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Dr. Axel Hahn

Recent Government Briefs

(NIOSH NTRC) – In February, the National Institute for Occupational Safety and Health (NIOSH) Nanotechnology Research Center (NTRC) released a report entitled, *Progress Toward Safe Nanotechnology in the Workplace*. The report reviews the progress of the NTRC from its inception in 2004, through 2006. The four primary goals of the Center are the following: determine if nanoparticles and nanomaterials pose risks for work-related injuries and illnesses; conduct research on the application of nanotechnology for the prevention of work-related injuries and illnesses; promote healthy workplaces through interventions, recommendations, and capacity building; and enhance global workplace safety through national and international collaborations on nanotechnology research and guidance. The NTRC receives redirected funds for its work (\$3 million in 2005, \$3.7 million in 2006, and \$4.6 million in 2007). Despite such “budgetary constraints,” the report notes that the Center has made progress toward each of its four primary goals. For example, the NTRC has conducted research in nanotoxicology, particularly the toxicity of carbon nanotubes because of their emerging technological importance in a variety of different industries. In addition, the NTRC has published more than 70 peer-reviewed scientific papers since 2004. The report also outlines the resource needs of the Center for future work. The full report can be found at: <http://www.cdc.gov/niosh/docs/2007-123/pdfs/2007-123.pdf>

Reports, Reviews, White Papers, and Books

Nanotechnology White Paper

US EPA, Science Policy Council, Nanotechnology Workgroup
<http://www.epa.gov/osa/nanotech.htm>

The authors of this paper explain that the purpose of the document is to “inform EPA management of the science needs associated with nanotechnology, to support related EPA program office needs, and to communicate these nanotechnology science issues to stakeholders and the public.” Previously released as an external review draft in December 2005 and revised following peer review and public comments, the white paper includes an expansive introduction on what nanotech/nanomaterials are, potential uses of nano materials and products, the role of national and international agencies, and what the EPA has been involved in within this field. There is also a brief review of the potential environmental benefits of nanotech, followed by a chapter on nano risk assessment. The risk assessment chapter is a useful composite of the topic on the whole, and also includes some analysis, for example, of current toxicity test methods and toxicological databases. The chapter on EPA's research needs for nanomaterials provides a list of questions that remain to be addressed. Finally, specific and general recommendations are made, such as, that EPA's Office of Research and Development (ORD) should lead the development of a set of standard methods for the sampling/analysis of nanomaterials of interest in environmental media and that ORD should lead research to determine the health effects resulting from exposure to nanomaterials and their byproducts.

Nano Risk Framework – Draft

Environmental Defense–DuPont Nano Partnership
<http://nanoriskframework.com/page.cfm?tagID=1081>

In February, Environmental Defense and DuPont, in a joint effort, published a draft of their Nano Risk Framework, asking for comments from others in the field. The goal of the Framework is to establish a process for the responsible development and production of nanomaterials and products throughout their lifecycle. Further, the authors wanted to develop the Framework itself transparently, with input from others in industry and various organizations. The Framework will be used to evaluate, address, document, manage, and communicate potential EH&S risks of nanomaterials. The draft document provides a comprehensive, step-by-step explanation of the Framework, as well as other considerations such as the costs associated with implementing it. Worksheets

are provided to facilitate implementation of the Framework. Once feedback has been received (due at the end of March), the Framework will be revised and finalized in the summer.

Upcoming Meetings and Conferences

3rd International Symposium on Nanotechnology, Occupational and Environmental Health

Taipei, Taiwan; Aug. 29- Sept. 1, 2007

<http://nano-taiwan.sinica.edu.tw/EHS2007/index.htm>

This symposium will bring together international nanotech experts with a variety of backgrounds to discuss how to identify, assess, and manage/govern risks associated with nanotech in both occupational and environmental health settings. Topics that will be addressed at the meeting include environmental and exposure monitoring, environmental applications of nanotech, and good working practices. In addition to keynote lectures and research presentations, there will be tutorials that provide basic training in areas such as nanoparticles dosimetry in the lung and exposure assessments of nanoparticles.

