Miller, Diane M. (CDC/NIOSH/EID)

From: Samantha Dozier [SamanthaD@peta.org]
Sent: Friday, February 15, 2008 11:03 AM
To: NIOSH Docket Office (CDC)
Cc: Samantha Dozier
Subject: NIOSH Interim Guidance for Medical Screening of Workers Potentially Exposed to Engineered Nanoparticles

Attachments: NIOSH Nano Testing Guidance (2-08).pdf

NIOSH Nano esting Guidance (2.

Please find our comments attached to this email.

Sincerely,

Samantha Dozier, Ph.D.
Nanotechnology Policy Advisor
Regulatory Testing Division

NIOSH Docket Office
Robert A Taft Laboratories
4676 Columbia Parkway, MS C-34
Cincinnati, Ohio 45226

PETA
PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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

Submitted via email at nioshdocket@cdc.gov

National Institute for Occupational Safety and Health (NIOSH) Interim Guidance for the Medical Screening of Workers Potentially Exposed to Engineered Nanoparticles

People for the Ethical Treatment of Animals represents more than 1.8 million members and supporters who are concerned about the suffering of animals used in laboratory experiments and the ramifications of using animal experiments rather than relevant human-cell-based methods to assess the risks of nanomaterials. We appreciate the opportunity to comment on NIOSH's proposed plans and offer relevant testing alternatives to ensure worker safety.

The best and most reliable methods of preventing exposure to nanoparticles and screening for the effects of nanoparticles is to use high-throughput, real-time analytical devices capable of measuring nanoparticles in the workplace and by using available screening and toxicity testing methods based on human cells. Resorting to animal experiments will result in the same lack of worker protection that was seen for decades with asbestos, arsenic, mercury, and any number of other dangerous substances. Workers went unprotected for many years because animal experiments did not replicate what was seen in humans and the same lack of correlation has been seen thus far in animal experiments on nanomaterials. We urge NIOSH to proctively embrace non-animal test methods that are more sensitive, reliable, reproducible, and relevant to humans.

Characterization of Nanoparticles

Use of appropriate, available metrological devices as well as continued advances in device development are needed so that purity, size, shape, distribution, structure, and surface area assessment is determined using high-resolution instruments. In order to reduce batch-to-batch variation in nanomaterials produced within the same manufacturing facility, as well as variability found in the same nanochemical made by different manufacturers, the use of high-throughput methods of synthesis and analysis are recommended. In this way, consistent composition and quality of product would be greatly improved.

The best methods available to measure and test the purity of nanomaterials include scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) analysis that measures nanomaterial size and detects dispersion, non-carbon-based contamination, and geometry of the nanomaterial. Transmission electron microscopy (TEM) answers questions pertaining to particle/bundle size, morphology and purity of the nanomaterial's surface while thermogravimetric analysis (TGA) analyzes a wide range of parameters,

including compositional analysis, decomposition temperature, rate of decomposition – a quantitative measure of mass change associated with transition and thermal degradation and nanomaterial oxidation. Raman spectroscopy is able to identify carbon spectral features while x-ray photoelectron spectroscopy (XPS) can be used to determine surface elemental group composition and provides chemical state information for the first several layers of the sample surface.

Instruments such as scanning mobility particle sizers (SMPS) that can measure the size of particles between 3 and 1000 nanometers (nm) and scanning electron microscopes (SEM) that utilize an electron dispersive spectrometer (EDS) make nanoparticles countable an their chemical compositions discernable. The Electrical Dekati Industrial Hygiene Particle Sensor (EDiPS) is a portable sensor that offers real-time nanoparticle measurements to ensure workplace safety.

In addition to the problems of standardizing nanoparticle metrics and tracking nanoparticles in biological systems, there are additional issues relating to particle state, such as agglomeration/dissaglommeration, impurities (differing depending on which factory manufactured a particular type of nanoparticle), and the presence of adsorbed species on the surface of nanoparticles.

Each of the variables described above affects the state of the nanoparticle and contributes to the added unpredictability of translocation and cellular uptake. It is for these reasons that standards for the field should be put in place and human cell-based methods for predicting toxicity should be used preferentially for this field. These methods are relevant to human health and can be performed quickly and inexpensively.

Nanotoxicity Testing

In a move towards progressive testing that would ensure human protection, we recommend that NIOSH use non-animal test methods for toxicity testing. Methods such as cytotoxicity studies, cell culture, and microarray assays that seek to measure specific cellular pathways and monitor whether changes indicative of cellular stress or other signs of exposure are generated would give NIOSH a clear sense of whether a given chemical caries risk.

