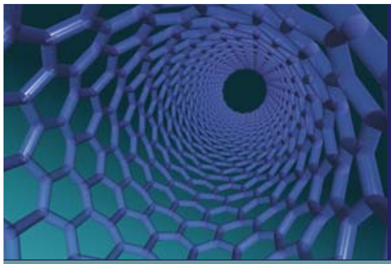

Gradient CORPORATION

EH&S Nano News

Volume 3, No. 05 www.ehsnanonews.com October 2008

Editor: Barbara D. Beck, Ph.D., DABT

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

Associate Editor: Chris Long, Sc.D

In This Issue

Recent Government Briefs

NSF and EPA Establish Two Centers for Environmental Implications of Nanotechnology

http://www.nsf.gov/news/news_summ.jsp?cntn_id=112234&org=NSF&from=news

UCLA and Duke University have been chosen by the National Science Foundation as the recipients of grants to establish two Centers for the Environmental Implications of Nanotechnology (CEIN). The centers will study how nanomaterials interact with the environment, information which will be used to develop nanotechnology risk assessment and mitigation strategies. The centers will also serve as a network to integrate nanomaterials science with molecular biology, toxicology, and environmental science. The UCLA CEIN will focus on the impact of nanomaterials on the environment while the Duke CEIN will examine the relationship between nanoparticles and their potential environmental exposures, biological effects and ecological consequences.

Nanotube Manufacturer Receives Consent Order from US EPA

http://www.thomas-swan.co.uk/ASP/News_Events/News_Events.asp?Type=News&ID=195&Arc=&DLT=Swan%20pioneers%20nanomaterial%20controls%20with%20EPA

Thomas Swan & Co. Ltd., a manufacturer of carbon nanotubes, recently announced the approval of a nanomaterial Pre-Manufacturing Notice (PMN) by the US EPA. This consent order applies to Swan's multi-walled carbon nanotubes, which have potential uses in ultra-strength composites and high performance electronics. This approval represents a step forward in standardizing production of nanomaterials such as carbon nanotubes. This announcement is expected to be published in the Federal Register.

Reports, Reviews, White Papers, and Books

Silver Nanotechnologies and the Environment: Old Problems or New Challenges?

By Samuel N. Luoma, Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies

http://www.nanotechproject.org/process/assets/files/7036/nano_pen_15_final.pdf

The author of this report concludes that use of silver in nanomaterials will prompt regulators to balance benefits against potential environmental risk. Silver is classified as an environmental hazard because it can be toxic in large doses, is persistent in the environment, and can bioaccumulate in organisms. Due to its properties as a broad-spectrum biocide, nanosilver is being incorporated into plastics, surfaces, and fabrics. Commercial products that contain nanosilver are one of the most rapidly growing classes of nanoproducts. As a result of its widespread usage, scientists have expressed concern about nanosilver releasing large amounts of silver ions into the environment. In this review, the author discusses the state of knowledge of silver in the environment as a starting point for making science-based decisions that will affect nanotechnology policy. Luoma highlights several research and policy needs, including establishing a nanosilver dialog among government agencies and stakeholders, integrating international research programs, and having clear rules for defining nanosilver in product ingredients. He presents several recommendations for managing environmental risks from nanosilver, including the necessity of further studying the dose-response effects of silver nanoparticles; investigating the effects of silver nanoparticles in aquatic ecosystems and during embryonic development, conducting toxicity testing at realistic exposure levels; and conducting nanosilver risk assessments.

Nanotechnology: Health and Environmental Risks (book review)

By Jo Anne Shatkin, CRC Press, 2008

http://www.crcpress.com/shopping_cart/products/product_detail.asp?sku=53639&isbn=9781420053630&parent_id=&pc=

This book, one in a series of four books exploring various nanotechnology issues, is intended to provide an introduction, for a non-technical audience, to key concepts and approaches for evaluating and managing health and environmental risks of nanotechnology. In contrast to familiar environmental contaminants such as PCBs, for which exposure primarily occurs via contact with contaminated media or via the diet, exposure to nanomaterials can potentially occur via use of nanomaterials in consumer products. Noting an urgent need to evaluate risks associated with nanotechnology despite limited data regarding exposure and health effects, this book highlights use of a NANO Life Cycle Risk Analysis (LCRA) approach as a framework for evaluating health and environmental risks of nanotechnology. NANO LCRA is described as an "adaptive screening-level life cycle risk assessment framework" that prescribes taking a conservative approach to risk management for making order of magnitude estimates when data are limited. Such an approach is intended to stimulate additional research, which would then allow a re-evaluation of the initial, conservative risk management decisions. Dr. Shatkin advocates use of the NANO LCRA framework as a tool that will allow "decision making under uncertainty" and that will present "a path forward to address the uncertainties."

