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

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Recent Government Briefs

US EPA Nanoscale Program Draft Documents Released on July 12, 2007, for Public Comment

<http://www.epa.gov/oppt/nano/nmspfr.htm>

On July 12, EPA released long-awaited documents that provide details on the Agency's intended approach for handling nanoscale materials under the Toxic Substances Control Act (TSCA) and its plans to develop key scientific information and appropriate risk management practices for nanomaterials under its voluntary Nanoscale Materials Stewardship Program (NMSP). The draft documents released for public comment include "TSCA Inventory Status of Nanoscale Substance General Approach," "Concept Paper for the Nanoscale Materials Stewardship Program Under TSCA," and "Information Collection in Support of EPA's Stewardship Program for Nanoscale Materials; Supporting Statement." Importantly, EPA clarifies its intended approach for determining whether nanoscale materials are considered as "new" vs. "existing" chemical substances under TSCA, observing that determinations will be made on a case-by-case basis. Inventory and molecular identity will be key factors underlying these determinations, rather than physical attributes such as particle size. The 60-day public comment period is scheduled to end on September 10, 2007, with a public meeting scheduled for August 2, 2007, to allow for open discussion of the NMSP and the draft documents.

Cambridge Convenes Nanomaterials Advisory Committee (NAC) to Examine Nanomaterial Policy Options

The City of Cambridge, Massachusetts, has convened its Nanomaterials Advisory Committee (NAC) to begin the process of identifying a set of policy options for local oversight of facilities involved in the manufacture or processing of engineered nanomaterials. Formed in response to the Cambridge City Council's request in January 2007 that city officials review the Berkeley (CA) nanomaterial ordinance, the Cambridge NAC consists of a diverse stakeholder group which includes representatives from Cambridge-area nanotech companies, local universities, the general public, local consulting companies, and policy groups. The first of a series of monthly meetings of the NAC is scheduled for mid-August, where members will begin the process of discussing the health and policy challenges posed by nanotechnology. This process is expected to continue through 2007, with the Cambridge Public Health Department ultimately recommending a course of action to the Cambridge City Council regarding the level, if any, of local regulatory oversight needed for Cambridge-area nanotechnology manufacturing and processing facilities to protect public and employee health.

Reports, Reviews, White Papers, and Books

EPA and Nanotechnology: Oversight for the 21st Century

By Dr. J. Clarence Davies, of the Project on Emerging Nanotechnologies, Woodrow Wilson Center for International Scholars

<http://www.nanotechproject.org/124/>

This paper by Dr. J. Clarence Davies identifies the actions that should be taken to establish an adequate nanotechnology oversight system. Davies identifies the US EPA as the key agency capable of performing this function. Davies views this as important due to the large increase in manufactured goods that involve nanotechnology; he also considers that there is a limited amount of time to establish an adequate oversight system. He identifies the Toxic Substances Control Act (TSCA) as the only law "potentially capable of providing general oversight for nanotechnology," and explains his belief that this law is currently insufficient and needs to be amended in order to become a legal authority applicable to the nanotechnology industry. In this paper, Davies details how an effective oversight system can be implemented using fewer resources and resulting in less intrusion than current forms

of regulation. These tools include adjusting existing regulatory programs, information tools, industry- and government-initiated voluntary efforts, economic tools, liability tools, state and local input, public participation, and reform of existing regulation. Davies concludes that US EPA can become involved in reforming nanotechnology oversight by becoming stronger in science, integrating with outside and international agencies, and measuring its own progress; he additionally notes that US EPA can overcome its current problems by taking the lead on nanotechnology regulatory issues. The paper also includes 25 steps that should be taken by executive agencies, Congress, and nanotechnology collaboratives.

Risk Assessment and Risk Management for Synthetic Nanomaterials, 2006-2009

Published by the Swiss Federal Office for the Environment and the Federal Office of Public Health

<http://www.innovationsgesellschaft.ch/images/publikationen/GrundlagenberichtSummaryEnglisch.pdf>

This publication outlines the Swiss government's call for the study of potential human health risks posed by nanoparticles and appropriate measures to mitigate these risks. This action plan is based on a similar measure taken by the European Union (EU) in 2005 and is based on studies showing the health risks of nanoparticles, including penetration into the deep lung, bloodstream, and internal organs. One of the goals is to collect data on how nanoparticles could accumulate in organisms and ecosystems. In addition, there is an estimate of risk based on "physico-chemical, toxicological, and exposure data" in order to set priorities for future research and regulation. The report makes 26 recommendations for actions based on promotion of research, establishment of legislation, communication, and technology assessment.

