
# Measurement and Standardization Needs for Predicting Nano-Biointeractions

*Kristen M. Kulinowski, PhD*

*9 June 2008*

*Washington, DC*

CENTER FOR BIOLOGICAL AND ENVIRONMENTAL NANOTECHNOLOGY
CBEN
RICE UNIVERSITY

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# Addressing Applications & Implications


**http://cben.rice.edu**

## The Center for Biological & Environmental Nanotechnology

*An NSF center of excellence in nanotechnology research & education*

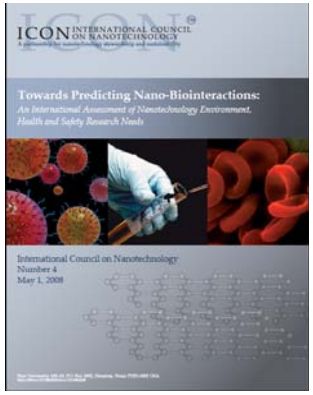

**http://icon.rice.edu**

## The International Council on Nanotechnology

*A unique multi-stakeholder forum for addressing the potential risks of nanotechnology*

# Recent Knowledge Transfer Highlights

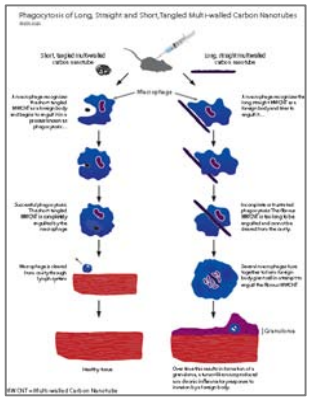
## NanoEHS Research Needs




**Towards Predicting Nano-bio Interactions**

## Backgrounders on High-Impact Research


**Nanoparticles and Amyloid Diseases**




**Nanotubes and Mesothelioma**

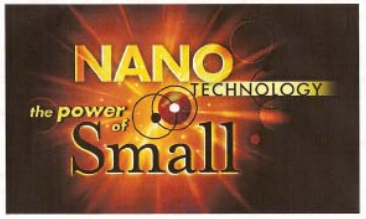
## CBEN Testimony

**Colvin at House Science Committee**

## New Resource for OEHS Practitioners

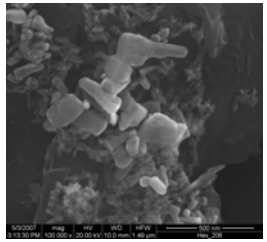

**ICON Wiki on occupational practices**

## CBEN in Nationally Syndicated TV Show




**Power of Small series on PBS**

## Partnership with Consumers Union



**Research on NPs in sunscreens**

Our 2007-8 highlights illustrate diverse, effective and innovative accomplishments

# Risk: Three Areas of Concern

End-of-use issues:
Ecological impacts

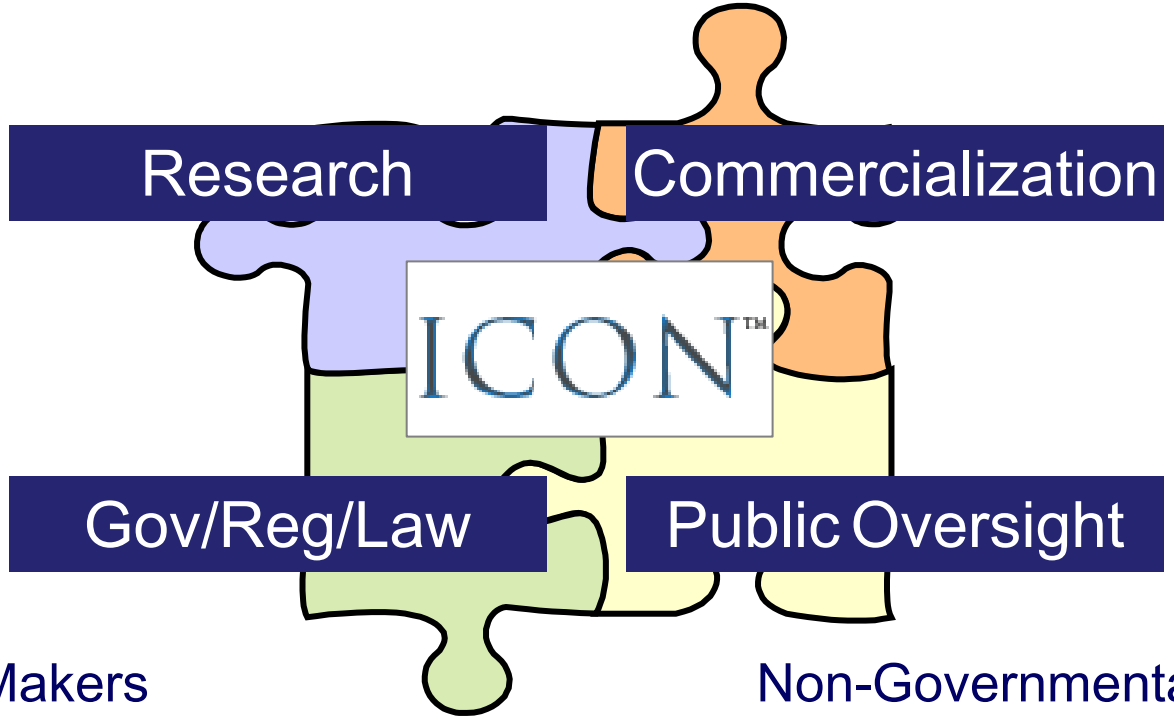

Direct consumer contact

Worker and laboratory safety

*What do we know about nano's impacts on EHS?*
*What are the barriers to effective risk management of nanomaterials?*

# ICON: A New Model for Interaction

Academics
Industry
Government




Industry
Trade Groups

Gov Policy Makers
Regulators
Lawyers

Non-Governmental Organizations
Social/Ethical Researchers

Information regarding potential environmental and health risks of nanotechnology to foster risk reduction and maximize societal benefit.

