

March 8, 2006

NTP Nanotechnology Working Group
c/o: Kristina Thayer, Ph.D.
Executive Secretary
NTP Liaison and Scientific Review Office
NIEHS/NIH
P.O. Box 12233, MD A3-01
111 TW Alexander Drive
Research Triangle Park, NC 27709



PEOPLE FOR THE ETHICAL
TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

Re: Solicitation of Comments pertaining to NTP Nanotechnology Working Group

The National Toxicology Program (NTP) is an interagency program whose primary responsibility is to evaluate chemicals for public health, while utilizing and developing modern diagnostic tools. The NTP was created to coordinate toxicity testing programs within the government, strengthen the science base in toxicology research, develop and validate improved testing methods, and to use the findings of these studies to protect citizens from toxic substances by educating health, regulatory, research and scientific Agencies of its findings. In recent years, nanotechnology has presented itself as a novel field in need of a standard set of toxicity tests and regulations to protect workers and consumers.

On May 19, 2003, the NTP received a letter from Vicki Colvin, PhD, Director of Rice University's Center for Biological and Environmental Nanotechnology. In her letter, Dr. Colvin listed the many types of nanomaterials already found in research laboratories at that time, and urged the NTP to begin formally studying the toxicity of nanomaterials. Dr. Colvin advised that nanomaterial toxicity studies be completed before the industry gains unregulated momentum. Further, Colvin reminded the NTP that, as nanotechnology developed, "the public would be exposed to increasingly high amounts of diverse forms of nanostructured materials."

The Nanotechnology Working Group (NWG) held its first meeting on June 24, 2005. There were twenty-seven attendees. Of the twenty-seven people present, seven were NTP NWG members, and sixteen others represented the NIEHS, FDA, National Nanotechnology Initiative (NNI), NIOSH/CDC, and the National Cancer Institute. Sherry Ward, PhD, contributed the only written comments received by the Working Group and represented Physicians Committee for Responsible Medicine (PCRM). Dr. Ward urged the NWG to include a scientist who would represent the animal protection perspective. I hereby reiterate Dr. Ward's statement. A direct quote from the NTP website regarding the stated structure of the Nanotechnology Working Group reads, "NWG's membership shall be sufficiently broad to promote input and exchange of ideas and information." The description goes on to include suggested membership from non-profit organizations and specifies that the NWG may include up to twelve people. Please consider my application as a member of this important Working Group.

The NWG is charged with advising the Board of Scientific Counselors on matters of scientific content of the nanotechnology research program. Specifically, the NWG is required to review the research program and advise on the merit and quality of the research as it relates to the needs of U.S. regulatory agencies, as well as issues of product development as they relate to public health.

The importance of this responsibility cannot be overstated. The NWG must learn from the mistakes of previous NTP research programs and prevent the problems now faced by other regulatory agencies, such as those the FDA is facing in the field of drug discovery. The NTP should avoid, up front, the uninterpretable and irrelevant data that have characterized other NTP projects.

A large-scale example of such an NTP-funded program is the rodent cancer bioassay program. These studies have resulted in reams of incongruent data sets. The faulty premise that an animal model can predict human carcinogenic effects is one of the root causes for this dilemma. The rodent cancer bioassay project attempted to predict whether a given chemical is carcinogenic to humans by testing each chemical on rats and mice. Yet, when the data from rats and mice are compared, the results are frequently in conflict. Rats and mice share a relatively recent common ancestor (between 12 and 24 million years ago) compared to the more distant common ancestor shared by humans and rodents (75 million years ago). When an experiment on two relatively closely related rodents results in conflicting data, it becomes increasingly problematic to extrapolate these conflicting findings further, to humans. Curiously, as scientists, we are trained to suspend this reality and base our predictions of what might happen in humans on a relative who has evolved with its own selective pressures for 75 million years. With current technology and increased understanding at our disposal, we can and must do better.

