Experiences with Assessing the Risks of Nanomaterials, and Implications for Research

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Overview

Examples of U.S. Regulatory Authorities

Examples of questions which regulatory authorities encounter as part of product reviews ‡ Research
  - Material Identification
  - Hazard Data: Ecological & Human Health
  - Exposure Data: Environment & Workplace
  - Risk Assessment & Risk Management

Charge to the Breakout Groups

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Regulatory Authorities, and Associated Data Needs

• **The Toxic Substances Control Act (EPA) -- “Industrial Chemicals”**
  – NMIs not on the TSCA Inventory are new chemicals; a Pre-Manufacture Notice is required before commencement of manufacture
  – Information required as part of a PMN: chemical identity, use, anticipated production volume, byproducts, exposure & release information, disposal practices, existing available health & environmental effects test data
  – **E.U. Equivalent is REACH** (Regulation on Registration, Evaluation, Authorization, and Restriction of Chemicals): information on old and new substances is registered through substance dossiers

• **The Federal Insecticide, Fungicide, and Rodenticide Act (EPA) -- “Pesticides”**
  – Data required to support a registration include toxicology, chemistry, exposure, efficacy, environmental fate, and ecological data
  – Most of the discussions to date for nano-pesticides involve nano-sized versions of already registered, conventionally sized pesticides, such as nanosilver

• **The Federal Food, Drug, and Cosmetic Act and the Public Health Service Act (FDA)**
  – Products subject to premarket authorization include drugs, biological products, devices, and food and color additives
  – Data requirements, which vary with product lines
  – Research needs include (1) evaluating the adequacy of testing approaches for assessing safety, effectiveness, and quality of products containing nanomaterials, and (2) method development which allows the accurate characterization, measurement, and detection of nanomaterials.
  – FDA Nanotechnology Task Force: Nanomaterials present challenges similar to those posed by products using other emerging technologies. However, these challenges may be magnified:
    • Properties of a material relevant to the safety and effectiveness of regulated products might change repeatedly as its size enters into, or varies within, the nanoscale range
    • Size can affect biological interaction, but other factors are also important
Regulatory Authorities, and Associated Data Needs (con’t)

- **The Federal Hazardous Substances Act (CPSC)**
  - Assess a product’s potential chronic health effects to consumers when distributed in commerce
  - Research needs exist for hazard, exposure, and risk assessment related to consumer health

- **The Occupational Health and Safety Act (OSHA)**
  - Addresses Worker Safety for Employers engaged in Interstate Commerce
  - Research needs include development of control techniques to reduce/eliminate potential exposures to nanomaterials, as implemented through a 2010 IAG with NIOSH; working with EPA and NIOSH to develop sustainable manufacturing practices that promote good technology stewardship

- **Federal Meat Inspection Act, Poultry Products Inspection Act, and Egg Products Inspection Act (USDA)**
  - Meat, poultry, and egg products
  - Prevent adulterated or misbranded products from entering commerce

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Types of Products

- **Pesticides**: Applications to date include materials preservatives (e.g., wood treatment, and textiles), but anticipate antimicrobial additives and crop protection uses in the future.

- **Products regulated by FDA**:
  - Drugs, medical devices, cosmetics, dietary supplements
  - Near term/future uses -- food applications, targeted medical therapies, device materials


<table>
<thead>
<tr>
<th>Nanomaterial Classes</th>
<th>General Uses</th>
<th>Approximate Range of Nanomaterials, per Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-walled, and multi-walled carbon nanotubes; carbon nanofibers; and other carbon particles</td>
<td>Enhanced electrical conductivity, mechanical reinforcement, and/or color additives</td>
<td>Less than 50</td>
</tr>
<tr>
<td>Fullerenes with variable carbon number</td>
<td>Enhanced electrical conductivity, &amp;/or mechanical strength; reduces friction</td>
<td>Less than 10</td>
</tr>
<tr>
<td>Other metal oxides (modified silica, titanium, and alumina), modified metals, and other metal-containing particles</td>
<td>Coating additives for scratch resistance, barrier films, self-cleaning surface; lighting applications; detection systems, additives in electrochemical systems</td>
<td>Less than 35</td>
</tr>
<tr>
<td>Other nanomaterials not listed above</td>
<td>Intentionally left blank due to confidentiality considerations</td>
<td>Less than 15</td>
</tr>
</tbody>
</table>

