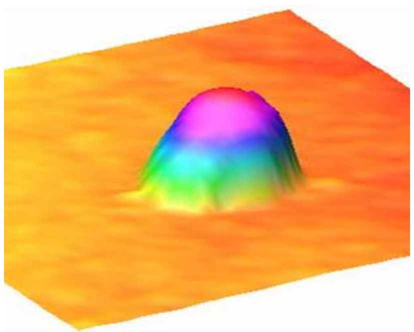
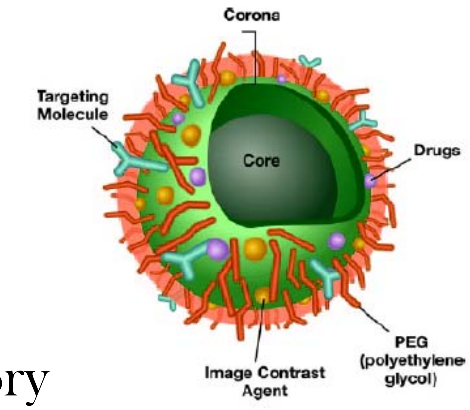
NNI NanoEHS Workshop: Nanomaterials and the Environment & Instrumentation, Metrology, and Analytical Methods - Oct 6-7, 2009

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**.

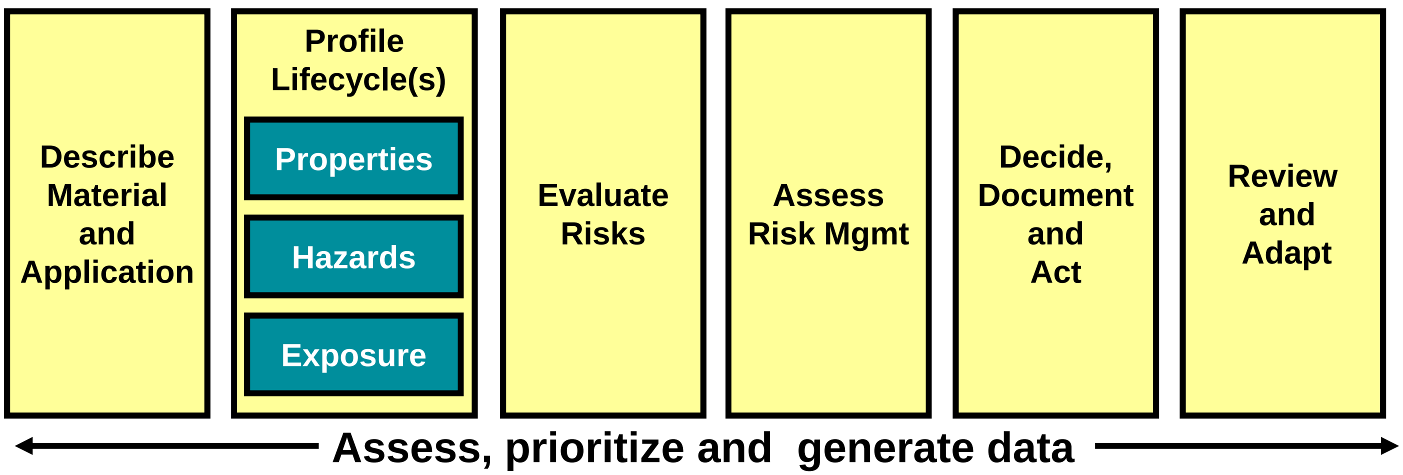
Chairman Committee on Science and Technology

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)*

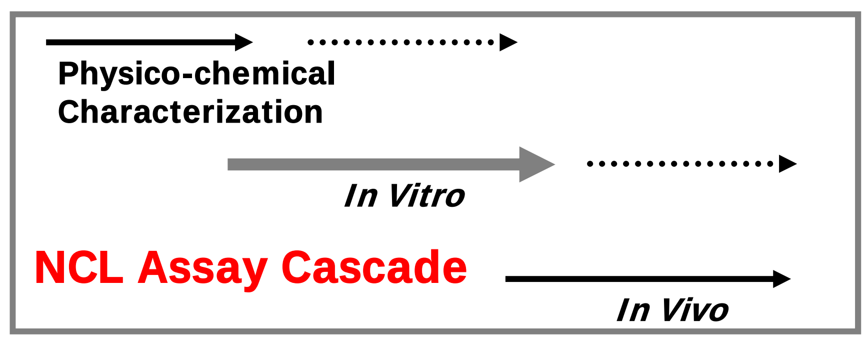


Linkages between P-C properties and beneficial/functional effects are essential:

e.g., NCI Nanotechnology Characterization Laboratory (Public release January 2005)