Problems in the field of drug discovery stem from the same fallacy and have been heavily reported in the news. The field of drug discovery has seen a litany of lawsuits and a 92% failure rate for drugs that have passed animal testing trials but are later found to be ineffective or dangerous while undergoing clinical trials. The failures of numerous other government testing programs can be largely attributed to the same root problem. Animals are not suitable models for the human condition. When animal tests in mice, rats, dogs, and others all give different answers to the same question, it is time to rethink the methods being used. Since the field of nanotechnology is not encumbered by half-century-old testing protocols, these problems can be avoided from the very beginning.

Many of the human-relevant, high throughput assays developed in related, modern fields, such as particulate matter toxicology, appear to be applicable to nanomaterials. Studies from this field have shown that a subset of cellular responses will predict potential chemical toxicity. I would like to call your attention to some of the most relevant of these assays.

Nanoscale materials have unique characters due to their minute size. As the size of the particle decreases, its surface area increases, thus allowing a greater proportion of its reactive groups to be on the surface of the molecule. This greater reactivity may be responsible for some of the beneficial characteristics of nanomaterials, but is also the reason for such concern regarding their potential toxicity. Reactive surface groups determine whether a chemical is hydrophobic or hydrophilic, lipophobic or lipophilic, catalytically active or passive. Chemical characteristics that lead to human cellular toxicity center on electron state – whether the molecule has the propensity to be an electron donor or acceptor determines whether there is a likelihood it will form superoxide radicals and reactive oxygen species (ROS). Currently, according to Shvedova *et al.* 2005, Oberdorster *et al.* 2004, and Donaldson and Tran 2002, among others, the most reliable paradigm for nanomaterial toxicity that accounts for the mechanism of human cell toxicity is the generation of ROS (Donaldson and Tran 2002; Shvedova, Kisin *et al.* 2005).

Cellular responses at each level of oxidative stress have been used successfully as indicators for toxicological effects in screening assays of ambient particulate matter. Proteomics and genomics have helped substantiate mechanistic hypotheses. Pathways of biological stress begin with the induction of antioxidant and detoxification enzymes. Next in the process are Nrf-2 associated responses. Nrf-2 is a transcription factor that binds to the promoters of phase II genes, which take part in the antioxidant response element (ARE). This is a protective response and any defects in this response can lead to cell cytotoxicity. MAPK (mitogen activated protein kinase) is the cascading response that leads to inflammation. Alternatively, programmed cell death can be predicted when mitochondrial perturbation is assayed. Nanomaterial toxicity testing should focus on the human-relevant cellular markers that have been identified and utilize *in vitro* assays that are most capable of detecting those responses in cell culture or microarray experiments.

A review entitled, “*Toxic Potential of Materials at the Nanolevel*,” published in *Science* on February 3 2006, describes many of the relevant assays and lists some of the short-term goals that toxicity studies of nanomaterials should attempt to attain (Nel, Xia *et al.* 2006). The author, Andre Nel PhD, is a well-respected nanomaterials expert. He summarizes the most important aspects of toxicity testing by explaining that generation of ROS is among the most predictive of tests that can be done. These assays can show injury to proteins, DNA, and cellular membranes due to oxidative stress. Oxidative stress can be measured by mitochondrial perturbation, specifically inner membrane damage, permeability transition, energy failure, and apoptosis. Table 2 of this review lists thirteen cellular responses to toxic chemicals and the corresponding assays by which these effects can be measured. In addition, Nel specifies that the ultimate goal of the predictive approach to toxicity testing “would be to develop a series of toxicity assays that can limit the demand for *in vivo* studies, both from a cost perspective as well as an animal use perspective.” This notable scientist seems to recognize that animal experimentation has severe limitations and is problematic in this modern era. He goes on to reference a number of *in vitro* studies that are human-relevant, high-tech, reliable, and humane.