International Symposium on Nanotechnology in Environmental Protection and Pollution (ISNEPP) 2007

Fort Lauderdale, Florida; Dec 11-13, 2007

<http://www.isnepp.org/ISNEPP07/front1.htm>

This conference will address nanotechnology in the context of environmental protection and remediation, public health, energy resources and production, and standards and regulation. Recent research developments, as well as outstanding data and research needs, will be highlighted and discussed in the symposia, round tables, and panel sessions. The program is still in the planning stages, but the abstract submission deadline is not far off (May 10).

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

1. **Sayes, *et al.*, 2007. Assessing toxicity of fine and nanoparticles: Comparing *in vitro* measurements to *in vivo* pulmonary toxicity profiles** *Toxicological Sciences*, doi:10.1093/toxsci/kfm018 (Advanced publication). Abstract: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=17301066

Synopsis:

- This study by Sayes and colleagues included both *in vitro* and *in vivo* components and sought to assess the ability of *in vitro* screening studies of fine and nanoparticles to predict *in vivo* pulmonary toxicity in rats. This is a highly relevant objective given the great benefit that could be gained from reasonably accurate, predictable *in vitro* screening tests.
- The *in vivo* aspect of the study involved intratracheal instillation of 1 or 5 mg/kg of the following particles: carbonyl iron (negative control; 358 nm), crystalline silica (Min-U-Sil 5 a-quartz) particles (positive control; 452 nm), amorphous silica (354 nm), nano-sized zinc oxide (90 nm), and fine-sized zinc oxide (111 nm). At 24 hr, 1 week, and 1 and 3 months post-exposure, the authors examined lung inflammation and cytotoxicity endpoints. There were 5 rats/group/dose/timepoint.
- For the *in vitro* work, the authors incubated 3 cell culture types (rat L2 lung epithelial cells; primary alveolar macrophages; and alveolar macrophages-L2 lung epithelial co-cultures) with the same particle types as described above. The cells were then evaluated for inflammation and cytotoxicity endpoints.
- The overall size range of the particles was 90–500 nm. It is important to note that the authors characterized the particles and found that for all particles, the sizes reported by the manufacturer were significantly different than the sizes measured by the authors. For example, the supplier of the nano-sized zinc oxide system reported a particle size of 50–70 nm, though “three independent sizing techniques indicated that the nano-sized zinc oxide particles ranged from 90–283 nm, which was similar to the size of the fine-sized zinc oxide particles.” In general, the fine- and nano-sized zinc oxide particles were similar to one another (the surface areas were 9.6 and 12.1 m²/g, respectively; both had a density of 5.6 g/ml, and the crystallinity of both is hexagonal), though the fine-sized particles tended to have somewhat larger aggregates.
- Briefly, the *in vivo* results were the following: carbonyl iron produced little toxicity; the crystalline silica yielded sustained inflammation and cytotoxicity; the amorphous silica produced reversible/transient inflammation; and both the fine- and nano-sized zinc oxide resulted in “potent but reversible inflammation” (resolved by 1 month post-exposure). In contrast, the *in vitro* results were much more variable and dependent on cell type, length of incubation with particles, and particle dose. For example, chemotactic (pro-inflammatory) factors were released from crystalline and amorphous silica-exposed alveolar macrophages, as well as from the co-cultures of alveolar macrophages and L2 cells, *in vitro*, though the L2 cells alone did not release such factors *in vitro*.
- The fine- and nano-sized zinc oxide particles often yielded similar results, perhaps due to their similarity in size and other characteristics. For example, *in vivo* experiments showed that high doses of both fine- and nano-sized particles caused substantial lung inflammatory responses at 24 hours post-exposure, which resolved by one month later; *in vitro* experiments demonstrated that at different incubation times, the two particle types had the same effects on cytotoxicity endpoints in L2 cells (measured via increases in lactate dehydrogenase, or LDH).

- Overall, the authors found little correlation between their *in vitro* and *in vivo* experiments and call for the further development of screening tests.