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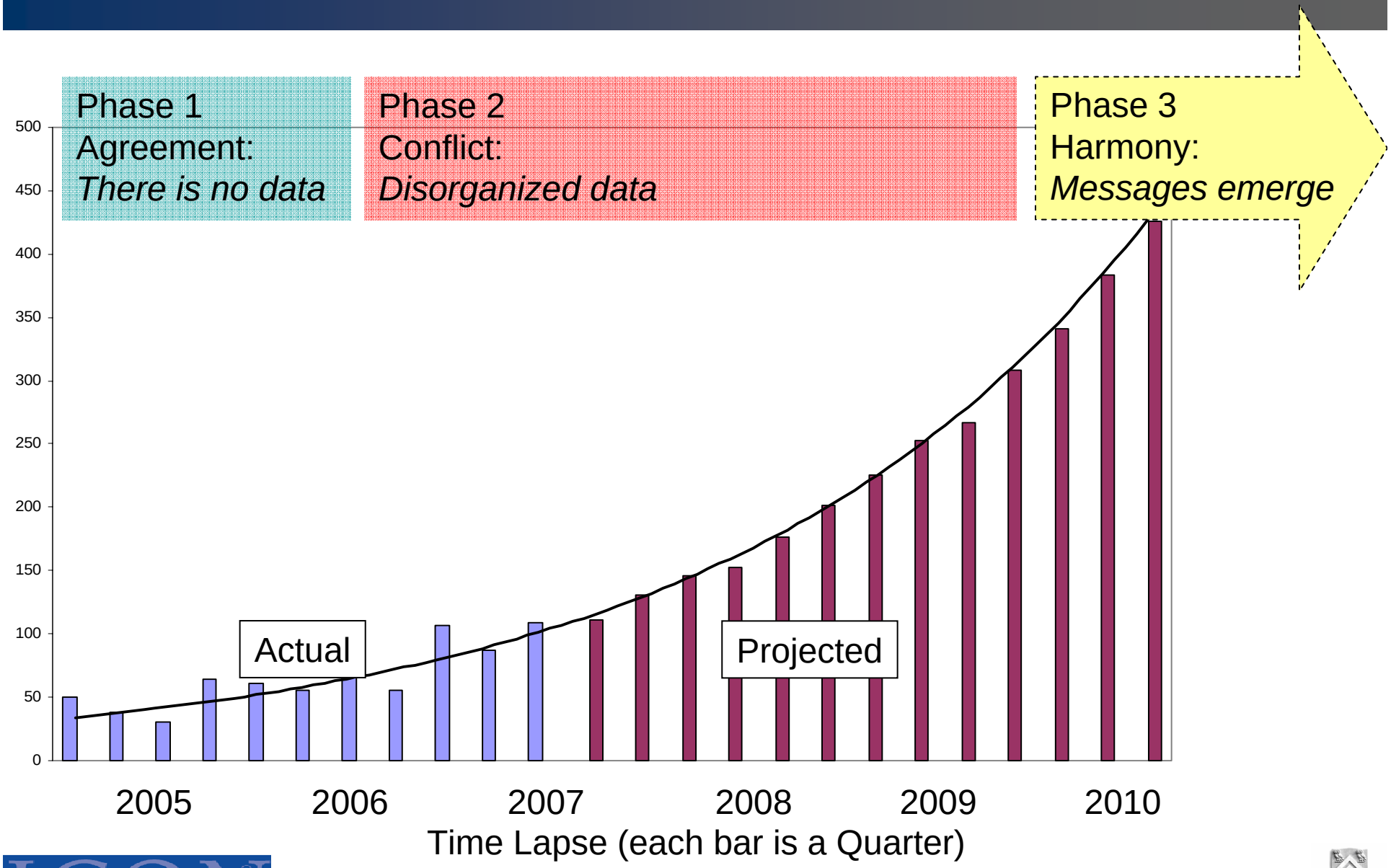
# Virtual Journal of NanoEHS


**Database of citations to peer-reviewed nanoEHS papers**

- Database launched August 2005
- Monthly updates
- Familiar, user-friendly format
- RSS feed
- Over 2000 records
- Backgrounders on key literature


http://icon.rice.edu/virtualjournal.cfm

# Growth Rate of EHS Publications





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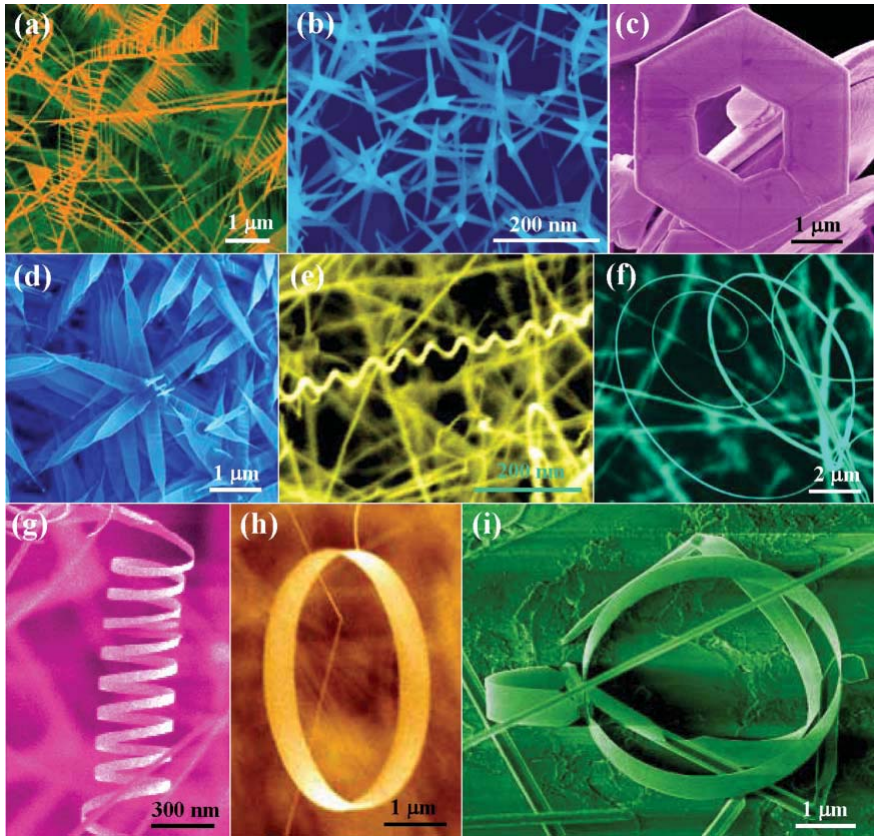
# Risk Assessment for Nano is Complicated