Table: This table provides many of the TSCA applications reviewed to date in the PMN (new chemicals) review process.
Pulmonary and Systemic Immune Response to Inhaled Multiwalled Carbon Nanotubes

Leah A. Mitchell,*† Jun Gao,* Randy Vander Wal,* Andrew Gigliotti,† Scott W. Burchiel,* and Jacob D. McDonald†,†,†

*College of Pharmacy, University of New Mexico, Albuquerque, New Mexico 87131-0001; †Lovelace Respiratory Research Institute, Albuquerque, New Mexico 87108; and ‡The National Center for Microgravity Research, c/o The NASA-Glenn Research Center, Cleveland, Ohio 44135

FIG. 2. Images of MWCNT bulk material by (A) SEM and (B) TEM. MWCNT are provided from the vendor as agglomerated powders. As shown in (A), the MWCNT are not completely rigid and bend together into a mesh. Panel B illustrates the diameter and structure of individual MWCNT, showing an approximately 20-nm–wide MWCNT possessing a herringbone-shaped carbon lamella.

Inhalation of multiwalled carbon nanotubes (MWCNTs) at particle concentrations ranging from 0.3 to 5 mg/m³ did not result in significant lung inflammation or tissue damage, but caused systemic immune function alterations. C57BL/6 adult (10- to 12-SWCNTs. Muller et al. (2005) reported the only MWCNT results and showed an increase in lung pathology and inflammation at approximately 10 mg/kg, but not in the 2-mg/kg range. MWCNT toxicity was increased when physically ground
Ecotoxicity Guidelines

- The Guidelines found at [http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/](http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/) were evaluated. Conclusions included the following:
  - Species and Endpoints:
    - Species tested are generally adequate
    - The endpoints targeted in the test guidelines -- including survival, reproduction, growth, and others -- are integrative of multiple mechanisms of toxicity, and should be as reflective of MNs toxicity as they are of soluble chemicals and formulations
      - Additional nanomaterial-specific endpoints might require modification of existing, or drafting of new, test guidelines, to be incorporated into regulatory testing
    - Methods and approaches for preparing exposure media, as well as measuring and characterizing materials both prior to testing and in prepared exposure media are absent in all test guidelines. Consider development of NM-specific guidance such as the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures [ENV/JM/MONO(2000)6]

- For details, see “Review of OECD/OPPTS-Harmonized and OPPTS Ecotoxicity Test Guidelines for Their Applicability to Manufactured Nanomaterials” EPA/600/R-09/065
Acute Ecotoxicity of Nanomaterials, and Ability to Begin to Develop Predictive Tools

| No. | Group of organisms | Inorganic nanoparticles | | | | | Organic nanoparticles | | | | | Most toxic NP |
|-----|-------------------|-------------------------|---|---|---|---|---|---|---|---|---|---|---|
|     |                   | mg TiO₂/l | mg ZnO/l | mg CuO/l | mg Ag/l | mg/l SWCNT | mg/l MWCNT | mg/l C60 |               |               |               |               |
| 1   | Crustaceans       | 67.7 (10) | 0.62 (3)  | 2.65 (2)  | 0.040 (1) | 15.0 (3)   | 8.7 (1)     | 35.0 (5)   | Nano Ag      |               |               | C60           |
| 2   | Bacteria          | 603 (4)   | 20 (3)    | 71 (2)    | 7.60 (5)  | 163 (2)    | 500 (1)     | 0.81 (4)   | C60          | Nano ZnO     |               |               |
| 3   | Algae             | 65.5 (4)  | 0.068 (2) | 0.87 (1)  | 0.23 (2)  | 1.04 (1)   | NF          | 100.0 (1)  | C60          |               | Nano ZnO     |               |
| 4   | Fish              | 300 (4)   | 1.9 (2)   | NF        | 7.1 (1)   | NF         | NF          | NF         | C60          |               |               |               |
| 5   | Ciliates          | NF        | 5.4 (1)   | 156.5 (1) | 39.0 (1)  | 6.8 (1)    | NF          | NF         | C60          |               | Nano CuO     |               |
| 6   | Nematodes         | 80.1 (1)  | 2.24 (1)  | NF        | NF        | NF         | NF          | NF         | Nano ZnO     |               |               |               |
| 7   | Yeasts            | 20000 (1) | 121.2 (1) | 20.5 (1)  | NF        | NF         | NF          | NF         | Nano CuO     |               |               |               |
| 1-7 | No. of data       | 24        | 13        | 7         | 10        | 7          | 2           | 14         |               |               |               |               |
| 1-7 | Lowest L(E)C50    | 65.5      | 0.068     | 0.87      | 0.040     | 1.04       | 8.7         | 0.25       |               |               |               |               |
| 1-7 | Most sensitive organisms | Algae | Algae | Algae | Crustaceans | Algae | Crustaceans | Ciliates |               |               |               |               |
| 1-7 | Classification (1-7) | Harmful | Extremely toxic | Very toxic | Extremely toxic | Toxic | Toxic | Very toxic |               |               |               |
| 1-3 | Classification (1-3) | Harmful | Extremely toxic | Very toxic | Extremely toxic | Toxic | Toxic | Very toxic |               |               |               |

