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

Recent Government Briefs

German Federal Institute of Risk Assessment (BfR)

(BfR) Germany's Federal Institute of Risk Assessment (BfR) has commissioned the University of Stuttgart (ZIRN, Center of Interdisciplinary Risk-science and Sustainable Development of Technology), to conduct a Delphi-survey (a series of surveys using questionnaires that build and are modified with each round) on risks associated with the use of nanotechnology in food, cosmetics, and other consumer products. Approximately 100 experts from the scientific community, industry, public authorities, and non-governmental organizations are expected to participate in two Delphi rounds (May and September) and deliberate on the current and future applications of nanotechnology, as well as the potential risks to consumers. In two subsequent workshops, BfR plans to consolidate the results of the Delphi-surveys into a "risk-barometer" that will be used to better inform public authorities. For more information visit: [http://www.dialogik-expert.de/en/list/archiv/Issue_01_\(May_2006\).pdf](http://www.dialogik-expert.de/en/list/archiv/Issue_01_(May_2006).pdf)

Reports, Reviews, White Papers, and Books

The Ethics and Politics of Nanotechnology

United Nations Educational, Scientific, and Cultural Organization, June 2006

This report from UNESCO focuses primarily on the ethical issues associated with nanotechnology, from an international perspective. After an overview of the basics and the history of nanotech, various ethical and political considerations are discussed. Interestingly, the report reviews how the top ten applications of nanotechnology for developing countries (e.g., water treatment and disease diagnosis) might also address the UN's Millennium Development Goals. There is also a brief discussion of ethical issues that "aren't" — attention-grabbing topics that only distract people from real concerns. One example discussed is the "post-humanism" debate, where concerns are raised regarding augmentation and repair of human characteristics (such as new forms of plastic surgery or performance enhancement for athletes). For more information visit: <http://unesdoc.unesco.org/images/0014/001459/145951e.pdf>

Research Strategies for Safety Evaluation of Nanomaterials, Part VIII: International Efforts to Develop Risk-Based Safety Evaluations for Nanomaterials

Thomas et al., 2006. *Toxicological Sciences*. 92(1):23-32.

Part VIII in the series on research strategies for nanomaterial safety evaluation, this review highlights government efforts in the US, Europe, and Japan that are aimed at improving the scientific basis for evaluation of human health and environmental risks associated with nanomaterials. In addition, research needs are discussed throughout the review. The US EPA draft Nanotechnology White Paper (<http://www.epa.gov/osa/nanotech.htm>) provides the framework for the discussion of the US efforts, with a primary focus on research needs. For the European discussion, Thomas *et al.* review completed, ongoing, and future EU nanomaterial EH&S projects. Finally, the discussion of the Japanese work emphasizes government funded projects, with research efforts deriving from the various government ministries in the country (e.g., the Ministry of Environment and the Ministry of Health and Welfare). The US, Europe, and Japan are each discussed separately, though the authors conclude that there are numerous commonalities in the research of the three countries' efforts. For more information visit: <http://toxsci.oxfordjournals.org/cgi/content/abstract/92/1/23>

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Upcoming Meetings and Conferences

Nanotechnology: Entrepreneurship & Research Opportunities in the Chemical Industry

Chemical Solutions 2006 Workshop Series: Cambridge, MA, Sept. 18 - 19, 2006

As part of the workshop series of the Synthetic Organic Manufacturers Association (SOCMA), this two-day meeting will include an up-to-date review of new developments in nanoparticle synthesis and recent patent data, in addition to talks with a focus on future markets. Speakers at the workshop will include experts from government, industry, and academia. One of the key topics of the workshop will be regulatory issues relevant to the chemical industry. For more information visit: <http://www.socma.com/conferences/chemicalsolutions.htm>

International Conference on Nanotechnology Occupational and Environmental Health and Safety: Research to Practice

Cincinnati, OH, Dec. 3 – 8, 2006

NIOSH, the University of Cincinnati, US EPA, and others are the co-sponsors of this conference—considered one of the pre-eminent EH&S nano meetings. The meeting will address the future of nanotechnology from two distinct perspectives of health and safety: the promotion and protection of worker safety throughout the life cycle of nano products, and the use of nanotech to prevent, detect, and treat occupational and environmental diseases. For more information visit: <http://www.uc.edu/noehs/>