Upcoming Meetings and Conferences

2nd International Conference on the Environmental Effects of Nanoparticles and Nanomaterials

London, United Kingdom; Sept. 24-25, 2007

<http://www.sebiology.org/Meetings/pageview.asp?S=2&mid=107>

The UK branch of the Society of Environmental Toxicology and Chemistry (SETAC) and the Society for Experimental Biology (SEB) are organizing the 2nd international meeting on the potential biological and environmental effects of nanoparticles and nanomaterials. The meeting will cover a range of themes on this topic such as ecotoxicology, life cycle analysis, environmental applications of nanomaterials, and regulatory and policy issues. Abstract submission is now open for oral and poster presentations.

NanoTX'07 – Conference and Expo

Dallas, Texas; Oct. 2-4, 2007

<http://www.nanotx.biz/>

The Semiconductor Industry Association (SIA) is presenting NanoTX'07 to highlight the world's commercial micro- and nanotechnology initiatives. The event is sponsored by several academic institutions, corporations, and government agencies with an interest in researching and promoting nanotechnology. The meeting will cover topics such as nano-health, environmental health and safety, advanced nanomaterials, and nanotechnology applications in computing and electronics. The conference aims to attract an audience from private industry, capital groups, government agencies, universities, and research and development fields.

Nanotechnology and Health: Evidence and Impact

<http://www.sph.umich.edu/riskcenter/2007%20symposium.htm>

Ann Arbor, Michigan; Oct. 25-26, 2007

Presented by the University of Michigan Center for Risk Science and Communication, this conference will bring together leading scientists and policymakers to examine the emerging field of nanotechnology, its potential impact on human health, and the growing debate over whether to regulate nanotechnology products. Keynote speaker Christine Todd Whitman, former Governor of New Jersey and Administrator of the Environmental Protection Agency (2001-2003), will examine nanotechnology through her address, "Risky, Riskier, Riskiest: The Pitfalls, Perils, and Possibilities for Policymakers." Other national and international scientific and policy experts will explore the implications, challenges, and opportunities provided by this emerging technology, including Gradient Principal Pertti (Bert) Hakkinen, who will examine nanotechnology through his address, "The European Perspective on Risk, Science, Policy, and Practice."

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

Limbach LK, et al. 2007. "Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress." *Environmental Science and Technology* 41(11): 4158-63. Abstract:<http://pubs.acs.org/cgi-bin/abstract.cgi/esthag/2007/41/i11/abs/es062629t.html>

Synopsis

- There is a growing volume of engineered nanomaterials on the open market. Many of these nanomaterials have unique physical and chemical properties, some of which are not well understood. A particular concern for some nanomaterials is that their degree of catalytic activity could contribute to oxidations inside living cells, resulting in aggressive and long-term toxicity in cells and tissues.

This study investigated *in vitro* evidence of oxidative stress, specifically formation of reactive oxygen species (ROS), associated with the same materials as widely used in industrial oxidation processes, namely iron-, cobalt-, manganese-, and titania-containing silica nanoparticles.

- The authors conducted several sets of *in vitro* assays, including experiments where human lung epithelial cells were exposed to nontoxic concentrations of either 0.5 or 1.6 weight percent of iron-, cobalt-, manganese-, and titania-containing silica nanoparticles or pure metal oxide nanoparticles. All nanoparticles were thoroughly characterized for primary particle size, hydrodynamic agglomerate size distribution, morphology, and chemical composition prior to exposures in the form of diluted (*i.e.*, 30 ppm) nanoparticle suspensions. Reference cultures of corresponding amounts of soluble transition metal salts (*i.e.*, without particle exposition) were conducted for comparison with the nanoparticle exposures. These same experiments were also repeated under cell-free conditions to investigate the effects of cells on the promotion of ROS formation. For each experiment, oxidative stress, specifically ROS generation, was measured using an established fluorescence assay following a four-hour exposure.
- *In vitro* assays involving exposures of the four transition metal oxides of 20-75 nm size demonstrated significant ROS generation after four hours. Importantly, response increases up to eight-fold were observed in the case of cobalt or manganese compared to the reference cultures exposed to aqueous salt solutions (with smaller effects for both titanium and iron). This supports the role of nanoparticles as carriers for heavy metal cellular uptake. Although pure nanoparticle cobalt and manganese metal oxides demonstrated the greatest ROS generation (*e.g.*, up to 50 times higher than in cultures exposed to silica or pure saline controls), ROS levels were not observed to be linearly related to the amount of introduced transition metals.
- In the cell-free experiments involving nanoparticle exposures, similar ROS responses were generally observed as for the culture assays. However, both iron and manganese aqueous salts elicited greater ROS formation without any living cells present, indicating that cells inactivate some of the effects of these metal ions.
- In an additional set of experiments, the authors exposed cell cultures to an extended series of iron-silica particle concentrations (0-10 weight percent iron in silica) to “systematically investigate the role of catalytic effects.” In these experiments, greater ROS levels were typically observed for nanoparticle exposures relative to exposures to iron ions at the same concentrations. Maximal ROS production was observed with 1-3 weight percent iron/silica, with higher iron amounts not resulting in increased ROS production.