A few of the barriers

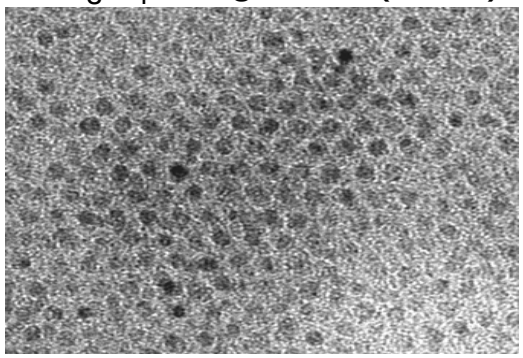
- Distinctive properties
- Nanomaterial diversity and variability
- Lack of standards
- Measurement tools

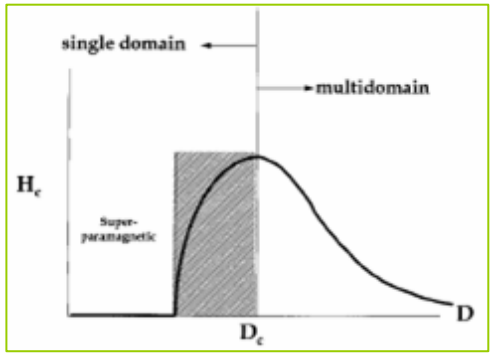
Credit: Zhong Lin Wang



# Nanomaterials: Distinctive Properties

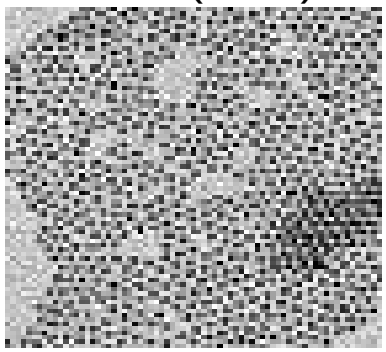
$Fe_3O_4$, Magnetite (4 nm)

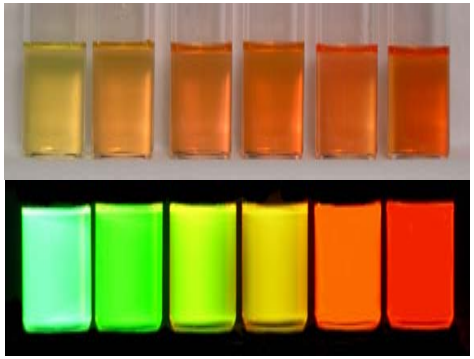





**Size-Dependent Magnetism**

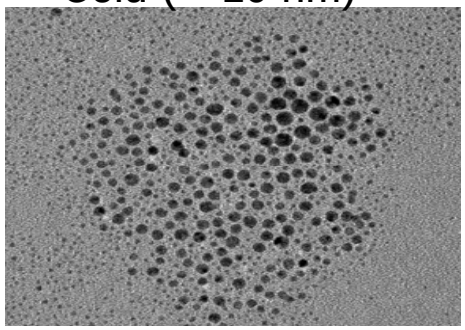
CdSe (8 nm)

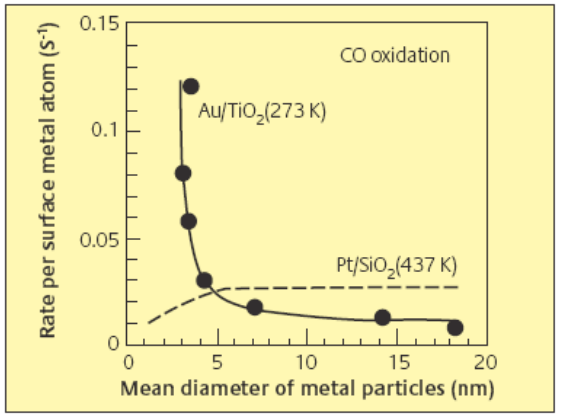




**Size-Dependent Emission**

Gold (~ 10 nm)



