamate is a major excitatory neurotransmitter. Excessive increases can trigger the death of neural cells. Excess glutamate can also affect the immune system by causing the invasion of macrophages and T-cells. This study demonstrated that nanoscale carbon black can increase glutamate levels; these increases can be further exacerbated by injection of LTA, which is found in bacterial cell walls.
- This and other studies demonstrate a potential for environmental toxins to induce neural inflammation, although it is unknown as to whether environmental exposures could be of sufficient magnitude or duration to have such effects. It is speculated that these toxins may play a role in triggering neurodegenerative diseases such as Parkinson's and Alzheimer's disease, although much research is needed to determine if there could be a causal link between neurotransmitter and proinflammatory cytokine expression in neurodegenerative diseases.
- Nonsynaptic communication plays a role in regulating immune responses. Increases of proinflammatory cytokines in CB-treated groups demonstrate that immune cells are modulated by released neurotransmitters. This suggests that there are potential immuno-modulating effects of certain nanoparticles such as CB, although much research is necessary to evaluate the relevance of these findings to human exposures.

Guest Contributor

By Anna A. Shvedova, PhD, D.Sc, FATS
Senior Scientist, Health Effects Laboratory Division, National Institute for Occupational Safety and Health

Nanotechnology Environment, Health, and Safety from the Research Bench

Nanomaterials, with their unique physico-chemical properties, form the basis for numerous novel technologies and applications that are expected to drive the next industrial revolution. Fast propagation of nanotechnologies into different industries and consumer products is causing exponential growth of nanomaterials production. As a consequence, large amounts of nanomaterials may reach the environment, intentionally or not, thus dictating the need to assess potential impacts and consequences. These same unusual properties may underlie unique biological activities, but insufficient research has been undertaken to explore their potential effects on human health and in particular on immunological effects.

There are considerable gaps in our knowledge concerning the potential hazardous effects of engineered nanomaterials (ENs), as noted by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) of the European Commission. In an effort to harmonize particle characterization and hazard assessment efforts, a consortium team from several European Union member countries (Sweden, Finland, Germany, United Kingdom), associated countries (Switzerland), and the United States was recently granted an award by the European Commission (the 7th Framework program, CP-FP 214281-2 NANOMMUNE) to conduct much-needed toxicological and risk assessment studies. Reinforced international cooperation in response to the EU 7th Framework Program is of critical importance since a reliable basis for the assessment of safety of nanomaterial-based products and technologies is the use of standardized test materials and the development of predictive assays/methodologies.

This collaborative effort between US and EU investigators aims to address the following topics: physico-chemical characterization of standardized ENs; assessment of cellular and molecular mechanisms of adverse effects of ENs on cells and tissues of immune origin; development and validation of high-throughput, array-based methodologies for the assessment and prediction of outcomes ("nanotoxicity-signatures"); evaluation of the biological behavior of different classes of ENs in animal models, and the use of data for hazard characterization and risk assessment. Overall, the international consortium plans to perform a comprehensive assessment of adverse immune effects of ENs in order to understand how the benefits of the emerging nanotechnologies can be realized while minimizing potential risks to human health.

A critical factor for the sustainable development and commercial success of nanotechnologies is determination of safe levels of exposure and the public acceptance of these emerging technologies. Therefore, an important aspect of the grant is to disseminate the findings concerning potential risks of ENs not only to government, industry, and academia to assist in the development of safe environmental and workplace practices and controls, but also to the general public, through the organization of public hearings and workshops of information-sharing. Finally, filling the gaps in our understanding and assessment of risks and potential impacts on the environment by nanomaterials will require a large multidisciplinary effort.

Coming In the Next Issue

Review of a recent publication reporting on the effectiveness of inexpensive low-efficiency filters in recirculation systems for removal of airborne nanoparticles.

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