A Perspective on Measurement Challenges and Needs for EHS Assessment of Engineered Nanomaterials

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Overview

- Defining the Scope of the Problem
- Four Measurement Challenge Areas
- A Role for NIST & Reference Materials
Nano Risks: Defining the problem

Nanomaterials and products that incorporate nanomaterials pose unknown risks throughout all stages of their life cycles, from initial sourcing through manufacture, use, disposal or recycling, and ultimate fate.

Scope of EHS risk of engineered nanomaterials: Real risks and perceived risks
- The environment (E)
- Health (H) and safety (S) of workers, consumers, and the general public

Who or what does the problem affect?
- The Economy: the unknown risks stifle innovation and commercialization
- The Public: the unknown risks generate fear
- Our Society: the known benefits may not be realized
- The Regulatory Agencies: the unknown risks limit the abilities of EPA, FDA, OSHA, and CPSC to carry out their missions

Magnitude of the problem:
DuPont suspended development of zero-valent iron, a “poster child” nanomaterial for environmental remediation, because the risks could not be sufficiently assessed.

Congressman Bart Gordon
Chairman Committee on Science and Technology
Lux Research, August 2008
What is required for solution?

Sets of accurate data for physico-chemical properties, hazards, and exposure to enable science-based, lifecycle risk assessment and risk management of nanomaterials and products that incorporate them.

**Linkages between P-C properties and EHS risks are essential:**
e.g., Environmental Defense-DuPont Nano Risk Framework *(Press release June 2007)*

- **Describe Material and Application**
  - Profile Lifecycle(s)
  - Properties
  - Hazards
  - Exposure

  **Assess, prioritize and generate data**

- **Evaluate Risks**
  - Assess Risk Mgmt
- **Decide, Document and Act**
- **Review and Adapt**

**Linkages between P-C properties and beneficial/functional effects are essential:**
e.g., NCI Nanotechnology Characterization Laboratory *(Public release January 2005)*

**Assay Cascade Protocols for:**
- Physico-chemical properties (NIST/NCL)
- *In vitro* and *in vivo* biological properties (NCL)

**National Institute of Standards and Technology**
Sets of accurate data for physico-chemical properties, hazards, and exposure to enable science-based, lifecycle risk assessment and risk management of nanomaterials and products that incorporate them.

**Recommended Minimum Physico-Chemical Parameters for Nano-Toxicity Studies**

- **Physical Attributes**: particle size/size distribution, agglomeration state/aggregation, shape
- **Chemical attributes**: Overall and surface composition (chemical and crystal structure), purity
- **Interactions with media**: surface area, chemistry (reactivity, hydrophobicity), charge
- **Overarching considerations**:
  - **Stability**: solubility, rate of release
  - **Media**: property changes with media
  - **Dose metrics**: mass, surface area, number concentration

MinChar Initiative, Woodrow Wilson Center
Sets of accurate data for physico-chemical properties, hazards, and exposure to enable science-based, lifecycle risk assessment and risk management of nanomaterials and products that incorporate them.

Other similar “lists” have been developed (e.g., ISO 229, REFNANO in the UK)

<table>
<thead>
<tr>
<th>P-C PROPERTY ENDPOINTS</th>
</tr>
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<tbody>
<tr>
<td>Agglomeration</td>
</tr>
<tr>
<td>Aggregation</td>
</tr>
<tr>
<td>Water solubility</td>
</tr>
<tr>
<td>Crystalline phase</td>
</tr>
<tr>
<td>Dustiness</td>
</tr>
<tr>
<td>Crystallite size</td>
</tr>
<tr>
<td>Representative TEM picture(s)</td>
</tr>
<tr>
<td>Particle size distribution</td>
</tr>
<tr>
<td>Specific surface area</td>
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</tbody>
</table>
Four Measurement Challenge Areas

- Dispersion and Aggregation Effects
- Measurement in Complex Matrices
- Characterizing the Surface State of Bound Species
- Sample Preparation Prior to Conducting Tests
Measurement Challenges for Nano EHS #1

Dispersion and Aggregation Effects
• many NMs unstable in biological test media
  - charge stabilized NMs unstable in high salt
  - PEGylated NMs disperse better, are stable
• aggregation alters effective NM size
• impacts transport properties, cellular interactions
• may alter delivered dose in tox measurements
• agglomerates also pose problems for P-C quantification
  - harder to define size, morphology, surface area

National Institute of Standards and Technology
Solution: Biocompatible Dispersion Medium?

NIOSH Lung Fluid Mimic:
Phosphatidylcholine + serum albumin in PBS
Porter et al., *Nanotoxicology* (2008)

\[
\text{1,2-Dipalmitoyl-sn-glycero-3-phosphocholine}
\]

*DPPC is major component of pulmonary surfactant*

Composition: PBS with 0.6 mg/ml SA + 10 μg/ml DPPC

Dispersion medium for P-C and Tox measurements
- works for different NM types (MeO, silica, carbon black, CNTs)
- does not appear to significantly alter tox profile
- can be standardized
- proposed for on-going interlab validation studies (IANH)
Measurement Challenges for Nano EHS #2

P-C Measurements in complex biological/environmental matrices
- relevant conditions: pH/buffer, salts, temp, chemical composition
- differentiation between NMs and natural bio/envirn components
- optical detection interference (scattering, fluorescence, absorption)
- protein absorption, interactions with other bio/env constituents


