IMA Research need #8: Developing methods to detect nanomaterials and determine exposure routes

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Case study: finding the cause

How would you go about identifying the one of potentially many nanomaterials in the air, soil, water, or organisms that could have caused this ecological catastrophe?

In affected populations, digest tissues and analyze to determine whether elevated concentrations of particular elements occur in these tissues.

How would you find the nanoparticles, or their by-products, in the suite of natural and synthetic nanomaterials?

**Need to refine techniques to:**

1) Measure low concentrations in water and tissue
2) Distinguish engineered, natural, and incidental sources
3) Distinguish various sources within a class of engineered nanoparticles
4) Minimize sample disturbance so that the observation reflects the unperturbed environmental state
<table>
<thead>
<tr>
<th>Nanoparticle properties</th>
<th>Microscopy and related techniques</th>
<th>Chromatography and related techniques</th>
<th>Centrifugation and filtration techniques</th>
<th>Spectroscopic and related techniques</th>
<th>Other techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregation</td>
<td>e.g. STEM, TEM, SEM, AFM, STM</td>
<td>e.g. ANUC</td>
<td>e.g. XRD, SANS</td>
<td>e.g. Zeta potential</td>
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<tr>
<td>Chemical composition</td>
<td>AEM, CFM</td>
<td></td>
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<tr>
<td>Mass concentration</td>
<td>AEM, CFM</td>
<td>√</td>
<td>√</td>
<td>e.g. Gravimetry, thermal analysis</td>
<td></td>
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<tr>
<td>Particle number</td>
<td>AEM, CFM</td>
<td></td>
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<td></td>
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<tr>
<td>concentration</td>
<td>e.g. STEM, TEM, SEM, AFM, STM</td>
<td>e.g. FlFFF-SLS, SedFFF-DLS</td>
<td>e.g. UC</td>
<td></td>
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<tr>
<td>Shape</td>
<td>e.g. STEM, TEM, SEM, AFM, STM</td>
<td>√</td>
<td>e.g. DMA</td>
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<tr>
<td>Size</td>
<td>e.g. STEM, TEM, SEM, AFM, STM</td>
<td>√</td>
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<tr>
<td>Size distribution</td>
<td>e.g. STEM, TEM, SEM, AFM, STM</td>
<td>e.g. FFF, HDC, SEC</td>
<td>e.g. CFF, UC, CFUF</td>
<td>e.g. UCPC, SMPS</td>
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<tr>
<td>Dissolution</td>
<td>e.g. STEM, TEM, SEM, AFM, STM</td>
<td>Dialysis, CFUF</td>
<td></td>
<td>Voltammetry, diffusive gradients in thin films</td>
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<tr>
<td>Speciation</td>
<td>e.g. SEC-ICP-MS</td>
<td></td>
<td>e.g. XAFS, XRD</td>
<td>e.g. Titration</td>
<td></td>
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<tr>
<td>Structure</td>
<td>e.g. STEM, TEM, SEM, AFM, STM</td>
<td></td>
<td>e.g. XRD, SANS</td>
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<tr>
<td>Surface area (porosity)</td>
<td>e.g. STEM, TEM, SEM, AFM, STM</td>
<td></td>
<td></td>
<td>e.g. BET</td>
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<tr>
<td>Surface charge</td>
<td>e.g. CE</td>
<td></td>
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<td>e.g. Zeta potential</td>
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<tr>
<td>Surface chemistry</td>
<td>AEM, CFM</td>
<td></td>
<td></td>
<td>e.g. XPS, Auger, SERS</td>
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</tbody>
</table>

From Tiede et al. (2008)
Dynamic light scattering (DLS) and other common methods provide good results for monodisperse samples.

Nanopowders in clean water aggregate and become polydisperse.

Nanoparticles in the environment end up bound with extracellular polymers and other natural organic matter.

Also become bound with natural (e.g. clays, oxyhydroxides, sulfides) and incidental nanomaterials (soot).

May undergo oxidation, reduction, precipitation, dissolution in response to changes in geochemistry along their flowpath.

The resulting “clump” needs some separation to make the signals from the various detection methods useful.
The same complications apply in tissue, likely to an even greater extent

http://cben.rice.edu/highlights.aspx
Goal: Separation of polydisperse samples

Methods:

Field flow fractionation (FFF)
- Cross-flow fields separate nanoparticles by size, charge, density, etc.
- Low-invasive technique (only bounding membrane or surface)
- Multiple field strategies (fluid drag, electrical, gravitational, thermal, acoustic)
- Concerns: effect of carrier solution. Distribution represents in-situ?

Size-exclusion chromatography (SEC)
- Separate via hydrodynamic size in internally porous media
- Concerns: low peak resolution, loss to media

Hydrodynamic chromatography (HDC)
- Separate according to hydrodynamic size in solid sphere porous media
- Concerns: low peak resolution

All of the above can be coupled to various detectors; e.g., UV, MS, etc.
State of the Science - continued:

Reviews by Tiede et al. (2008, 2009); Klaine et al. (2008):

FFF very promising as a separation technique

Especially if coupled to mass spectrometer to give elemental analysis (over large mass range) across size/charge/other distribution

many ~ 6 nm

Some ~ 2 nm

As, Hg dissolved?