Kahru & Dubourguier. 2010. *Toxicology* 269
Complexity of Assessing Toxicity: Effects of Environmental Modifications, and Available Chronic Aquatic Toxicity Data

Table 1. Multiwalled nanotube (MWNT) particle characterization and *Daphnia magna* 96h acute toxicity results

<table>
<thead>
<tr>
<th>Nanoparticle suspension</th>
<th>pH</th>
<th>Zeta potential</th>
<th>Hydrodynamic diameter</th>
<th>LC50 (mg/L)</th>
<th>95% CI (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWNT in 18.8 mg/L DOC (SR-NOM)</td>
<td>8.08</td>
<td>NA</td>
<td>706.4 ± 35.9*</td>
<td>2.48</td>
<td>2.00, 3.07</td>
</tr>
<tr>
<td>MWNT in 15.2 mg/L DOC (SR-NOM)</td>
<td>8.23</td>
<td>-28.9 ± 3.7</td>
<td>629.7 ± 26.8</td>
<td>1.90</td>
<td>1.59, 2.28</td>
</tr>
<tr>
<td>MWNT in 10.4 mg/L DOC (SR-NOM)</td>
<td>8.29</td>
<td>NA</td>
<td>655.7 ± 27.6</td>
<td>2.25</td>
<td>1.72, 2.95</td>
</tr>
<tr>
<td>MWNT in 5.1 mg/L DOC (SR-NOM)</td>
<td>8.25</td>
<td>-21.1 ± 3.8</td>
<td>655.5 ± 27.6</td>
<td>2.06</td>
<td>1.66, 2.57</td>
</tr>
<tr>
<td>MWNT in 2.0 mg/L DOC (SR-NOM)</td>
<td>7.86</td>
<td>-26.5 ± 4.6</td>
<td>NA</td>
<td>2.78</td>
<td>2.18, 3.55</td>
</tr>
<tr>
<td>MWNT in 15.1 mg/L DOC (ER-NOM)</td>
<td>8.61</td>
<td>-32.8 ± 4.14</td>
<td>703.3 ± 19.1*</td>
<td>4.09*</td>
<td>3.41, 4.91</td>
</tr>
<tr>
<td>MWNT in 15.7 mg/L DOC (BR-NOM)</td>
<td>8.14</td>
<td>-30.6 ± 5.04</td>
<td>528.0 ± 24.2*</td>
<td>1.91</td>
<td>1.40, 2.62</td>
</tr>
</tbody>
</table>

**D. magna** first 21-day chronic toxicity study with a coated titanium np in absence of photoactivation showed reproduction to be a more sensitive endpoint than mortality (EC50 = 26.6 mg/L for repro.). Ref: Weinch, et al. 2009 *Chemosphere* 76

Fig. 2. *Ceriodaphnia dubia* reproduction (% control) during exposure to multiwalled nanotube-natural organic matter particle (MWNT-NOM) (nanoparticle NP; mg/L). Significant decreases were observed in all concentrations greater than 0.25 mg/L. Capital letters denote statistical groupings (p < 0.05).