- impacts tox profile, alters transport properties, changes size
- material dependent uptake (function of size, charge, composition)
- time dependent and complex
  - different uptake profiles with time - min to hrs
  - common plasma proteins adsorb fast, displaced by others
Measurement Challenges for Nano EHS #3

State of Surface-Bound Species

- proteins (e.g., serum albumins, TNF, IgG)
- surfactants (e.g., SDS, CTAB, TWEEN, phospholipids)
- ligands, complexing agents (e.g., citrate)
- polymers (e.g., PEG, PVP, starch)
- natural organic matter (e.g., humic acids)

- influence dispersion, aggregation
- uptake by reticuloendothelial system (RES)
- functionality (e.g., targeting moieties)
- biocompatibility, alter tox profile of ENMs
- transport properties and fate

Surface Characterization Needs

- adsorption, density, conformation, distribution on surface
- single particle analysis as well as ensemble average
- stability of surface complexes
- linkage to other surface properties, particle size & stability

PEG-thiol on Au NP protects from RES
BSA conjugated NP prevents aggregation
Solutions: Hyphenated Methods

Combine particle fractionation with in-line or downstream analytical and/or property measurements

- Fractionation: Size Exclusion Chromatography (SEC), Field Flow Fractionation (FFF), Dynamic Mobility Analyzer (DMA)
  - in situ (flow mode, fluid phase and aerosols)
  - separate particles by size, mass or other physical properties

- Elemental analysis / composition
  - ICP-Mass Spectrometry

- Physical property measurements
  - Dynamic Light Scattering (DLS - hydrodynamic size)
  - Multi-Angle Light Scattering (MALS - MW, Rg)
  - Optical absorption or fluorescence detectors (chemical ID)
  - Condensation particle detector (particle concentration/number)
  - Differential refractometer / viscometer (MW)

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Measurement Challenges for Nano EHS #4

Sample Preparation for Particle Analysis -> Artifacts and Uncertainty

- Sample prep is often the dominant source of uncertainty & bias for particle characterization measurements
  - material dependent
  - method dependent
  - subject to user bias, errors
- Ex situ microscopy methods are especially susceptible to artifacts
  - nearly impossible to differentiate between artifacts and aggregates formed in solution
- Introduction of contaminants (both chemical and particulate)
  - NPs are highly sensitive to low level contamination

What is needed to mitigate these effects?

- sample prep protocols defined specifically...
  - for different classes of NMs
  - for different measurement applications and media
- validation & standardization of protocols (ISO, ASTM)
- reference materials for validation, qualification
A Role for NIST in NanoEHS

Create and disseminate critical measurement solutions for determining physico-chemical properties of nanomaterials and products that incorporate nanomaterials throughout the stages of their life cycles.

- **Standards**: reference materials, documentary standards
- **Instruments and methods**: transferable to others
- **Data**: evaluated and measured using advanced methods

Provide data and methods for partners to establish *linkages* to hazards and exposure data

Drivers
- Economic benefits from nanotechnology
- Regulatory agencies need data to make rational laws and regulations

Define solution requirements with industry and regulatory agencies:
- Needed levels of accuracy and precision
- Materials used in large volume in multiple products
- A coordinated effort needed
- Strong partnering across industry sectors and with other agencies
Reference Materials Are Enablers

Nanoscale Reference Materials can be used for:

- Instrument calibration and laboratory qualification
- Method development and validation
  - physical, chemical and biological characterization
- Interlaboratory comparisons
  - standards development, precision determination
  - confidence building
- Benchmarking and controls
- Quality assurance and traceability in industry
  - GMP, GLP
- Hypothesis testing and research
- Implementation of standard practices
  - linked to RMs
Currently Available Nanoscale RMs

From the German Federal Institute for Materials Research and Testing (BAM) compilation (http://www.nano-refmat.bam.de/en/)

65 nanoscale RMs available
15 RMs possibly relevant to EHS applications

Reference materials (with National Metrology Institute traceability)
- SRM 1963a: polystyrene spheres (100 nm) USA
- SRM 1964: polystyrene spheres (60 nm) USA
- RM 8011: gold nanoparticles (10 nm) USA
- RM 8012: gold nanoparticles (30 nm) USA
- RM 8013: gold nanoparticles (60 nm) USA
- IRMM-304: silica nanoparticles (40 nm) EC
- GBW 12011: polystyrene spheres (61 nm) CN
- SC-0030 to -0100: polystyrene spheres (30 to 100 nm) JP
On-Going NIST Nanoscale RM Activities

- **Nano Titania - SRM 1898 (in production)**
  - Industrial nanocrystalline powder
  - Same material chosen for OECD testing program
  - Collaborating with NIOSH on certification studies

- **Single Wall Carbon Nanotubes (in production)**
  - SRM 2483 (raw powder) certified on elemental composition
  - RM 8281 (3 length sorted populations in liquid dispersions)
  - “bucky paper” (under development)

- **Nano Silver - Feasibility Study (initiated in FY09)**
  - Need to address stability issues, identify relevant forms
  - Cooperating with FDA, EPA, NIOSH labs on research / materials
  - Alignment with OECD testing program on silver
  - Joint NIST-ERDC Workshop on Nano-Ag (Apr 2009)
  - Nano Silver Wiki (http://nanobiology.ncifcrf.gov/groups/silver/)