Fe, Cu, Zn, Pb, Mo Sulfides

Size range 0.9 – 7.5 nm

Site 3510

NNI-EHS-Research-Strategy-2008:

“as there is a low number of projects [funded 2006] for determination of particle size, particularly in biological, environmental, and other complex media, efforts could be stronger in this area”

“the ability to accurately measure particle size is critical”

“detection in solid media (soil, solid waste streams) is not well addressed”

“IMA is supportive to the other four research categories”

The EHS priority research needs listed in table 3 (IMA needs) are:

- Develop methods to detect nanomaterials in biological matrices, the environment, and workplace.
  1) Evaluate the scope and suitability of technologies to quantify nanomaterials across biological media indicative of exposure.
  2) Develop common commercially available samplers for measuring mass concentrations of nanoparticles in air (indoor and outdoor).
  3) Develop instruments to measure nanomaterials in water
  4) Develop samplers for personal monitoring of nanomaterials and biomarkers indicative of exposure
Research Priorities On Target? Yes – but personally – I would focus this to:

Investigation of success in separation into “primary” particle sizes
  Carrier choice
  Surfactants – how well do we break them apart?
  Enzymes – digest organic matrix without dissolving nanoparticle
  Allows sending samples to off-site laboratories?
  The criterion is not to reflect the in-situ distribution (impossible?), but rather, to determine what’s in the “clump”.

Determination of “fingerprints” of distinction among sources via elemental and isotopic signatures
  Contaminant elements
  Stable isotopes

Development of robust methods for integration from larger volumes for monitoring low concentrations in water
  SPLITT continuous binary separation
  SPLITT in series
  Integration via nanomembranes
Elemental signatures

Plata et al. (2008)

All available commercial SWCNT sources

All had “unexpected” trace metals

Ni:Y ratios showed distinct ranges for commercial SWCNTs (2.9 to 4.9) relative to coal (1.3), petroleum (66 000), and continental crust (0.9)

Co:C ratios for commercial SWCNTs were relatively high (0.31 to 0.88) relative to coal ($5.6 \times 10^{-6}$), petroleum ($4.0 \times 10^{-6}$), and continental crust ($\text{Co:C} = 3.7 \times 10^{-3}$).

“... metal-to-carbon content of SWCNTs can be useful for tracing these emerging contaminants as they are released to the environment”. 

Plata et al. (2009) Nanotechnology 19 185706 doi:10.1088/0957-4484/19/18/185706
Isotopic signatures

Plata et al. (2008)

SWCNTs manufactured by carbon monoxide chemical vapor deposition have distinct $\delta^{13}C$ (−51.7 to −49.8‰) relative to other SWCNTs

Reflects $^{13}C$-depleted feedstock

“... this unique isotopic signature may be a useful tracer of CO-SWCNTs and their metabolites in both laboratory and field studies”.

Distinct from the bulk isotopic content of marine seawater and sedimentary organic matter (−30 to −20 ‰)

SWCNTs Synthesized via:
arc-discharge (●), CVD (○), CO-CVD (●)
Starting materials (●●●)
Dichloromethane-extracted C (○)
Case study: preventing another disaster

What biological and instrument methods could have been used to test all the nanomaterials produced by this company?

Characterize elemental and isotopic signatures associated with distributions (size, charge, etc.) in the product using FFF and MS methods.

Are current paradigms for inorganic or organic chemicals suitable for use with nanomaterials?

Not in terms of current analyses used in environmental monitoring, which distinguish dissolved versus particulate with an arbitrary cutoff filter. Need to refine FFF and SPLITT methods coupled to MS methods to characterize distributions in size, charge, etc. across the range from nano to micro.
Which research needs should be addressed in the near- (< 5 years), medium- (5 – 10 years) and long-term (> 10 years)?

NNI-EHS-RS-2008 states (page 27):

   Needed short term is the development of air samplers
   Needed mid-term are technologies for monitoring nanomaterials in water.

My opinion: technologies for monitoring in water are needed in the near term. Developing this capability is critical to the other research needs, for example, other goals in IMA:

1) Understand the effect of surface function on mobility and transformations in water
2) Evaluate correlation of microscopic with other size-measurement techniques.
3) Evaluate or modify microscopic and mass spectrometric approaches for determination of shape and structure of nanomaterials
4) Explore methods beyond isothermal adsorption for nanomaterial surface area determinations.

These goals cannot be attained without robust methods to characterize nanoparticle size (charge, elemental) distributions in aqueous suspensions and tissues.
Thank You
**State of the Science** - microscopy-based techniques

**Goals:**
- Visual identification
- Aggregation, size, shape

**Methods:**
- Scanning electron microscopy (SEM)
- Transmission EM (TEM)
- Atomic force microscopy (AFM)
- Coupled to EDS gives elements

**Concerns:**
- Analysis-driven aggregation
- SEM and TEM under vacuum
  (some success in liquid via ESEM and others)
- AFM in solution
- Limited scale raises question of representativeness

State of the Science (continued):

Ultracentrifugation, nanofiltration, cross-flow filtration
  Allows separation of solutes (e.g. < 1nm)
  Concerns: pore clogging.

Gel phase separation via voltammetry, diffusion gradients in thin films (DGT), diffusion equilibration in thin films, and the permeation liquid membrane method (PLM)
  Allows separation of very small sizes
  Concerns: sample is in gel or other medium
Isotopic signatures (continued)

Isotopic signatures have been used to study sources and transport of pollutants and the geochemical mechanisms operating in soil and groundwater systems.

Natural terrestrial $\delta^{114}$Cd/$^{110}$Cd range very low ~ 0.4 ‰  
Anthropogenic $\delta^{114}$Cd/$^{110}$Cd shows large range  
-0.64 ‰ (dust sample issued from a lead smelter)  
+0.50 ‰ for NIST SRM 2711 (metal-rich soil)

Cloquet et al. (2005), Geostandards and Geoanalytical Research, v. 29, p. 95-106.