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

Long TC et al., 2006. “Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity.” *Environ. Sci. & Technol.* **40(14): 4346 - 4352**

Abstract: <http://pubs.acs.org/cgi-bin/abstract.cgi/esthag/asap/abs/es060589n.html>

Synopsis:

- This article describes the cellular response of mouse brain microglia (BV2) to nanoscale titanium dioxide (TiO₂) particles. The purpose of these experiments was to study the neurotoxic potential of TiO₂ on microglia, which are phagocytic cells in the central nervous system (CNS) known to respond to potentially damaging exogenous stimuli.
- The researchers incubated BV2 cells with 2.5 to 120 ppm (mg/L) concentrations of Degussa P25 (~30 nm in size) TiO₂ particles, and measured intracellular reactive oxygen species (ROS) production using fluorescent probes. The ability of BV2 cells to phagocytose the TiO₂ particles, as well as the physicochemical properties (aggregation potential, zeta potential) of the nanoparticles in the cell culture media, were also evaluated.
- ROS were generated through two distinct mechanisms: oxidative burst (rapid response occurring within minutes of exposure) and alteration of mitochondrial electron transport chain activity (sustained response occurring approximately after one hour of exposure).
- TiO₂ nanoparticles aggregated into larger particles in the cell culture media, ranging in size from 826 to 2368 nm, depending on the concentration of the TiO₂ particles (5 to 120 mg/L) in media. Aggregation was observed within 3 – 5 minutes, with aggregation into the larger particle sizes corresponding to higher concentrations of the TiO₂ particles.

- Phagocytosis of TiO₂ aggregates by BV2 cells was observed using transmission electron microscopy, at 6 and 18 hours post-exposure to 2.5 mg/L of the nanoparticles.
- The TiO₂ particles exhibited negative zeta potentials (or charge) in both cell culture media and physiological buffer.
- The concentrations of TiO₂ were not toxic to the microglial cells.

Implications:

- This study demonstrates that microglial cells in culture, exposed to fairly high concentrations of nano TiO₂ particles, produce ROS via two distinct mechanisms.
- ROS generated in these experiments were not cytotoxic to the microglial cells.
- The authors hypothesized that the negative zeta potential of TiO₂ particles may be responsible for ROS generation by the microglial cells.
- Nanoscale TiO₂ particles formed aggregates in cell culture media, which were then phagocytosed by the microglial cells. The authors note, however, that the high osmolarity of the cell culture media promoted aggregation.
- The extrapolation of these study results to actual human exposures remains unclear at this point; however, it is important to note that the study indicated that nanoparticles can rapidly agglomerate in cell systems and that ROS can be generated without causing cytotoxicity. Thus, there is a need for caution when making inferences regarding potential toxicity based on particle size and on generation of ROS in cell culture.

Templeton RC et al., 2006. “Life-cycle effects of single-walled carbon-nanotubes (SWNTs) on an estuarian meiobenthic copepod”. *Environ. Sci. & Technol.*, ASAP Article.

Abstract: <http://pubs.acs.org/cgi-bin/abstract.cgi/esthag/asap/abs/es060407p.html>

Synopsis:

- The authors of this study investigated the aquatic toxicity of single-walled carbon nanotubes (SWNTs). Acute and chronic toxicity of SWNTs were evaluated on the estuarian copepod *Amphiascus Tenuiremis* (a type of crustacean) using full lifecycle bioassays, where mortality, development, and reproduction of the copepods were evaluated.
- The copepods were exposed for 28 days to: preparations of crude SWNTs, the highest molecular weight purified (electrophoretically) fractions, and fluorescent nanocarbon byproducts of the crude SWNTs. Concentrations of SWNTs tested were 0, 0.58, 0.97, 1.6, and 10 mg/L.
- Statistically significant increased mortality rates were seen only in copepods treated with the highest concentration (10 mg/L) of the crude SWNT and the fluorescent SWNT fraction. In contrast, purified fractions of the SWNTs did not affect the mortality rates of these organisms.