Implications:

- Many individuals and organizations have called for the development of standardized, validated *in vitro* screening tests in order to either avoid *in vivo* work or to accelerate initial toxicological research on nanoparticles. In general, the findings by Sayes *et al.* are consistent with attempts to find correlation between *in vitro* and *in vivo* experiments – *i.e.*, little correlation was shown.
- The study authors highlight several key areas of concern stemming from their own work as well as the work of others. For example, they note that no *in vitro* cell culture system can simulate the recruitment of inflammatory cells into the lung – an important biomarker for *in vivo* studies. The use of cytokine generation in cell culture as an indirect marker of inflammation is used, but has not been tested or validated.
- In conclusion, the work by Sayes and colleagues demonstrates the significant amount of work that remains if there is to eventually be a well-tested *in vitro* screening system for fine and nanoparticles toxicity assessment.
- Given the finding of a similar size range for the fine- and nano-sized zinc oxide particles, the importance of careful characterization of nanomaterials prior to toxicity testing, even those with manufacturer-provided properties, is apparent.

2. Lacerda, *et al.*, 2006. “Carbon nanotubes as nanomedicines: From toxicology to pharmacology” *Advanced Drug Delivery Reviews* 58(14):1460-1470. Abstract: <http://www.aapspharmaceutica.com/search/view.asp?ID=81297>

Synopsis:

- Lacerda and colleagues provide a review of all currently available *in vivo* toxicological and pharmacological studies of carbon nanotubes (CNTs), in the context of using CNTs in/as nanomedicines. The key properties of CNTs that underlie their potential use in areas such as biomedicine include their ultralight weight, high mechanical strength, high surface area, and high thermal conductivity, though one important obstacle in their use is that pristine CNTs are hydrophobic, therefore requiring surface modification. Both single-walled (SWNT) and multi-walled (MWNT) nanotubes are considered in this review.
- The authors note the importance of understanding each parameter and barrier that affects the fate of any material administered as a medicine (*e.g.*, blood flow dynamics, interstitial pH, and crossing the cell membrane). While the safety and toxicological aspects of CNTs as nanomedicines are key, the pharmacological parameters that have proven to play a determinant role in *in vivo* fate must also be addressed – as much for nanomedicines as for any medicine or therapeutic component.

- With respect to the toxicity of CNTs, the authors review 11 *in vivo* studies that used either rodents or human volunteers, and the studies provide a range of outcomes to consider. For example, two recent studies in rats showed that CNTs modulate the immune response, clearance kinetics, and bioavailability regardless of whether the particles had been functionalized. A study by Warheit *et al.* (2004) led those authors to conclude that while agglomerated SWNTs in the major airways of rats can cause death, any inherent toxicity of the particles themselves did not play a role in mortality. Another study, by Huczko *et al.* (2001), found no association between soot with high CNT concentration and dermatological irritation or allergic reaction in human subjects.
- The authors report only two studies to-date that have examined the biodistribution of CNTs, both of them having used water-soluble functionalized particles. Wang *et al.* (2004) administered SWNT (functionalized by oxidation) via intraperitoneal, subcutaneous, oral, and intravenous administration to male mice. They found that route of administration did not significantly affect biodistribution and that the particles quickly distributed throughout the body; accumulation was noted in the stomach, kidneys, and bone. Importantly, 94% of the SWNTs were excreted in urine and 6% in the feces; no tissue damage or distress was reported. The other study, by Singh *et al.* (2006), administered functionalized SWNTs and MWNTs via intravenous administration to female mice. This study showed the following: no accumulation in the body; the biodistribution of both particles was similar; all nanotubes were rapidly cleared from all tissues (the max blood circulation half-life was 3.5 hr); and excretion was via the renal route.