At a minimum, NIOSH should require that modern, non-animal-based OECD Test Guidelines (TGs) be used for nanomaterials testing. Use of reliable methods is imperative at the outset of NIOSH's guidelines for protecting human health.

Relevant OECD Test Guidelines:

- **Skin sensitization/irritation:** Please specify that OECD Test Guideline 429 (Reduced LLNA) is a partial replacement for the guinea pig tests often used to test skin sensitization and that EPISKIN™-SIT is a complete ECVAM-validated replacement for *in vivo* skin irritation tests for skin irritation.

- **Skin penetration:** The *In Vitro* Skin Absorption Test is a full replacement for the *in vivo* skin penetration test under OECD TG 428.
- **Genotoxicity:** Specify the Ames test for bacterial mutation (OECD TG 471), testing for mutagenicity by *in vitro* chromosomal aberration (OECD TG 473), Unscheduled DNA synthesis (OECD 482), Sister Chromatid Exchange (OECD 479) each of which can be a partial or full replacement for *in vivo* methods. Negative results for these assays preclude the use of additional *in vivo* test confirmation.
- **Skin Corrosivity:** Using CORROSITEX (OECD TG 435) as a full replacement for the *in vivo* skin corrosion test for acids, bases, and acid derivatives. EpiDerm and EPISKIN each serve as full replacements for the *in vivo* skin corrosion test (OECD TG 431).
- **Phototoxicity:** The 3T3 Neutral Red Uptake Test is considered a replacement for the *in vivo* photoirritation tests (OECD TG 432) and has gained FDA preference.
- **Ocular Corneal Opacity-Permeability Test:** ICCVAM and EU approved for use as a positive screen for ocular corrosivity and severe irritation and is acceptable for use based on the sequential testing strategy supplement to OECD TG 405.
- **Acute Neutropenia:** In order to predict chemical-induced neutropenia, the CFU-MG assay has been validated for use by ECVAM and endorsed by ESAC.
- **Pyrogenicity:** The PBMC/IL-6, WB/IL-1, CryoWB/IL-1, WB/IL-6, and MM6/IL-6 tests are now under ICCVAM peer review and should be considered as replacements for the rabbit and LAL tests.
- **Embryotoxicity:** Partial replacements for the *in vivo* developmental toxicity test exist and include tests such as: Embryonic Stem Cell Test, Rat Limb Bud Test, and Micromass Test.

In addition to OECD-approved tests for toxicity testing, there are many test methods specifically designed to assess nanotoxicity. For example, several reproducible, reliable, and human-relevant *in vitro* assays based on human cell lines have been developed [1-7]. There are many research groups working to tailor cell culture protocols to the field of nanotechnology. Barbara Panessa-Warren of the Department of Energy's Brookhaven National Laboratory, for example, has had success using ultrasound imaging to assess the cellular toxicity of various carbon-based nanomaterial in human lung and colon cells [8].

Many groups have used human cell culture in concert with microarray experiments and cytotoxicity analyses, to detect early signs of cellular toxicity. Known cellular stress responses, such as the generation of reactive oxygen species (ROS) can be measured before and after exposure to nanomaterials thereby giving scientists an accurate and reproducible measure of cellular responses [7, 9-11]. Cell types typifying exposure routes (dermal fibroblasts, lung epithelial cells, and colon cells); movement across barriers (blood brain barrier, lung epithelia); target effects (astrocytes, macrophages, T-cells, liver cells, kidney cells); and diseased tissues (liver carcinoma cells, B2-microglobulin cells, and lung cancer cells) have each been the focus of nanotoxicity studies and method development [3, 5-9, 11-15]. These important studies illustrate that toxicity testing can be done rigorously *in vitro*.

In Nel *et al.* 2006, "*Toxic Potential of Materials at the Nanolevel*," a series of established *in vitro* assessments of ROS activity is proposed as a paradigm for nanomaterials toxicity testing. The authors reflect the sentiment of a growing number of toxicology researchers that animal experimentation has severe limitations in their statement 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"** [16].

In a study entitled "*Nano-C_{60} cytotoxicity is due to lipid peroxidation,*" Sayes *et al.* demonstrate that fullerene toxicity can be tested by means of cost-effective, predictive, and relevant *in vitro* assays of established cellular stress responses. 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"** [17]. The above studies clearly show that the most efficient (and humane) means of toxicity testing lie in modern, high-throughput *in vitro* assays.