- Similarly, adverse effects on development and reproduction were seen only in copepods treated with the highest concentration of the crude SWNTs. Purified fractions of the SWNTs had no adverse effect on either of these parameters at any of the administered doses. Copepods treated with the fluorescent SWNT fraction, however, exhibited decreased developmental and reproductive success at all treatment doses.
- Examination of the guts and excretory products of the copepods showed that SWNTs were present as aggregated clusters. SWNTs were present as more tightly packaged material in the feces of the copepods.

Implications:

- Crude SWNTs were shown to have adverse effects on the survival rate, reproductive rate, and development of copepods only at very high (environmentally unrealistic) concentrations.
- Copepods are among the most sensitive of aquatic species to environmental insult. Thus, other marine organisms will most likely be less sensitive to the toxic effects of SWNTs. Similarly, findings regarding limited risk for copepods would suggest limited risk for other aquatic organisms.
- Since the crude SWNTs and the fluorescent nanocarbon fraction of the SWNTs exhibited toxicity at the same concentration (10 mg/L), the authors hypothesized that the fluorescent fraction of the crude SWNTs is likely to be the toxicologically relevant constituent of SWNTs.
- Characterizing the fate and transport of materials in the SWNT fluorescent fraction in the aquatic environment may be important since it is this fraction of the crude SWNT material that will most likely be released as waste byproducts; whereas the purified fractions, being more valuable, are less likely to be released to the environment.

Guest Contributor

Assessing Health Effects of Inhaled Nanomaterials

By David B. Warheit and Christie M. Sayes

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Nanotechnology is an emerging multidisciplinary science that is associated with the synthesis, production, and application of particles in the nanoscale (10^{-9} meter) size range. The term “nanotechnology” is derived, in part, from the Greek word “nano,” meaning “dwarf”. The development of new products using nanomaterials is exciting from a materials science perspective, because as one moves into the nanoscale size regime (i.e., reducing the particle size range below ~100 nm), the laws of both classical and quantum mechanics apply – often yielding completely new physical properties. For example, white titanium dioxide particles lose their color and become transparent at sizes below 50 nm. This feature may be useful in the production of cosmetics and other applications. Moreover, at the nanoscale level, some particles which have been utilized for electrical insulating may become conductive; or insoluble substances can become more soluble

below 100 nm. As a consequence, these changes in physical properties enhance versatility and efficacy in product development, thus resulting in new industrial and medical applications.

One challenge that scientists are currently addressing is the language variances in each of the multidisciplinary fields. The research associated with probing the implications of nanomaterials (nanotoxicology) is one notable example of this dispute. The terms “ultrafine” and “nano,” have been used interchangeably, to describe the size of a particle, with the latter being the current nomenclature. This has led to some confusion, as some investigators have considered the term “ultrafine” to pertain to particles primarily generated as byproducts from combustion sources. Alternatively, nanoparticles are known to be intentionally engineered to achieve specific characteristics. To reconcile the issues related to definitions, a number of international technical-based committees (ANSI, ASTM, and others) are currently attempting to standardize the nomenclature.

Assessing the potential health risks related to exposures to engineered nanomaterials is an emerging area in toxicology, exposure assessment, and health risk evaluations. The development of toxicity data sets as well as methodologies for facilitating exposure assessments for various nanoparticles and nanomaterials is commencing (slowly) as new particles and materials are being developed.

Dating back to the mid 1990s, the few pulmonary toxicity studies that were conducted with ultrafine particles in the rat have demonstrated that lung exposures to ultrafines or nanoparticles produce greater adverse inflammatory and fibrotic responses when compared with larger-sized particles of similar or identical composition at equivalent doses/mass concentrations. Virtually all of the particle studies were conducted using rats (a known sensitive species) and under particle overload conditions. Contributing to these effects is the high size-specific deposition of nanoparticles when inhaled as singlets rather than aggregated particles. Limited evidence suggests inhaled ultrafines or nanoparticles which deposit in the lung will, to a greater degree, escape alveolar macrophage surveillance and gain access to the pulmonary interstitium and the systemic vasculature. Therefore, results from the limited toxicological database have fostered the perception that all nanoparticles are likely to be more toxic than fine-sized particulates.