Toxicity Data for Manufactured Nanomaterials

– Respirable poorly soluble particle concerns via inhalation route
  • First publicly-available 90-day inhalation study results for MWCNTs, with the LOAEL = 0.1 mg/m³ (R. Landsiedel et al, Tox. Sci., 2009)
  • Second available subchronic study for MWCNTs, with the NOAEL = 0.1 mg/m³ (J. Paulhun, et al. 2010. Tox Sci, 113(1))
  • First publicly-available 90-day inhalation results for C60 fullerenes: no significant effects for 50 nm particles up to 2.5 mg/m³ (N. Walker et al, SOT Poster, 2009)
  • Few Chronic inhalation studies on Manufactured Nanomaterials
  • Protocols: Subchronic inhalation study alternatives
  • Biodistribution: inhalation and i.v. injection of different sizes of gold nanoparticles (Semmler-Behnke, et al 2008 Small, 4:12)

– Scarce data showing penetration of healthy intact human skin by nanomaterials (in absence of additional solvents)
  • A dermal penetration study in minipigs indicated that there is no significant penetration of either nano- or submicron-size titania particles when administered in sunscreen formulations (Sadrieh et al., 2010)
  • Studies in Humans with 16 nm ZnO, however, indicate small amounts of soluble and/or particulate Zn enters the blood stream and urine when applied outdoors in a sunscreen (Gulson, et al. 2010. Tox Sci 118:1).

The FIFRA Science Advisory Panel 2009 on the Evaluation of the Hazard and Exposure Associated with Nanosilver and other Nanometal Pesticide Products was asked whether hazard or exposure data developed for 1 to 20 nm silver particles or silver composites be used to assess the risks of 51 to 100 nm silver particles or composites.

“The lack of a clear understanding of how particle size and other physical properties affect hazard profiles led most Panel members to be unsupportive of bridging amongst silver-based materials with different properties.”

- Bridging is feasible for the portion of hazard due to silver ion release
- Many particle physicochemical properties may affect uptake, distribution and magnitude of toxicity for silver nanoparticle
- An appropriate set of metrics which incorporates size in conjunction with physicochemical or biological parameters such as surface area may be appropriate in bridging exercises

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Exposure Data are Limited

- Exposures in the Workplace
  - CNTs:
    - SWCNTs: 53 ug/m³ *Maynard et al. 2004. J. Toxl. & Envir. Health,*
    - MWCNTs: 0.018 – 194 tubes/cc *Han, et al. 2008 Inhalat. Tox.*
  - Data coming from NIOSH workplace monitoring studies
  - Findings confounded by measurement methods / Need for personal monitoring
- Environmental and General Population Exposures
  - Cerium oxide in Air: 0.0006 mg/m³ in ambient U.K. air (likely an underestimate)  (*Park, et al., 2008, Inhalat. Tox, 20*)
  - Titanium Dioxide in Wastewaters: 80% removal in WWTP / effluent in low ug/L levels  (*Kiser et al, 2009, EST*)
  - Nano Silver: High rate of leaching from socks, paints, textiles  (*EPA SAP, 2009*)
  - CNTs and Fullerenes in Natural Waters:
    - Presence of NOM increases dispersion of CNTs in freshwater to low ppm levels  (*Hyung, et al. 2007, EST 41*)
    - Presence of NOM changes particle size and morphology of Fullerenes, with implications for fate and transport  (*Xie, et al, 2008, EST*)

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At the moment, in vivo or clinical studies evaluating effects of nanoscale CeO$_2$ particles have not yet been published. In a recent Society of Toxicology abstract, however (Staal et al., 2010), preliminary results of a toxicity study in rats were described. Exposure by inhalation to nano-sized CeO$_2$ (caused pronounced effects at a mass concentration of 0.14 mg/m$^3$ (increased levels of biochemical parameters and leukocytes in bronchoalveolar lavage fluid).

In order to assess an exposure, the mass of the TiO$_2$ particles that deposits in the pulmonary region of the lung needs to be estimated. Assuming a peak TiO$_2$ aerosol concentration of 3.4 mg/m$^3$ with a MMAD of 836 nm (or a MMD of 395 nm), a minute ventilation rate of 20 L/min (33% sitting and 67% light exercise), a deposition fraction of 11.3% (ICRP, 1994), and a human alveolar epithelium surface area of 102 m$^2$ (Stone et al., 1992), the approximate lung burden after 1 min of spray application would be $-0.075 \mu g$ TiO$_2$ per m$^2$ alveolar epithelium. This is equivalent to a pulmonary dose of about 0.03 $\mu g$ TiO$_2$ in a rat (Stone et al., 1992).