Implications

- These study findings provide additional experimental evidence that exposure to specific types of nanomaterials may have a more deleterious effect on cells than bulk (*i.e.*, non-nano) materials comprised of the same elements. Specifically, cobalt- and manganese-containing silica nanoparticles were found to efficiently enter cells by a Trojan-horse type mechanism, provoking significantly greater ROS responses than aqueous solutions of the same metals.
- These experiments demonstrated the role of both delivery form (nanoparticle versus aqueous solutions) and composition (pure silica, transition metals in silica, pure transition metals) as factors influencing cellular oxidative stress.
- Based on their observation that ROS levels were not linearly related to the amount of introduced transition metals, the authors concluded that there was a non-compositional effect that was a key determinant of ROS levels. The authors attributed this non-mass correlating effect to “increased catalytic activity of transition metal sites consisting of single or few transition metal ions incorporated as clusters in an inert matrix.”
- As discussed by the authors, the similarity of findings for ROS promotion by nanoparticle exposures in cultures *versus* cell-free experiments provides support for the important role of a chemical pathway in nanoparticle-mediated ROS stimulation within cells, while comparison of cultures *versus* cell-free experiments for the case of aqueous solutions of transition metals provides collaboration for the well-established role of an intact cell membrane as an ion barrier.
- Findings from this paper thus emphasize the need to consider chemical and catalytic properties beyond physical properties such as size, shape, and degree of agglomeration when developing and assessing the risk of nanomaterials.

Helland, A., et al. 2007. “Reviewing the environmental and human health knowledge base of carbon nanotubes.” *Environmental Health Perspectives* 115(8): 1125-1131. Abstract: <http://www.ehponline.org/docs/2007/9652/abstract.html>

Synopsis:

- Combining both a review of the published scientific literature and expert interviews with seven leading scientists, the article reviews the state of the knowledge regarding the human health and environmental risk potential of carbon nanotubes (CNT). The article considers both studies of multi-wall CNT (MWCNT) and single-wall CNT (SWCNT), highlighting open research questions and recommending key research needs.
- Although very few studies are currently available to characterize the fate and exposure potential of CNT in

occupational settings or environmental media, Helland *et al.* cite evidence indicating that CNT are likely to accumulate along the food chain due to their low solubility, lipophilic nature, and low biodegradability. Helland *et al.* also cite evidence indicating that the aquatic mobility and deposition behavior of CNTs are highly dependent upon their form (e.g., whether they are released as pristine nanotubes, acid-treated nanotubes suspensions, surfactant-treated CNTs, functionalized SWNTs, etc.). The large surface area of CNTs is also discussed as a factor that could enhance their environmental impacts, since they can potentially pick up and transport other environmental pollutants.

- Drawing upon a toxicological and environmental impacts database of approximately 50 studies (mostly *in vitro*), the authors observe that the available scientific evidence shows a wide range of results that depend upon such factors as CNT physical and chemical characteristics (size, shape, surface area, impurities, functionalization, coating, and agglomeration state), how CNT are administered to cells (e.g., homogeneously dispersed or not, with or without surfactant, and type and concentration of surfactant), and how laboratory animals are exposed (primarily intratracheal instillation rather than inhalation). Despite the diverse and sometimes contradictory findings of these published toxicological studies, the authors conclude that adequate evidence currently exists demonstrating that sufficiently high doses of CNT can produce time and dose-dependent toxic responses including oxidative stress, pulmonary inflammation, cytotoxicity, granulomas, fibrosis, and wall thickening.
- The authors stress that the numerous physical and chemical properties affecting the exposure potential and toxicity of CNT can change during the CNT product life-cycle (e.g., from synthesis, to production of intermediates, to further processing, to product usage, and concluding with disposal), meaning that CNT may pose different health risks depending on the stage of the product life-cycle and fate in the environment.
- The authors highlight several outstanding research questions, including the extent to which CNT can enter the bloodstream (e.g., through the alveolar passage) and whether organisms can eliminate pristine CNT. Studies are also needed to evaluate whether food chain bioaccumulation does indeed occur for CNT, as hypothesized based on their properties.

Implications:

- Based on their observations that there are numerous different types of CNT and toxicology studies that have observed a wide range of results, the authors emphasize that CNT cannot be considered a uniform group of substances from a toxicological perspective. The study authors propose that the development of a set of toxic equivalency factors (TEFs), as exist for such compound classes as polycyclic aromatic hydrocarbons (PAHs), may be a useful approach for expressing the relative toxicity of different CNT types.
- As discussed by the authors, the wide range of results of

CNT toxicity studies is likely not only a result of the differing toxicities of various CNT types, but is also a function of differences in the test methods between studies. To better allow for the comparability of findings between different CNT toxicity studies, the authors highlight the need for standardization of CNT materials, test procedures, reference materials, and cell models/organisms employed.