Recently, we have sought to test the hypothesis that particle size and surface area are the major determinants which influence toxicity of nanoparticles. Herein we discuss the results of two published studies and one preliminary study where we have assessed 1) the toxicity of nanoscale TiO₂ particles vs. fine-sized TiO₂ particles; 2) the toxicity of nanoscale quartz particles vs. fine-sized Min-U-Sil quartz particles; and 3) evaluated the role of surface treatments on the potential pulmonary toxicity of low toxicity particulates, namely different formulations of TiO₂ particulates.

In the first study, we compared the pulmonary toxicity effects of intratracheally instilled nanoscale TiO₂ dots (anatase; avg. particle size ~10 nm in diameter, 169 m²/g in surface area) or rods (anatase, 200 nm x 35 nm in length and width, 26.5 m²/g

in surface area) with low toxicity, reference, fine-sized, rutile TiO₂ particles (300 nm in diameter, 6 m²/g in surface area) at two different dose concentrations. The lungs of vehicle or particle-exposed rats were assessed using bronchoalveolar lavage (BAL) fluid biomarkers, cell proliferation methods, and histopathological evaluation of lung tissue at 24 hrs, 1 week, 1 month, and 3 months post-instillation exposure. No differences in pulmonary inflammation, cytotoxicity, or histopathology endpoints were measured in the comparisons of two nanoscale versus fine-sized TiO₂ particles. The results with nanoscale dots and nanoscale rods were considered to be at variance with the “conventional wisdom” that nanoscale particles are always more toxic than fine-sized particulates of similar chemical composition (Warheit *et al.*, *Toxicological Sciences*. 91:227-236, 2006).

Studies have been conducted to compare the pulmonary toxicities in rats of two nanoscale quartz particle samples (12 nm and 50 nm in diameter) versus Min-U-Sil quartz particles (534 nm). Min-U-Sil quartz is a well-known cytotoxic reference particulate used as a positive control in many particulate toxicology studies. The International Agency for Research on Cancer (IARC) has classified quartz as a Group I carcinogen (human carcinogen). Accordingly, we have postulated that the nanoscale quartz particles should be even more toxic than Min-U-Sil, which consistently produces a sustained and progressive inflammatory response in the lungs of exposed rats. Substantial physicochemical characterization of the particle samples was conducted. Our findings indicate that the 50 nm nano quartz samples were significantly less inflammatory and cytotoxic when compared to the Min-U-Sil samples, whereas the 12 nm nano quartz samples demonstrated equivalent toxicity. It is interesting to note that the level of toxicity did not correlate with particle size or surface area but was consistent with hemolytic potential of erythrocytes. Our conclusions thusfar suggest that for consideration of lung toxicity of quartz samples, surface reactivity is more influential than particle size or surface area.

Surface treatments on particles can also influence or modify the toxicity of particulates. Accordingly, we assessed in rats the lung toxicity of inhaled or intratracheally instilled, fine-sized (300–400 nm) TiO₂ particle formulations with various surface treatments, ranging from 0–6% alumina (Al₂O₃) or alumina and 0–11% amorphous silica (SiO₂). Both inhalation and instillation studies were conducted with the same TiO₂ particle formulations. For both studies, the lung effects of different TiO₂ particle formulations were compared to reference TiO₂ particles and/or controls. Based upon the results of these studies, we concluded that surface treatments can modify the toxicity of TiO₂ particles, and the results of intratracheal instillation studies provided an effective preliminary screen for predicting the effects of inhalation studies (Warheit *et al.*, *Toxicological Sciences*. 88:514-524, 2005).

To briefly summarize our results on nanoparticle toxicity, in addition to particle size and surface area, several additional factors may play more important roles in influence the lung toxicity of nanoparticles. These include, but are not limited to: surface reactivity, surface treatments on particles, the aggregation/disaggregation potential of nanoparticles,

particle shape, surface charge, translocation potential of the nanoparticle, and the method of particle synthesis. Some additional concluding messages are the following: risk is a product of hazard and exposure, and, in general, one cannot assume that nanoparticles have the same chemistry or toxicity (i.e., biology) as their bulk counterparts. As a consequence, the hazards of nanoparticles cannot be generalized and should be tested on a case-by-case basis.

Coming Next Month

- Review of pulmonary toxicology of carbon nanotubes and implications for workplace safety
- Brief review of Dr. Andrew Maynard’s research strategy for nanotechnology risk issues



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