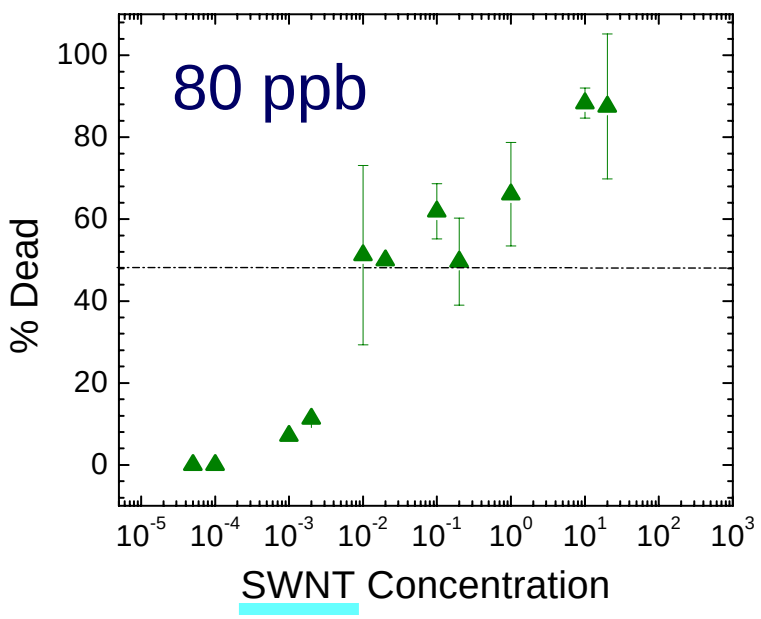



*Taken from M. Haruta Nature, 2005*

**Size-Dependent Reactivity**

# Nanomaterial Diversity

*Are single-walled carbon nanotubes toxic?*




Colvin, et al. unpublished

- 20 major types of SWNT
- 4 manufacturing types (trace impurities)
- Lengths ranging from 5 – 300 nm
- 5 methods of purification
- 10 possible surface coatings

*> 50,000 SWNT samples*

**How can better tools for predicting nanomaterial risk be developed?**

# International NanoEHS Research Needs

GRAND CHALLENGE: Computational Models that Predict Interactions of Engineered Nanomaterials with Organisms and the Environment

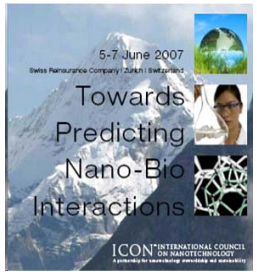
**Workshop 1: Towards Nanomaterial Classes**

- Develop classification of NMs based on material type
- Determine present and future applications
- Describe potential hot spots in lifecycle
- Describe properties important to biointeraction

NIH Campus Jan 2007

**Workshop 2: Towards Predictive Models**

- Elucidate mechanisms of nano-biointeractions
- Elucidate interactions at cell-free, cellular, tissue and whole animal levels
- Develop prioritized strategies and timelines




Swiss Re June 2007

OVERALL GOAL OF WORKSHOPS: Prioritized Research for Predicting Biointeractions for Nanomaterials of Commercial Relevance

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# Key Findings from Workshop 1

***CHALLENGE: Nanomaterial properties are not static throughout lifecycle***

Tools and models must be developed that can describe the dynamic nature of nanomaterials throughout their lifecycle.

***CHALLENGE: Chemical composition is not the ideal or sole property on which to focus***

A set of screening tools is needed to correlate the functional properties of nanomaterials with their potential for biological interaction.

***CHALLENGE: Exposure scenarios are too diverse to draw general conclusions***

Exposure assessment studies are needed to enable predictions about implications of physicochemical properties for net dose.

# Key Findings from Workshop 2

***CHALLENGE: Nanoparticle surfaces undergo changes during interactions in biological environments***

Quantitative models are needed to describe how the properties of NPs control the nature and extent of biomolecular interactions at their surface.

***CHALLENGE: Existing mass-based metrics of measuring dose and dose rate may underestimate NP impacts***

Dose and dose rate may need to be validated independently for NPs.

***CHALLENGE: In vitro assays are practical given nanomaterial diversity but may be poor predictors of in vivo endpoints***

Better biomarkers are essential to address the vast diversity of NP types and to develop strong correlative models for predicting in vivo endpoints based on in-vitro results.