- Given study findings showing that such engineered properties as degree of functionalization can influence both the degree of toxicity as well as environmental fate of CNT, the authors discuss how it may be possible to control the potential impacts of CNT on human health and the environment.
- Because studies have shown that the environmental fate and toxicity of CNTs are highly dependent on the characteristics of the particular CNT tested, the authors emphasize that it is vital that researchers study the form of the CNT to which organisms will be exposed under real environmental conditions. To identify relevant CNT forms, they highlight the need to characterize real-life exposure scenarios prior to conducting toxicological studies. Although not discussed by the authors, a parallel recommendation involves more closely matching laboratory test exposure routes (*i.e.*, inhalation rather than instillation) and exposure levels to potential real-life exposure conditions.
- Because most *in vivo* studies of CNT toxicity have employed instillation exposures and few exposure studies have studied the potential for SWNT to become airborne, there are especially large uncertainties regarding potential inhalation exposures and toxicity of airborne CNT. For example, inhalation studies are needed to confirm such *in vitro* findings as those indicating that short MWCNT are more toxic than long ones and that well-dispersed SWCNT are less cytotoxic than micron-sized SWCNT agglomerates.

Guest Author

By Dr. Barbara Rothen-Rutishauser and Dr. Peter Gehr

Institute of Anatomy, University of Bern, Switzerland
Nanoparticles – What do we know today?

By Dr. Barbara Rothen-Rutishauser and Dr. Peter Gehr
In addition to the generation of ultrafine particles from combustion processes in large amounts, there are progressively more nanoparticles, defined as manufactured structures with 1-100 nm size dimensions, being released into the air, water, and soil every year from other sources, *i.e.* nanotechnology. The potential human health benefits of nanotechnology are undoubted, however, occupational exposure, direct human exposure through medical applications and ambient air pollution may, depending on the dose and particle characteristics, cause adverse health effects. Most toxicity research so far has been carried out with mammals, focusing on the respiratory system. A huge internal pulmonary surface area of >140 m² in humans with a minimal air-blood tissue barrier of <1 μm is available for the interaction with inhaled particles. Little is known about the interaction of such particles with lung cells, how they may cross the epithelial barrier, and how

they may penetrate the capillaries in the pulmonary tissue. Over the last several years scientists have found that exposure to combustion-derived ultrafine particles can be associated with pulmonary and cardiovascular diseases. The mechanisms of ambient particulate matter that induce health effects are believed to include oxidative stress and inflammatory reactions in cells. The comparison of manufactured nanoparticles to combustion-derived ultrafine particles suggests that cellular effects and health effects in humans may be the same. Ultrafine combustion-derived particles and nanoparticles of various materials can cross cellular membranes. This may occur by an actin-based and endocytotic mechanism which results in vesicle formation, *i.e.* through an ingestion-type mechanism; however, other mechanisms are likely to account for individual nanoparticles translocation into the cell. We have found with inhalation experiments in whole animals using TiO₂ particles and with *in vitro* studies in cell cultures using different nanoparticles a transport mechanism that includes a non-specific penetration of particles into cells by adhesive interactions. Diffusion and penetration promoted by thermal capillary waves may also be considered as possible uptake mechanisms. These mechanisms may be largely independent from particle surface chemistry and charge. We were able to show that TiO₂ particles one hour after deposition translocated in a directed way from the alveolar surface into the connective tissue and 23 hours later into the capillaries where they can be found in erythrocytes. *In vitro* studies showed that nanoparticles easily penetrate into erythrocytes, and these particles were not membrane bound, *i.e.* they may have direct access to cytoplasmic proteins.

This knowledge of particle uptake may be used to develop drug delivery systems, as has been reported for functionalised carbon nanotubes which are able to cross cell membranes in a passive and endocytotic-independent way. On the other hand, the adverse potential of ultrafine combustion-derived particles and nanoparticles is greatly enhanced by their free location and movement within cells. They may cause several biological responses including the enhanced expression of pro-inflammatory cytokines (cellular proteins that enhance inflammation), the generation of reactive oxygen species, and induction of DNA double-strand breaks. Penetration of nanoparticles into mitochondria and the nucleus has been shown, suggesting a toxic and carcinogenic potential by interfering with the respiratory chain and by interacting with the DNA.

Thus, nanotechnology and its use in many applications has the potential to induce a spectrum of human health hazards, in a manner similar to ambient ultra-fine particles. Because we know little about the health hazards of newly manufactured nanoparticles, *in vivo* and *in vitro* toxicology studies and regulations are essential before the large scale industrial production of nanoparticles occurs.

Coming In the Next Issue

Review of a new research study assessing the tissue kinetics, persistence, and redistribution of quantum dots in mice.

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