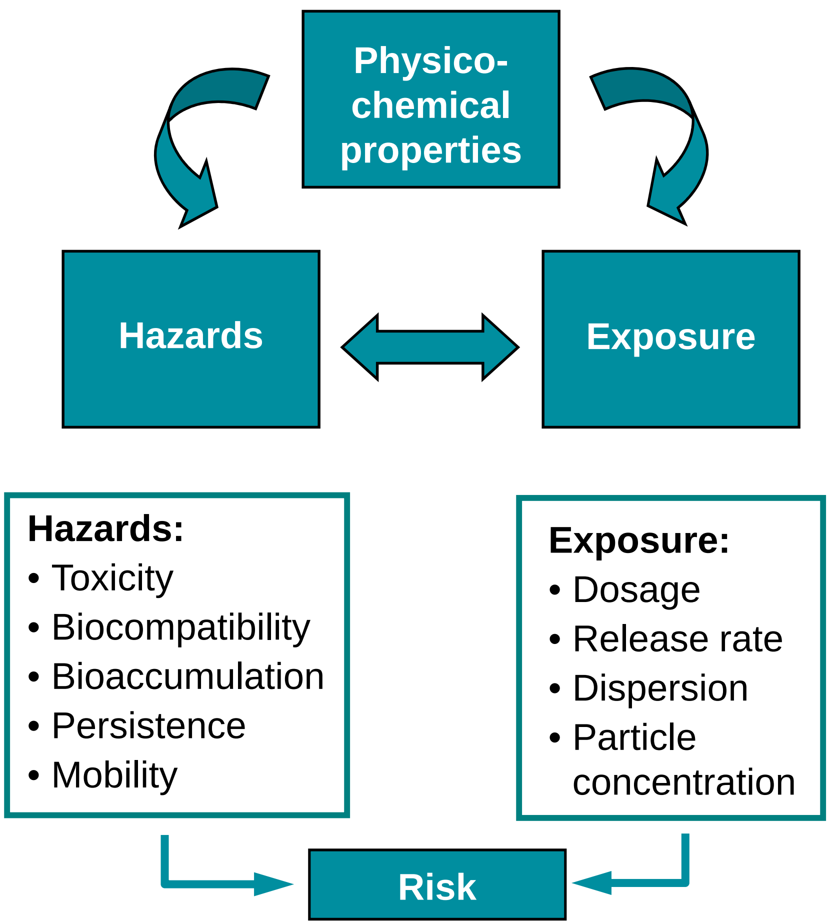
Assay Cascade Protocols for*:*

- Physico-chemical properties (NIST/NCL)
- *In vitro* and *in vivo biological* properties (NCL)



Minimum P-C Characterization

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

Minimum P-C Characterization

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)

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT OECD

P-C PROPERTY ENDPOINTS	
Agglomeration Aggregation	Zeta potential (surface charge)
Water solubility	Surface chemistry (where appropriate)
Crystalline phase	Photocatalytic activity
Dustiness	Pour density
Crystallite size	Porosity
Representative TEM picture(s)	Octanol-water partition coefficient
Particle size distribution	Redox potential
Specific surface area	Radical formation potential

ISO International Organization for Standardization

TC 229/WG3

IOM

REFNANO (IOC, UK, 2007)