Upcoming Meetings and Conferences

NanoRisk 2008

October 21-23, 2008, Paris, France

http://www.upperside.fr/nanorisk2008/nanorisk2008intro.htm

Due to the large potential impact and low public awareness of nanoparticles, many people expect that nanotechnology will become a major public, media, and political issue. Hosted by the nanotech conference organizer Upperside, this conference is intended to bring together researchers, industry officials, and government representatives to address in detail the questions of regulatory policies, risk governance, and methodologies to estimate toxicity. Sessions and presentations will cover the topics of nanoparticle occupational health, product life cycle, risk communication, and exposure assessment.

NanoSafe 2008: International Conference on Safe Production & Use of Nanomaterials

November 03 - 07, 2008, Grenoble, France

hhp://www.nanosafe2008.org

Hosted by the NANOSAFE2 Project, the European Technology Platform on Industrial Safety, and several European technology firms, this conference is intended to present NANOSAFE2 research and report progress on safe production and use of nanomaterials. Topics will include nanomaterials exposure, characterization, types of personal protection, toxicology, life cycle analysis, secure production, safety evaluation, and standardization. The five-day program will include lectures, as well as poster presentations.

International Conference on the Environmental Implications and Applications of Nanotechnology

June 9-11, 2009, Amherst, Massachusetts

http://www.umass.edu/tei/conferences/NanoConference/index.html

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 application 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.

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

1. Shvedova A.A., *et al*. 2008. Inhalation versus aspiration of single-walled carbon nanotubes in C57BL/6 mice: Inflammation, fibrosis, oxidative stress and mutagenesis. *Am J Physiol Lung Cell Mol Physiol.* In Press.

Abstract: http://ajplung.physiology.org/cgi/content/abstract/90287.2008v1

Synopsis:

- In a previous study where mice were exposed to suspensions of single-walled carbon nanotubes (SWCNT) via pharyngeal aspiration (*i.e.*, a single exposure to a bolus of SWCNT placed on the back of the tongue), Shvedova *et al.* reported evidence of dose-dependent granulomatous pneumonia, oxidative stress, acute inflammatory/cytokine responses, fibrosis, and decreased pulmonary function. To explore whether the observed responses were due to possible artifactual effects, such as the high single bolus exposure or the presence of large mats and agglomerates of SWCNT, arising from the instillation exposure technique, Shvedova *et al*. conducted the current inhalation exposure study using stable and uniform SWCNT dispersions generated by a novel aerosolization technique.
- A newly developed aerosol dispersion system was used to maintain stable, uniform SWCNT dispersions in whole-body inhalation chambers. Using non-purified SWCNT purchased from Carbon Nanotechnology, Inc. (iron content of 17.7% by weight, diameters of 0.8-1.2 nm, lengths of 100-1,000 nm, surface area of 508 m^2/g), both a powder

feeder and a knife mill were used to deliver a respirable SWCNT aerosol at a flow rate of 10 L/min to two animal exposure chambers. Mass concentration was measured in real-time using a DataRAM to allow feedback control, with gravimetric samples confirming a mean SWCNT concentration of 5.52±1.37 mg/m^3. Size distribution data indicated a count mode aerodynamic diameter of approximately 240 nm.