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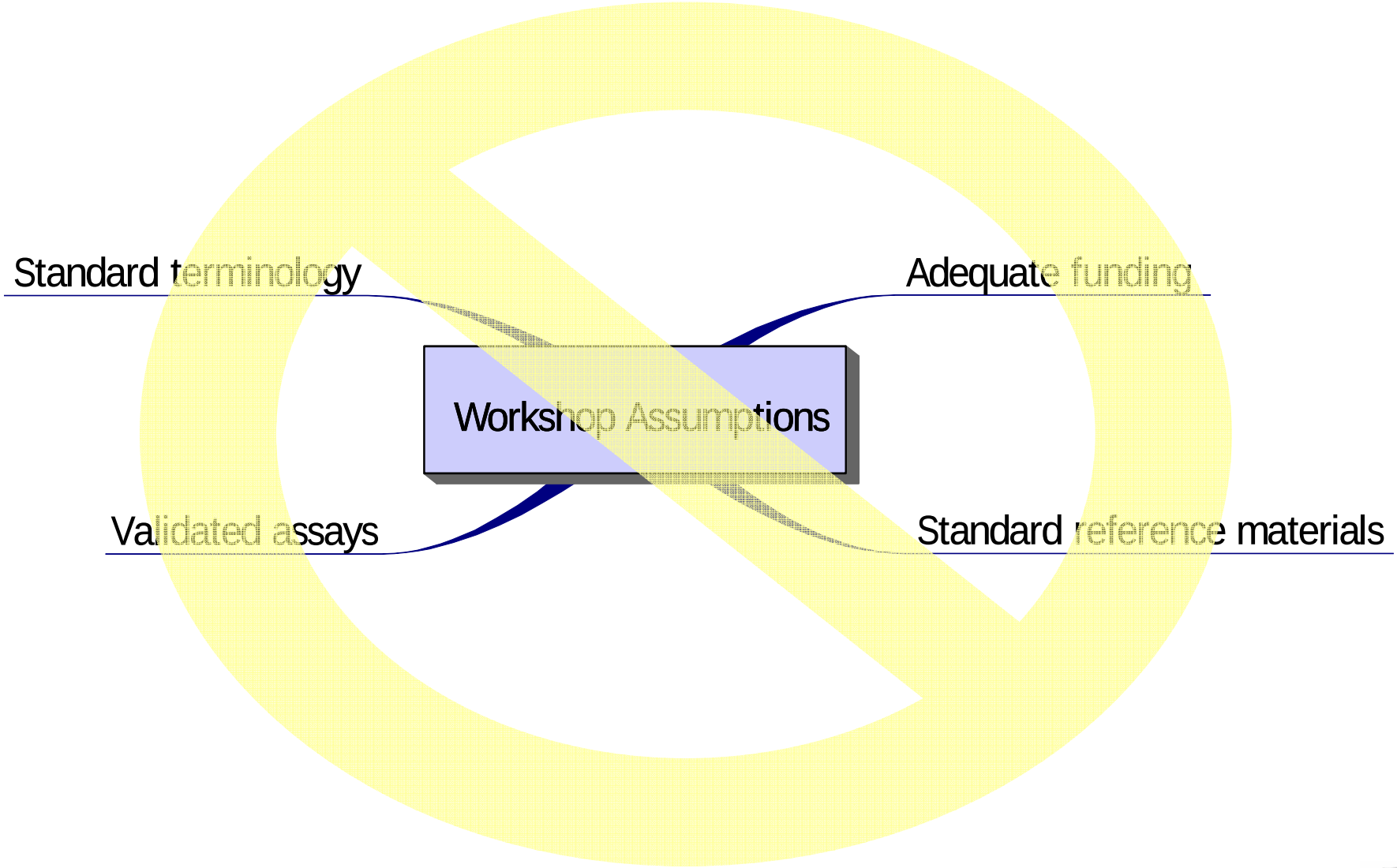
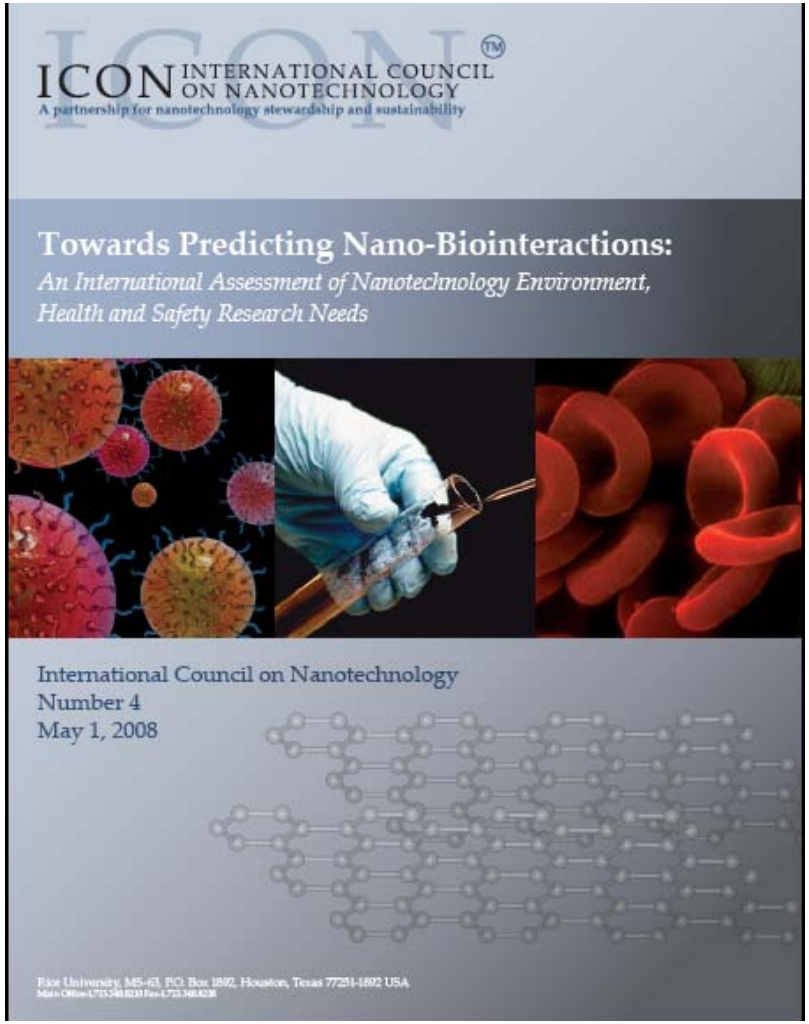
# Workshop 2 Assumptions



# Cross-Cutting Issues between WS 1 and 2




Many outstanding needs

- Lack of standards for terminology, characterization, and materials
- Metrology and tools to characterize and measure nanomaterials and to monitor their presence in the environment and in biological media
- Test methodologies to characterize potential mobility of embedded nanomaterials
- Evaluation of the appropriateness of in vitro tests to characterize nanomaterial interactions more broadly,
- Standardization of biological materials used in testing

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# Highest Priority Research (2-yr Goals)

## Research to Predict Nano-Biointeractions

- Understand which NM characteristics are most important for biointeractions
- Establish validated NMs that have been tested in vitro and in vivo
- Develop tools for in vitro testing that map onto in vivo endpoints
- ➡ Develop new techniques for imaging NMs in biological media and organisms
- Determine fate and interactions of NMs in reference organisms
- Design framework for data sharing and ontologies

# Highest Priority Research (2-5 yr Goals)

## Research to Meet Risk Management Needs

- Identify/develop tools for detecting and characterizing presence of NMs in the workplace and the environment
- Validate the effectiveness of personal protective equipment in limiting exposure
- Establish test methods for evaluating the stability and mobility of NMs in liquid and solid matrices
- Develop portable tools to monitor a wide range of nanomaterials in the workplace and environment
- Determine the bioavailability of nanomaterials throughout the lifecycle