Besides modeling, in vivo toxicity studies have been conducted in our laboratory by exposing Sprague-Dawley rats to ultrafine TiO$_2$ (Degussa, P25) aerosol via inhalation. A dose dependent, systemic microvascular dysfunction was found in rats following the exposure (Nurkiewicz et al., 2008) and the lung burden that produced 50% impairment ($ED_{50}$) was about 10 $\mu g$ (Nurkiewicz et al., 2009). Although the accumulated doses used in the animal studies were hundreds of times higher than those in the present study, there is a concern if repetitive sprays are conducted each day in a poorly ventilated environment. For this reason, CPSC and NIOSH plan to conduct an inhalation toxicological study by exposing rodents to TiO$_2$ aerosols generated with a spray can to obtain dose-response relationships, as well as, to establish a No Effect Exposure Level for setting guidelines.
EPA ChemSTEER and E-FAST:
Modeling Predicted Environmental and Human Exposures

Estimates workplace exposures to, and releases of, a chemical in the absence of monitoring data; or used to fill in gaps when some data such as workplace monitoring data are available from PMN or Literature. See:

http://www.epa.gov/oppt/exposure/pubs/chemsteer.htm

\[
\text{Exposure} \times \text{Hazard} = \text{RISK}
\]

ChemSTEER

Occupational

Environmental
(Aquatic, Terrestrial, Avian)

Non-occupational
(Consumer, General Population)

E-FAST

EXAMPLE INPUTS:
* Physical / Chemical Properties
* Stream Flows
* Consumer Profiles

EXAMPLE INPUTS:
* Physical / Chemical Properties
* Production volume, Batch Size
* Industry Specific profiles, Generic models, etc.
The Need for More Quantitative Risk Assessments

- Improved Material Characterization
- Better Health and Ecotoxicity data: realistic exposures and chronic toxicity data
- Improved estimates of Exposures to Workers, the General Public, and Environmental Receptors
- Dose-Response Metrics for Risk Assessment

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Examples of Activities in the E.U. with Potential Research Implications

- There is ongoing work to develop a definition of the term "nanomaterial" that is suitable for E.U. legislation
- SCENIHR, European Commission (JRC, DG ENV, ENTR, SANCO, and others)

- REACH Implementation Projects on Nanomaterials (RIP-oNs):
  - Objective is to provide scientific and technical advice on key aspects of the implementation of REACH for nanomaterials
  - RIP-oN 1: Substance Identification
  - RIP-oN 2: Information Requirements
  - RIP-oN 3: Chemical Safety Assessment
Longer-term Research Examples

1. High-throughput Screening Methods
2. Prediction of protein corona impacts on biodistribution and toxicity
3. Categories approaches ‡ SAR ‡ QSAR

Physical—Chemical Aspects of Protein Corona: Relevance to *in Vitro* and *in Vivo* Biological Impacts of Nanoparticles
Marco P. Monopoli,* E† Dorota Walczyk,† Abigail Campbell,† Giuliano Elia,‡ Isceult Lynch,† Francesca Baldelli Bombelli,*† and Kenneth A. Dawson*†

An index for characterization of nanomaterials in biological systems
Xin-Rui Xia, Nancy A. Monteiro-Riviere and Jim E. Riviere*
Regulatory Panel Members

- FDA: Carlos Pena
- CPSC: Treye Thomas
- USDA: Kerry Dearfield
- OSHA: Janet Carter
- EPA: Bill Jordan and Phil Sayre

- Dutch Ministry of Infrastructure and the Environment: Tom van Teunenbroek
- SCENIHR: Kenneth Dawson
- D.G. Environment: Andrej Kobe
- D.G. JRC: Hermann Stamm
- Austrian Ministry for Transport, Innovation & Technology: Alexander Pogany

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Charge to Breakout Groups

• Go to either the Human Health or Environment Breakout Groups
  – Health: Remains in Plenary Room
  – Environment: Linder Conference Room, 6th floor
• 2:15 – 2:45 Two 10-minute presentations in each Breakout Group & Discussion
• 2:45 – 3:40 Chair/Rappateur lead discussions, using Questions in Handouts + Slides
• Chair/Rappateur finalize Slides summarizing Findings of Breakout Group

Considerations/Questions for Breakout Groups:

– Consider the Plenary, and the Two Breakout Session, Presentations
– Identify the Top Three Nearer Term Regulatory Challenges that can be met in the next two to three years, and Data Needs to Address the Challenges
  • Suggested Topic: Inhalation Toxicology
  • Suggested Topic: Bioavailability
  • Identify Barriers to Implementation, and Areas of Near-term Cooperation for at least the No. 1 Regulatory Challenge
– Provide suggestions for Longer-Term Research (8-10 years)

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