- Mice were exposed to the non-purified SWCNT either by inhalation at a nominal mass concentration of 5 mg/m^3 for 5 hours/day for 4 days (estimated to be equivalent to a deposited dose of approximately 5 µg in the pulmonary region of the mouse respiratory tract), or by pharyngeal aspiration (suspensions of 0, 5, 10, or 20 µg/mouse). Whole body plethysmography was used to measure breathing patterns and assess respiratory changes after SWCNT exposures. Mice were sacrificed 1, 7, or 28 days post-inhalation. Bronchoalveolar lavage (BAL) fluid was collected to assess the level of inflammatory response, with measurement of total cell counts, cell differentials, and accumulation of cytokines; and to assess evidence of pulmonary cytotoxicity, with measurement of total protein and lactate dehydrogenase (LDH) activity. Both morphometric measurements and collagen deposition were used to assess the fibrogenic response, while genotoxicity of SWCNT was assessed by measuring accumulation of K-*ras* mutations.
- Similar chains of pathological events were observed for both exposure routes, consisting of an early inflammatory response and oxidative stress, followed by development of multifocal granulomatous pneumonia and interstitial fibrosis. Based on findings that included 2-4 fold greater cytokine production, 4-fold increases in fibrosis for SWCNT inhalation, and a higher frequency of K-*ras* mutations, the study investigators concluded that inhalation exposure was a more potent exposure route than aspiration for an equivalent mass of SWCNT.

Implications:

- Given that studies have shown that SWCNT are typically present as large mats and micron-sized agglomerates in the liquid suspensions used for intratracheal instillation and pharyngeal aspiration exposures, the authors hypothesize that their findings indicating the higher potency of inhaled SWCNT versus aspirated SWCNT may be due to the effects of a greater fraction of dispersed, nano-sized SWCNT reaching the lower respiratory tract. Although this hypothesis is supported by their measurement of a count mode aerodynamic diameter of approximately 240 nm, additional investigation is needed to further explore the role of dispersed, nano-sized SWCNT versus SWCNT agglomerates in the observed biological responses. In addition, as noted by the study investigators, real-world exposure data are needed to determine the relevance of the doses and conditions used in this experimental study.
- While the authors state that their findings "may be important for establishing a permissible exposure level for SWCNT," it is again important to emphasize the need for additional exposure data for real-world conditions to confirm how representative the experimental conditions (*i.e.*, 5 mg/m^3 exposure concentration, high fraction of dispersed nano-sized SWCNT) are of actual SWCNT exposures. Given findings from a prior study indicating the lesser toxicity of inhaled CNTs as compared to instilled CNTs, additional research is needed to investigate the relationship between various CNT properties and toxic potential prior to establishing workplace exposure limits. Work is needed to determine whether the properties of laboratory aerosolized CNTs are representative of real-world conditions, especially given the findings of a prior NIOSH study that reported evidence of rapid agglomeration, and low airborne respirable particle concentrations (in the range of 0.7 to 53 µg/m^3), in small-scale SWCNT production facilities.
- In addition to observing an increased rate of K-*ras* mutations that began shortly after SWCNT inhalation (on days 1-7) and that persisted through day 28, Shvedova *et al.* also reported the detection of a double mutation that had not been reported previously and which may be specific for SWCNT exposures. While these findings are consistent with previous research indicating an association between inflammation and pulmonary fibrosis and increased risk of lung cancer, it is important to note that additional research is needed prior to concluding that there is a causal association between K-*ras* mutations and SWCNT genotoxicity, let alone with fibrosis-associated lung cancer. As the authors note, there remains a poor understanding of the involvement of K-*ras* mutations in fibrosis-associated lung cancer.

2. Zhu, H., *et al.* 2008. "Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants." J Environ Monit. 10:713-717.

Abstract: http://www.rsc.org/publishing/journals/EM/article.asp?doi=b805998e

Synopsis:

- Nanoparticles may be present in soil due to intentional activities, such as use of nanoparticles for remediation, or unintentional activities, such as from spills. Compared to the number of studies evaluating effects of nanoparticles on animal species, relatively few studies have evaluated effects of nanoparticles on plants. Studies evaluating effects of nanoparticles on plants have demonstrated both beneficial effects, such as improving growth of seedlings, and harmful effects such as inhibiting root elongation. The aim of the study by Zhu *et al.* was to evaluate uptake and subsequent translocation and accumulation of magnetite nanoparticles (Fe_3O_4–$_{nano}$) in pumpkins *(Cucurbita maxima).*
- Pumpkins were selected for this study based on their large water uptake capacity. Fe_3O_4–$_{nano}$ was selected based on its