# ~~No~~ Few Standards

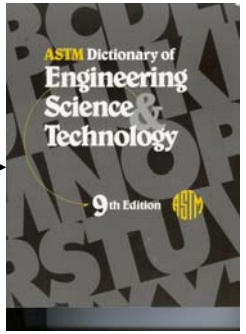
Standard: a technical document that is a basis for manufacturing, management, codes & regulations

Scientific data

Public reports

Industry practices

**Standards**

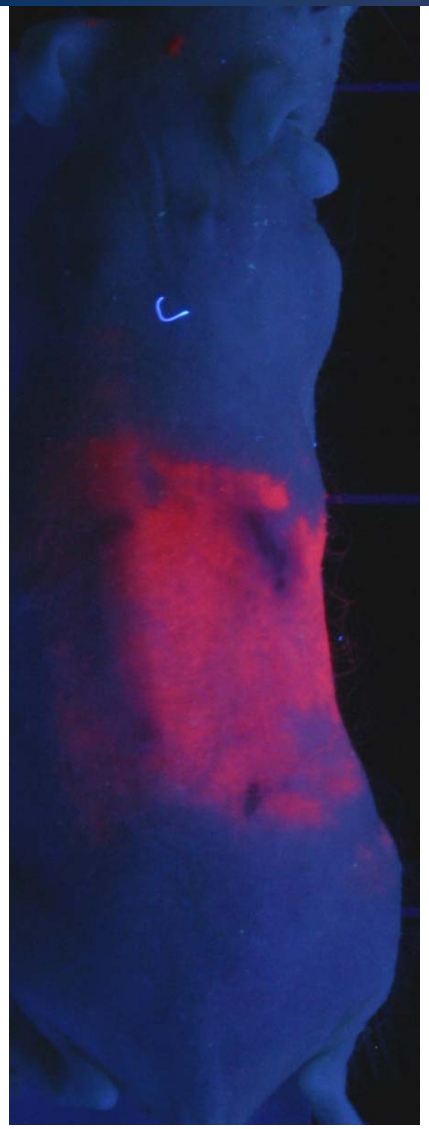


First goal: Terminology and nomenclature
Second goal: Product stewardship

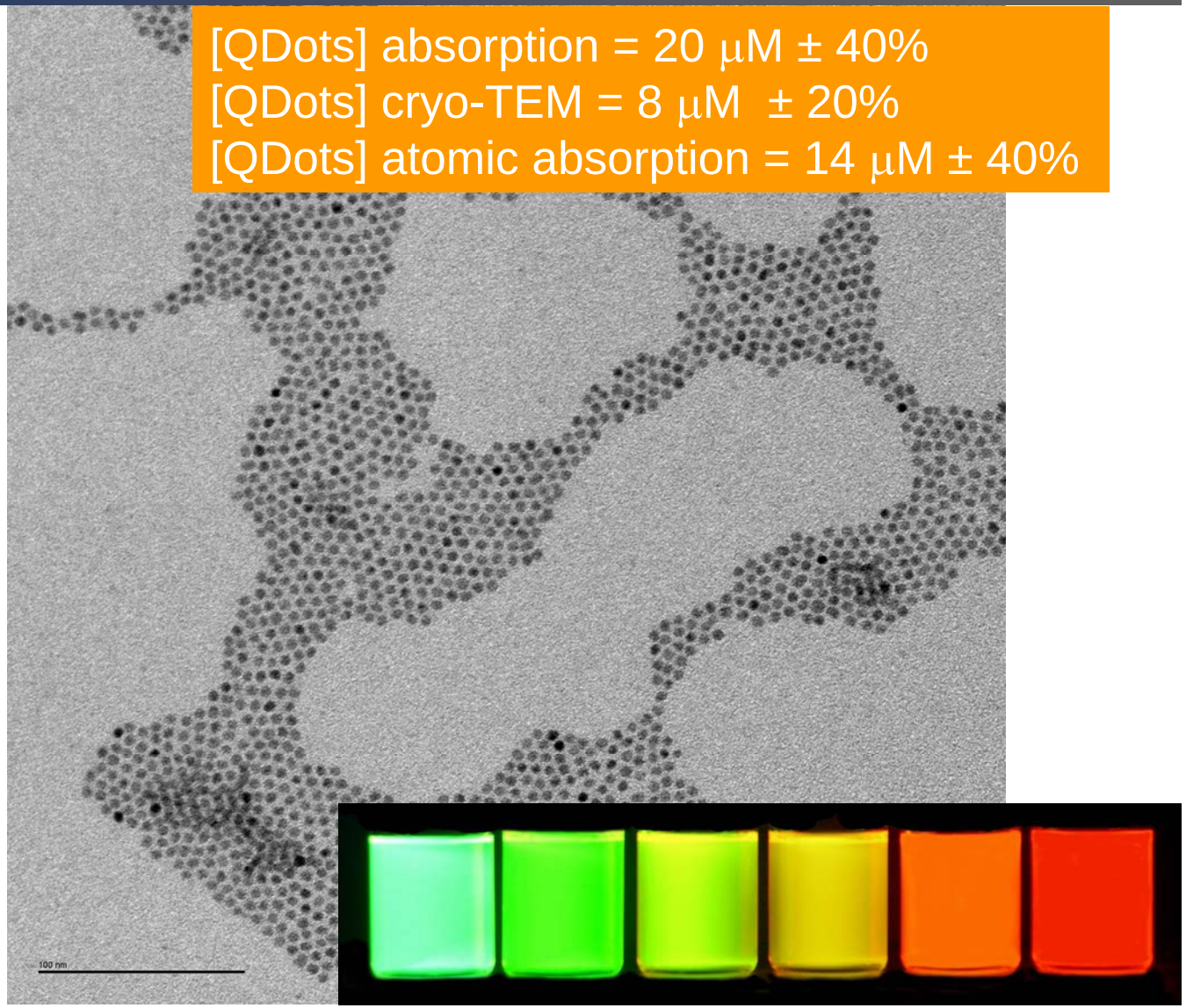
# How Standards Impact Risk Management

| | |
|---|---|
| Terminology | TSCA: new chemical?<br>OSHA: Material Safety Data Sheet<br>Data in literature can't be easily compared |
| Metrology/characterization | Occupational exposure: No standard detection equipment<br>Environmental monitoring not easy |
| EHS | No standard test battery for tox screening<br>No standard practices for occupational handling |