To assess the effects of pharmaceuticals on particular organs or the ability of drug delivery devices to target specific cell types, a novel *in vitro* multi-chambered microfluidic microchip of various human cell types has been developed. Sin *et al.* (2004, entitled *The Design and Fabrication of Three-Chamber Microscale Cell Analog Devices with Integrated Dissolved Oxygen Sensors*) have developed one such device, the HuREL, to address questions about nanomaterial toxicity, targeting, cellular interaction, and metabolism. Using this novel technology will save not only human and animal lives, but also time, money, and resources [18].

In addition, Walker *et al.* developed a nano-capable lab-on-a-chip for cytotoxicity testing able to test nine linear dilutions in parallel in human cell lines [19]. And, Gottwald *et al.* have developed a chip-based platform for the *in vitro* generation of three-dimensional tissue organization that is compatible with automation [20].

I look forward to further communication with NIOSH on this important matter. Please contact me with any questions by phone at 607-272-3143 or via email at SamanthaD@peta.org

Sincerely,

Samantha Dozier

Samantha Dozier, Ph.D.
Nanotechnology Policy Advisor
Regulatory Testing Division

References:

1. Veeriah, S., et al., *Apple flavonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics*. Mol Carcinog, 2006. **45**(3): p. 164-74.
2. Wachowicz, K. and R.E. Snyder, *A continuous-flow perfusion system for the maintenance and NMR study of small tissue samples in vitro*. Magma, 2005. **18**(1): p. 35-40.
3. Ma, S.H., et al., *An endothelial and astrocyte co-culture model of the blood-brain barrier utilizing an ultra-thin, nanofabricated silicon nitride membrane*. Lab Chip, 2005. **5**(1): p. 74-85.
4. Lyon, D.Y., et al., *Bacterial cell association and antimicrobial activity of a C60 water suspension*. Environ Toxicol Chem, 2005. **24**(11): p. 2757-62.
5. Lesniak, W., et al., *Silver/dendrimer nanocomposites as biomarkers: fabrication, characterization, in vitro toxicity, and intracellular detection*. Nano Lett, 2005. **5**(11): p. 2123-30.
6. Knoll, N., et al., *Genotoxicity of 4-hydroxy-2-nonenal in human colon tumor cells is associated with cellular levels of glutathione and the modulation of glutathione S-transferase A4 expression by butyrate*. Toxicol Sci, 2005. **86**(1): p. 27-35.
7. Jia, G., et al., *Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene*. Environ Sci Technol, 2005. **39**(5): p. 1378-83.
8. Panessa-Warren, B., Warren, J., Wong, S., Misewich, J., *Biological cellular response to carbon nanoparticle toxicity*. J. Phys.: Condens. Matter, 2006. **18**(33): p. S2185-S2201.
9. Magrez, A., et al., *Cellular toxicity of carbon-based nanomaterials*. Nano Lett, 2006. **6**(6): p. 1121-5.
10. Stone, V.a.D., K., *Signs of Stress*. Nature Nanotechnology, 2006. **1**(1): p. 23-24.
11. Sayes, C.M., et al., *Correlating Nanoscale Titania Structure with Toxicity: A Cytotoxicity and Inflammatory Response Study with Human Dermal Fibroblasts and Human Lung Epithelial Cells*. Toxicol Sci, 2006.
12. Bourgougnon, V. et al., *Effects of mineral nanoparticles on in vitro model of blood-brain-barrier*. Proc. INVITOX 2006, Belgium.
13. Geys, J. et al., *In vitro study of the pulmonary translocation of nanoparticles: a preliminary study*. Toxicol Lett. 2006. **160** (3):218-26.
14. Porter et al., *Visualizing the uptake of C60 to the cytoplasm and nucleus of human monocyte-derived macrophage cells using enegry-filtered transmission electron microscopy and electron tomography*. Environ Sci Technol. 2007. **41**(8):3012-7.
15. Linse, S., *Nucleation of protein fibrillation by nanoparticles*. PNAS, 2007. **104**(21):8691-6.
16. Nel, A., et al., *Toxic potential of materials at the nanolevel*. Science, 2006. **311**(5761): p. 622-7.
17. Sayes, C.M., et al., *Nano-C60 cytotoxicity is due to lipid peroxidation*. Biomaterials, 2005. **26**(36): p. 7587-95.
18. Sin, A., et al., *The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors*. Biotechnol Prog, 2004. **20**(1): p. 338-45.

19. Walker G., et al. *A linear dilution microfluidic device for cytotoxicity assays.* Lab Chip, 2007. **7**((2):226-32.
20. Gottwald, E. et al. *A chip-based platform for the in vitro generation of tissues in three-dimensional organization.* Lab Chip, 2007. **7**((6):777-85.