biological compatibility, structural stability, and ability to be used to quantify uptake, translocation and accumulation using non-invasive magnetometry. After allowing pumpkin seeds to germinate, the seedlings were transferred into an aqueous medium and grown hydroponically. Fe_3O_4–$_{nano}$ was added after the appearance of the third leaf, at a concentration of 0.5 g/L. Control seedlings were grown without Fe_3O_4–$_{nano}$. Uptake, translocation and accumulation of Fe_3O_4–$_{nano}$ were assessed when the pumpkin plants had reached an average height of 27 cm, at approximately 20 days. Concentrations of Fe_3O_4–$_{nano}$ were measured in plant tissues that had been dried in a vacuum dessicator for three days.

- There was no difference in the visual appearance of control vs. treated pumpkin plants, indicating that the Fe_3O_4–$_{nano}$ did not cause any overt toxicity. The highest concentration of Fe_3O_4–$_{nano}$, as assessed by the strength of the magnetic signal, was observed at the base of the plant, just above the roots, with the magnetic signal decreasing to 0.00 at the top of the stalk. Fe_3O_4–$_{nano}$ concentrations in the leaves were higher than in the stalk, with much less of a vertical concentration gradient – magnetization ranged from 1.76×10^{-3} to 1.29×10^{-3} electromagnetic units per gram, for leaves at the bottom and top of the stalk, respectively.
- The mass balance for Fe_3O_4–$_{nano}$ at the end of the experiment was 67.4%, with the majority (45.4%) associated with the root tissue, a small portion (0.6%) in the leaf tissue, and the remainder (21.4%) in suspension. According to the study authors, most of the Fe_3O_4–$_{nano}$ that was not accounted for was most likely lost with the xylem sap when the plants were processed for analysis, and a small portion may have been lost due to evaporation and gas exchange via the leaves. According to the study authors, Fe_3O_4–$_{nano}$ associated with the roots likely included Fe_3O_4–$_{nano}$ that had been absorbed by the pumpkin plants, as well as Fe_3O_4–$_{nano}$ adsorbed on the outside of the roots, that remained after the plants had been rinsed with water.
- Accumulation of Fe_3O_4–$_{nano}$ in pumpkin plants grown in sand was approximately 33% of that for pumpkin plants grown hydroponically. Pumpkin plants grown in soil did not accumulate any detectable Fe_3O_4–$_{nano}$. An initial screening test with lima bean plants did not detect a magnetic signal in any portion of the lima bean plant.

Implications:

- The findings from this study indicate that accumulation of Fe_3O_4–$_{nano}$ in plants is tissue-specific, with more accumulation in the leaves than in the stems. The majority of Fe_3O_4–$_{nano}$ was associated with the roots – either absorbed into the roots or adsorbed on the surface, which raises some concern for contamination of root vegetables. However, given that there was no detectable accumulation of Fe_3O_4–$_{nano}$ from soil, potential contamination of root vegetables might not represent a significant source of exposure for plants grown in soil.
- Results from previous studies indicate that uptake of Fe_3O_4–$_{nano}$ varies among different types of plants and soil types. No doubt, uptake would also vary among different types of nanoparticles. In this respect, the ability of soil to limit uptake of Fe_3O_4–$_{nano}$ into pumpkin plants may not necessarily apply to other types of nanoparticles. Factors that affect mobility of chemicals in soil, such as water solubility and octanol-water partitioning behavior, would likely also affect accumulation of nanoparticles in plants.
- The ability of pumpkin plants to accumulate Fe_3O_4–$_{nano}$, particularly under hydroponic conditions, suggests a potential for their use in phytoremediation of groundwater. Successful use of plants for phytoremediation of nanoparticles would depend, at least in part, on the extent to which nanoparticles would remain sequestered in plants, or whether they would tend to leach from the plants.

Guest Contributor

By Joseph D. Brain,

Drinker Professor of Environmental Physiology, Harvard School of Public Health, Boston, Massachusetts

The Promise And Dangers Of Nanotechnology

How do we balance the potential hazards from new and poorly characterized materials with the promise and power of nanotechnology? There is an explosion of nanomaterials and a parallel increase in the applications of these novel creations. The magnitude of investment in this new technology is enormous, and private companies, universities, and governments are supporting this rapid growth. How do we evaluate risk and benefit?