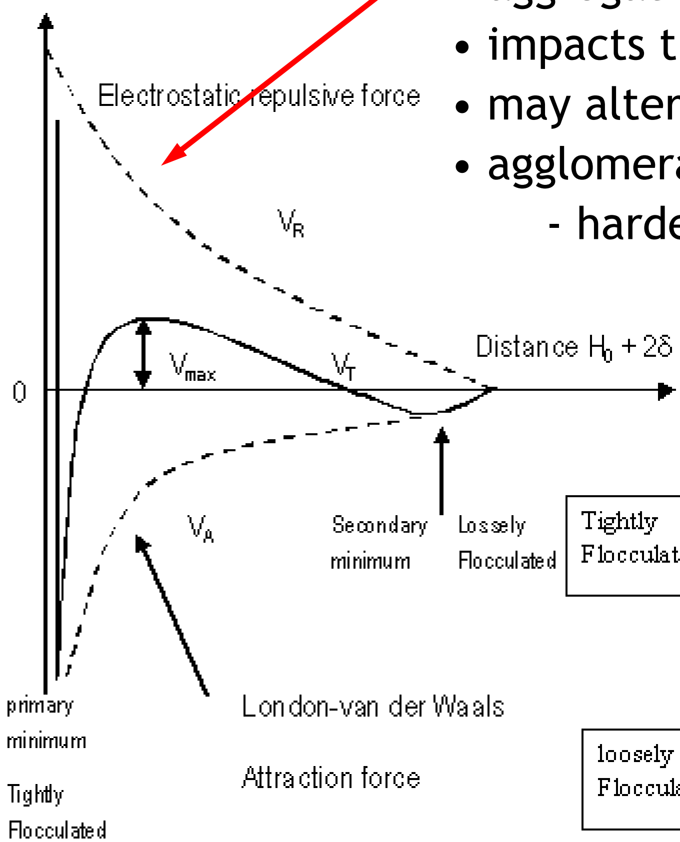
Four Measurement Challenge Areas

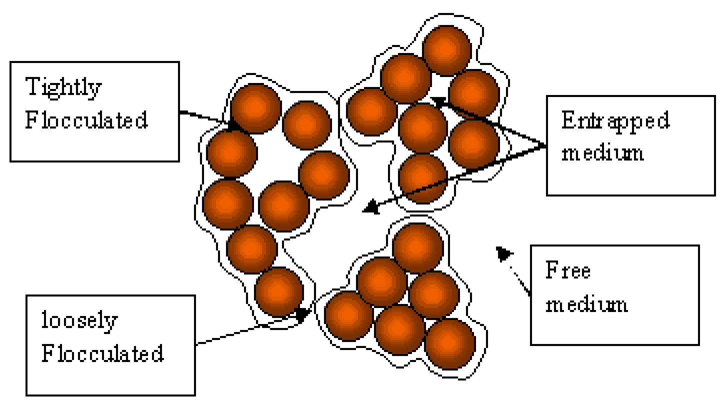
- ❖ Dispersion and Aggregation Effects
- ❖ Measurement in Complex Matrices
- ❖ Characterizing the Surface State of Bound Species
- ❖ Sample Preparation Prior to Conducting Tests

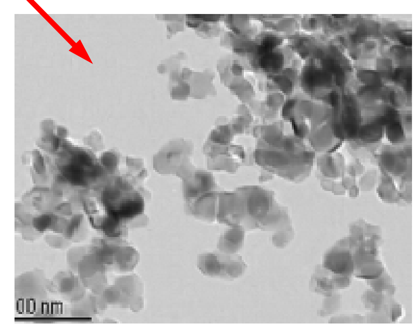
Measurement Challenges for Nano EHS #1

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



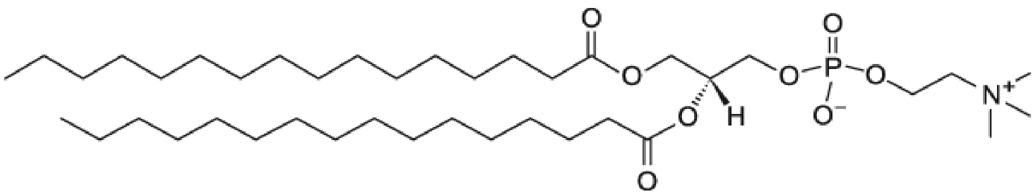




Solution: Biocompatible Dispersion Medium?

NIOSH Lung Fluid Mimic:
Phosphatidylcholine + serum albumin in PBS

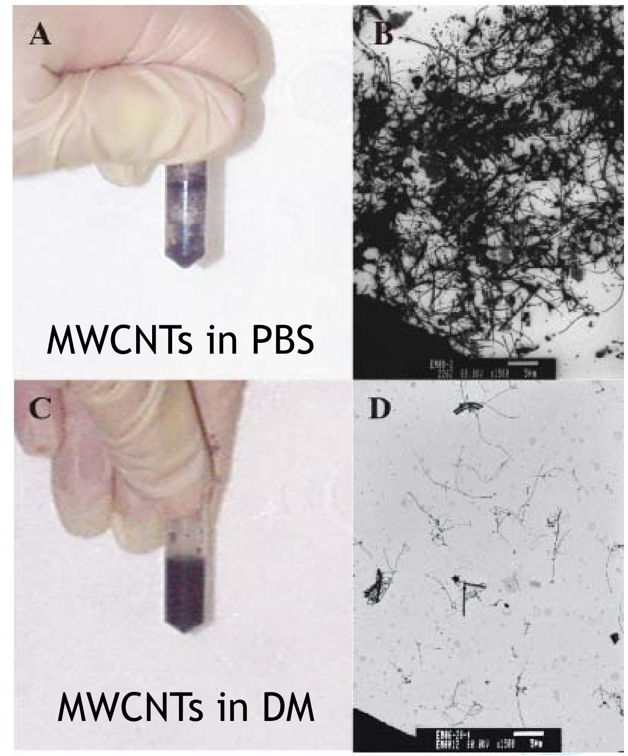
Porter et al., *Nanotoxicology* (2008)