# Example: Standard Characterization Methods Needed!



[QDots] absorption = 20 μM ± 40%
[QDots] cryo-TEM = 8 μM ± 20%
[QDots] atomic absorption = 14 μM ± 40%




V.L. Colvin w/ Paul Howard (NCTR), Nigel Walker (NIEHS)

# Nanotechnology Stds Activities

- Chair, Vicki Colvin

Subcommittees

- E56.01 Terminology & Nomenclature*
  - Colvin sub-chair
- E56.02 Characterization
- E56.03 Environment, Health, and Safety
  - Kulinowski sub-chair

Limited stakeholder engagement in these processes

- ANSI-Accredited U.S. Technical Advisory Group (TAG) to ISO/TC 229 *Nanotechnologies*
- www.ansi.org/nsp

- Technical committee on nanotech (ISO/TC 229)
  - Working Group (WG) 1 - Terminology and Nomenclature – Leadership assigned to Canada
  - Working Group (WG) 2 - Measurement and Characterization – Leadership assigned to Japan
  - Working Group (WG) 3 - Health, Safety and Environment – Leadership assigned to United States

# ASTM Nano Standards

**Active standards**

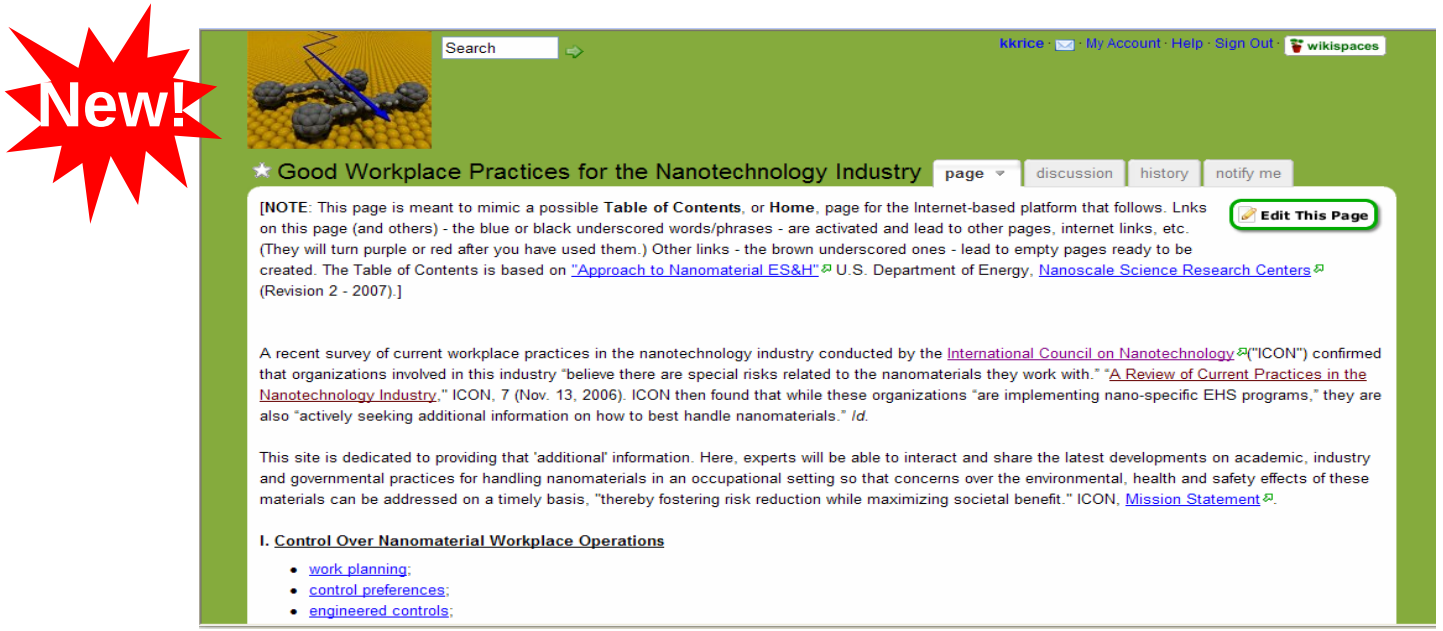
- **E2456-06 Standard Terminology Relating to Nanotechnology**
- **E2524-08 Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles**
- **E2525-08 Standard Test Method for Evaluation of the Effect of Nanoparticulate Materials on the Formation of Mouse Granulocyte-Macrophage Colonies**
- **E2526-08 Standard Test Method for Evaluation of Cytotoxicity of Nanoparticulate Materials in Porcine Kidney Cells and Human Hepatocarcinoma Cells**
- **E2578-07 Standard Practice for Calculation of Mean Sizes/Diameters and Standard Deviations of Particle Size Distributions**
- **E2535-07 Standard Guide for Handling Unbound Engineered Nanoscale Particles in Occupational Settings**