There are reasons to be concerned about nanomaterials, including particles and fibers. Importantly, most are new and untested. Some studies, have shown that injecting nanofibers into the pleural space produces responses similar to those caused by asbestos fibers. We know that flexible, durable, persistent fibers are dangerous in compartments like the pleural space or pulmonary connective tissue. It is also the case that nanomaterials have access to unexpected sites, *e.g.* the nucleus. They sometimes enter cells and organelles by nontraditional mechanisms. For example, larger particles are almost always found in the cytoplasm, usually membrane-bound within phagosomes or phagolysosomes. In contrast, multiple investigators have described the entry of nanoparticles into cells by a nonphagocytic mechanism; they appear in the cytoplasm not enclosed in membranes.

Progress has begun in characterizing the inherent toxicity and pharmacokinetics of nanomaterials in both in vitro and in vivo systems. Exposure assessment lags behind. To what extent do nanomaterials actually appear in air, water, and food? To what extent do the public and workers come in contact with nanomaterials? An enduring principle of toxicology is "the dose makes the poison." It is essential to remember that humans are rarely affected by toxic materials unless the materials enter the body. To do that, the materials must cross one of the interfaces between the outer and the inner environment: the skin, the gastrointestinal tract, and the respiratory tract. In addition to exposure assessment, we need better pharmacokinetic data which quantitatively describe the extent to which particles cross one of these body surfaces and enter the blood. After nanomaterials enter the blood, we need to know their ultimate anatomic fate and rate of clearance in critical organs.

There are factors which may mitigate the potential dangers of nanomaterials. First, the most exotic, unusual, highly engineered materials are very expensive, and are produced and used in small quantities. Some of these nanomaterials may be toxic, and yet human exposure may be minimal. We need information about the concentrations of nanomaterials in air, water, food, and other materials. Second, there are not as many nano products as is sometimes claimed. The word nano has become a marketing ploy. Some so-called nano products do not contain nanomaterials. They may be 500 nm, 1000 nm, or even greater in size.

We cannot hold back the ocean of nanotechnology. It is a powerful economic force, and will undoubtedly bring many benefits. But, in parallel fashion, we need to evaluate the potential toxicity of nanomaterials and technologies. In addition to assessing the toxicity of particular nanomaterials – particularly those which are widely used in consumer products – we need to examine the fundamental principles which govern the toxicity of nanomaterials. What are the rules of nanotoxicology? A promising approach will be to examine families of engineered particles and study the role of (1) size, (2) shape, and (3) surface coatings. For example, we should study the same material as we systemically vary its length and diameter. As is true for asbestos, we anticipate that long, narrow fibers will be more toxic than short fibers of the same volume. Moreover, biopersistence is a common feature of toxic materials. Materials that rapidly dissolve are less likely to produce disease than those that persist for longer periods of time. We can also examine families of spherical particles whose diameters vary by orders of magnitude. Finally, one could examine spherical particles with identical dimensions, but with a range of surface properties. This could be a family of proteins with varying mediator activities or surfaces that vary in pH or in reactive chemical groups. The field of nanotoxicology will be a fruitful one for decades to come.

Adapted from an abstract submitted to NanoTox 2008, Zurich, Switzerland.

Coming In the Next Issue

New research linking CNT inhalation with fibrosis in an asthmatic mouse model but not in a healthy mouse model

Publication Staff

Editorial
Barbara D. Beck, Ph.D., DABT
Noelle M. Cocoros, M.P.H.
Todd C. Hudson, M.S.P.H
Christopher M. Long, Sc.D.
Mara Seeley, Ph.D., DABT

Production
Matthew Braman
Ruth Buchman
Janelle Forbes

Contact Us
Gradient Corporation
20 University Rd
Cambridge, MA 02138
t: 617.395.5000
f: 617.395.5001
email: ehsnanonews@gradientcorp.com
website: www.gradientcorp.com

Science and Strategies for Safe Environments
www.gradientcorp.com