Studies such as those performed by Veeriah *et al.* 2005, show that human cell culture followed by microarray experiments assay whether particular chemicals help diminish the detrimental effects of cancer-causing oxidative stressors (Veeriah, Kautenburger et al. 2006). This study monitored cellular markers in human colon cancer cells that respond to damage and predict cancer. When human colon cancer cells were exposed to apple flavenoids, the cellular markers indicative of oxidative stress and cancer progression were greatly reduced and the genes responsible for protective responses were up-regulated. Microarray results were confirmed by qRT-PCR. Based on the pattern of differential gene expression found, apple flavenoids are able to modulate toxicological defenses against colon cancer risk factors. These assays are readily adaptable for nanomaterials toxicity testing and are more reliable than results from animal experimentation.

In another study, cultured *Arabidopsis* cells were treated with mitochondrial electron transport chain inhibitors (rotenone and antimycin A), resulting in increased transcript levels of import components. Microarray analyses detected the up-regulation of gene sets involved in mitochondrial chaperone activity, protein degradation, respiratory chain assembly, and division (Lister, Chew et al. 2004). These cellular responses indicate the ability to measure the cell's direct response to added chemicals. Assays such as these are also important and applicable to the study of nanomaterial safety.

Studies from Dr. Colvin's lab, (Sayes, Gobin et al. 2005) utilize a series of *in vitro* human cell culture assays predictive of cellular responses to toxic chemicals. In a study entitled "*Nano-C₆₀ cytotoxicity is due to lipid peroxidation,*" experiments were performed to assess cytotoxicity/cell viability, lactate dehydrogenase release, mitochondrial activity, DNA content, plasma membrane permeability, lipid peroxidation, glutathione production, and the ability to prevent oxidative damage by the addition of L-ascorbic acid. By changing the number of hydroxyl groups on the surface of the fullerene, toxicity dropped by several orders of magnitude. These telling experiments show that fullerene toxicity can be rigorously tested by means of these cost-effective, predictive, and relevant assays. In addition, potential toxicity of the fullerenes was lowered significantly by using these *in vitro* assays to target chemical aspects of the nanomaterials that contribute to toxicity. The author states that, "*in vitro* testing provides a cost-effective means for such studies, and as this report illustrates, cell culture experiments are well suited for developing mechanistic models to inform material development." In addition, the author explains that this study seeks "to set a standard for future efforts to characterize the environmental and health impacts of other classes of engineered nanoparticles." This study clearly shows that the most efficient (and humane) means of toxicity testing lie in modern, high-throughput *in vitro* assays.

For questions pertaining to the repercussions of chemical metabolites or how well a substance is targeted to a particular organ, the HuREL, a microfluidic device that allows the scientist to test a compound within a matrix of different cell types, linked by microfluidic channels, can answer questions regarding how nanomaterials or nanomedicine will interact with human tissues. Details of this work can be read in depth in Sin *et al.* 2004, entitled *The Design and Fabrication of Three-Chamber Microscale Cell Analog Devices with Integrated Dissolved Oxygen Sensors* (Sin, Chin et al. 2004).

Systems such as these will allow scientists to test whether their nanomedicine is effectively targeted to a particular organ, or cell, and whether a nanomaterial has detrimental effects on organs such as the kidney, liver, or heart. Using these modern systems will save not only human and animal lives, but also time, money, and resources. This system was unveiled this year and has been exciting for both researchers and investors alike.

As the nanomaterials market grows and the budget for its toxicity research is increased, we urge you to allocate funding for studying nanomaterials wisely. This is the time to set a precedent and use only the most modern, human-relevant assays at your disposal to set standards in this high-tech field.

Thank you in advance for considering these recommendations and please do not hesitate to contact me if you have any questions. I can be reached by phone at 607-272-3143 or by e-mail at SamanthaD@peta.org.

Sincerely,



Samantha Dozier, Ph.D.
Nanotechnology Research Liaison

References:

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