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



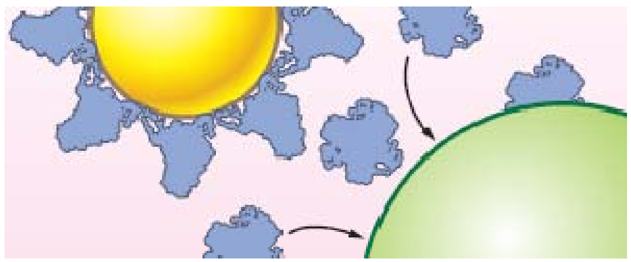
Dispersion medium for P-C and Tox measurements

- o works for different NM types (MeO, silica, carbon black, CNTs)
- o does not appear to significantly alter tox profile
- o can be standardized
- o proposed for on-going interlab validation studies (IANH)

Measurement Challenges for Nano EHS #2

P-C Measurements in complex biological/environmental matrices

o relevant conditions: pH/buffer, salts, temp, chemical composition
o differentiation between NMs and natural bio/environ components
o optical detection interference (scattering, fluorescence, absorption)
o protein absorption, interactions with other bio/env constituents



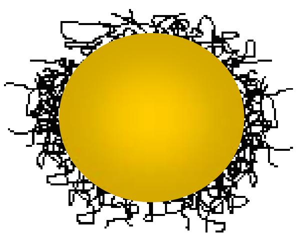
Lynch, Dawson and Linse,
Science STKE (2008)

- 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

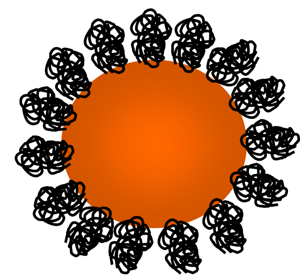
State of Surface-Bound Species

- ❖ 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)



PEG-thiol on Au NP protects from RES

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



BSA conjugated NP prevents aggregation

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

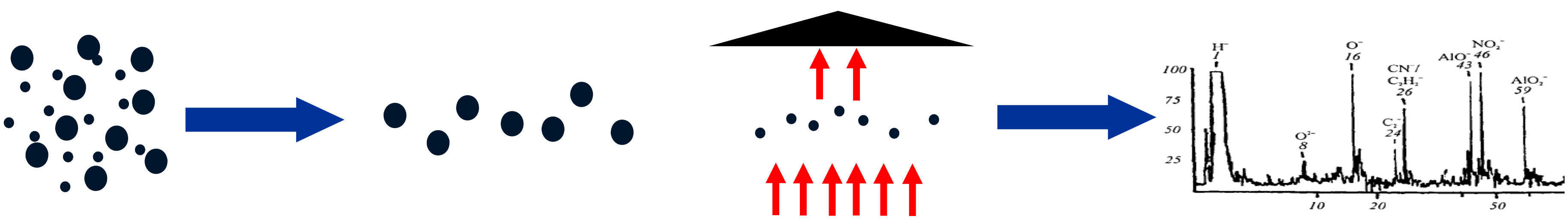
Solutions: Hyphenated Methods

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)



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…

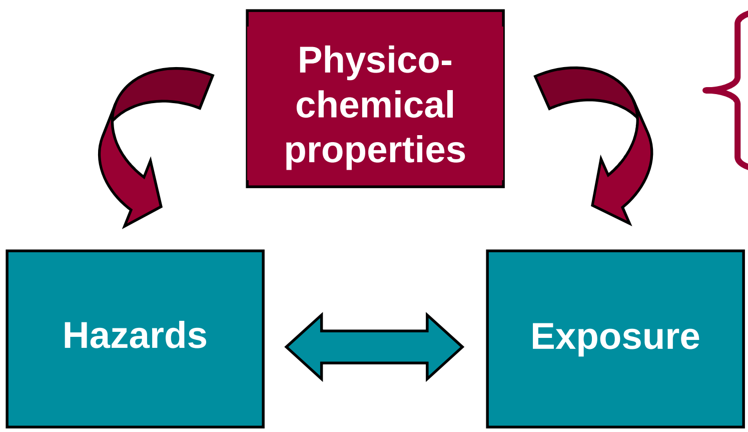
- for different classes of NMs
- for different measurement applications and media

❖ 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

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

Drivers

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

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/)