Implications:

- The studies discussed throughout the review by Lacerda and colleagues reveal the importance of functionalizing CNTs for use in biomedicine – both for literal functionality (making them water-soluble and biocompatible) and for better toxicological profiles (due to their ability to be rapidly excreted via the renal route).
- While more biodistribution studies are certainly needed for these and other forms of nanotubes, the two murine biodistribution studies discussed above reported no acute toxicity or adverse effects following administration of CNTs.
- The authors of the review find the studies on CNTs and nanomedicines to-date promising. Studies of both forms of CNTs loaded with peptides, proteins, nucleic acids, and drugs have shown that they can translocate into mammalian cells – and “moderate” biological effects have been observed, providing proof-of-principle support for further study. The two therapeutic studies they briefly assess (one using CNTs in vaccine delivery and one using CNTs to improve the bioavailability of erythropoietin) and the pharmacological studies indicate the vast promise held within CNTs. Although the toxicological studies indicate important health and safety concerns, the non-functionalized form of the particles appears to be the primary concern.

Guest Author

By **Dr. Axel Hahn**

Acute health impairments due to
"Magic Nano" sealing sprays in Germany

In Germany, a series of rapidly developing and sometimes severe cases of health impairment were observed after the correct use of 'nano' sealing sprays intended for the treatment of glass and ceramic surfaces. The first case was reported on 27 March 2006 and until noon of the following day, as many as 10 cases had been treated by physicians; a day later, the number of cases recorded had increased to 69. So far, the Federal Institute for Risk Assessment (BfR) has received reports of 150 cases. The cardinal symptom was strong cough, dyspnea, and in eight severe cases, edema of the lung.

Based on a rapid and complete documentation of cases in collaboration with the Poison Control Centers, the BfR, as an independent federal institute, immediately initiated a recall of the hazardous products. Therefore, three days after the product was marketed, no additional cases were observed. In parallel, information was communicated to the government authorities, to the EU rapid alert system for non-food products RAPEX, and to the WHO rapid information system, INTOX. The public as well as authorities and ministries were informed by timely publication of three press releases in German and English, which were mostly based on three expert meetings held at the BfR (April to May 2006), and also on an EU meeting held in Brussels, since one of the suppliers allegedly was located in Luxembourg.

Investigations into the composition of the product were considerably complicated by the fact that the distributor had no knowledge of the composition of his products, and the suppliers refused to provide information – they argued to keep trade secrets on three components of their preparations. In addition, the situation was complicated because some of the supplier companies of the product's components were located in different German federal Länder, though the basic component of the nanofluid had been produced in Luxembourg.

The components responsible for health impairment of exposed users were largely determined in the context of an expert meeting held at the BfR at the end of May 2006. The findings were as follows: the products concerned did not contain any nano-sized particles; the nano function suggested by the product name referred only to the thickness of the film of the active substance; due to unexpected chemical changes during the processing to produce aerosol sprays, the active silicon compounds in the active substances had obviously disappeared to a large extent; and with regard to their pattern, the hazardous manifestations associated with nano sealing sprays were very similar to the health problems documented in a number of earlier case clusters associated with leather and impregnating sprays (Germany, USA, Netherlands, Switzerland, Denmark).

The 2006 German case series associated with nano sealing sprays—characterized in some cases by severe health impairment of exposed consumers—has revealed considerable gaps in

the documentation of formulations for medical emergency purposes. When products not subject to compulsory notification are involved, neither the distributor nor any other party in the chain of supplier companies is sufficiently informed about the formulation of the final product. Knowledge of the complete formulation of a product will considerably enhance product safety. In cases of emergency, sufficient information about the formulation of the final product must therefore be available at least at one point of the production chain or from a neutral party where it has been deposited before marketing. It should also be considered whether products and preparations containing defined nano-sized particles should have to be labeled accordingly and listed in a register (*e.g.*, the BfR poison information data bank) for early detection of health impairments. The BfR expert meeting held on 5 July 2006 set out the requirements for further research: Currently the BfR is analyzing the case series' pattern of signs and symptoms and reviewing the case series reported from other countries. In parallel, a chemical analysis of the aerosols is being performed and animal studies will be carried out to examine the respirable components of the aerosol fractions in the sprays that caused the health impairments reported.

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Coming In the Next Issue

- Findings of cardiovascular-related effects in mice exposed to single-wall carbon nanotubes

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