Proposed New Standards

- WK8705 Measurement of particle size distribution of nanomaterials in suspension by Photon Correlation Spectroscopy (PCS)
- WK8997 Standard Practice for Analysis of Hemolytic Properties of Nanoparticles
- WK9326 Standard Practice for Evaluation of the Effect of Nanoparticulate Materials on the Formation of Mouse Granulocyte-Macrophage Colonies
- WK9327 Standard Practice for Evaluation of Cytotoxicity of Nanoparticulate Materials on Porcine Kidney Cells
- WK9952 Standard Practice for Measuring Length and Thickness of Carbon Nanotubes Using Atomic Force Microscopy Methods
- WK10417 Standard Practice for the Preparation of Nanomaterial Samples for Characterization

# Future Directions

- Identify promising metrology and methodologies for monitoring nanomaterials in the workplace and the environment
- Identify tools for detecting the presence and characteristics of nanomaterials in biological systems
- Develop a minimum set of experimental data to be submitted with a technical manuscript to allow for greater reproducibility and comparison of nano-biointeractions research
- Identify model biological systems and model nanoparticles for nano-biointeractions research
- **ICON Good Workplace Practices Wiki**



# Addressing Applications & Implications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# Prioritized Research Needs I

| Research to Predict Nano-Biointeractions | Near-term *2 years* | Mid-term *5 years* | Long-term *7-10 years* |
|---|---|---|---|
| ***Nanomaterial characterization*** | | | |
| 1. Establish minimum NM physicochemical properties for characterization. | ✓ | | |
| 2. Establish validated correlation between physicochemical properties and biointeractions. | | ✓ | |
| ***Standard terminology*** | | | |
| 3. Establish common vocabulary/terminology for materials and assays. | ✓ | | |
| ***Standard reference nanomaterials*** | | | |
| 4. Establish validated reference NMs that have been tested *in vitro* and *in vivo*. | ✓ | | |
| 5. Establish tight control of nanoparticle reference materials. | | ✓ | |
| ***Techniques for detecting Nanomaterials in biological media*** | | | |
| 6. Develop new techniques for imaging NMs in biological media and organisms to supplement TEM | ✓ | | |
| ***In vivo tests and correlation to in vitro tests*** | | | |
| 7. Quantitatively determine the fate and interactions of engineered NMs within reference organisms, including dose and dose-rate effects. | ✓ | | |
| 8. Develop a fundamental understanding of NM interaction with cell-signaling pathways. | | ✓ | |
| 9. Identify nano-biointeractions for chronic exposure. | | | ✓ |
| 10. Validate SARs based on *in vitro* and *in vivo* data. | | | ✓ |

# Prioritized Research Needs II

| | | | |
|---|---|---|---|
| ***In vitro test validation*** | | | |
| 11. Identify standard *in vitro* biological media and tests based on *in vivo* tests. | ✓ | | |
| 12. Evaluate interactions of a range of engineered NMs with standard *in vitro* tests and evaluate vs. *in vivo*. | ✓ | | |
| 13. Establish validated correlation between physicochemical properties and biointeractions. | | ✓ | |
| 14. Validate standard *in vitro* biological media and assays vs. *in vivo* tests. | | ✓ | |
| 15. Correlate engineered NMs *in vitro* and *in vivo* for model systems. | | ✓ | |
| 16. Explore interactions of a broad range of engineered NMs with complex coatings with standardized *in vitro* tests. | | ✓ | |
| 17. Complete mechanistically based QSAR studies. | | ✓ | |
| 18. Develop engineered NM-specific, high-throughput screening methods with supplemental modeling. | | | ✓ |
| 19. Validate SARs based on *in vitro* and *in vivo* data. | | | ✓ |
| ***Model development*** | | | |
| 20. Design framework(s) for data sharing and ontologies. | ✓ | | |
| 21. Explore applicability of established modeling algorithms. | ✓ | | |
| 22. Establish data-sharing structures. | | ✓ | |
| 23. Establish mechanistically based QSAR studies. | | ✓ | |
| 24. Validate SARs based on in vitro and in vivo data. | | | ✓ |
| 25. Validate algorithms and training sets for computational models. | | | ✓ |
| 26. Develop engineered NM-specific, high-throughput screening methods with supplemental modeling. | | | ✓ |

***NM = Nanomaterial***

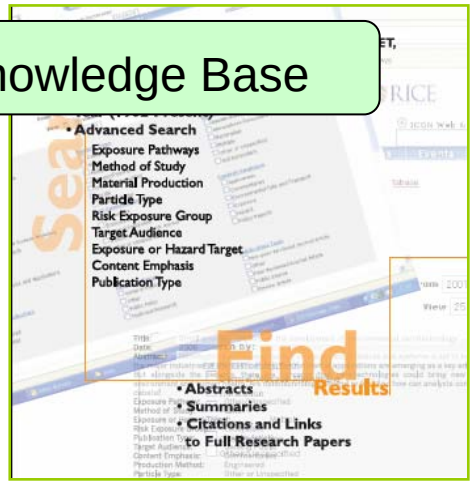
# Prioritized Research Needs III

| Research to Meet Risk Management Needs | Near-term *2 years* | Mid-term *5 years* | Far-term *7-10 years* |
|---|---|---|---|
| ***Metrology for risk management*** | | | |
| 1. Identify metrology techniques capable of characterizing the presence of engineered NMs in the workplace and environment. | ✓ | | |
| 2. Validate the effectiveness of personal protective equipment in limiting exposure. | ✓ | | |
| 3. Develop portable tools to monitor a wide range of NMs in the workplace and environment. | | ✓ | |
| ***Assessment of bioavailability*** | | | |
| 4. Determine the bioavailability of NMs throughout the lifecycle. | | ✓ | |
| ***Characterization of potential mobility of embedded NMs*** | | | |
| 5. Establish test methodologies to evaluate the stability and mobility of NMs in liquid and solid matrices. | ✓ | | |
| 6. Complete evaluation of stability and mobility of NMs in common liquid and solid matrices. | | ✓ | |

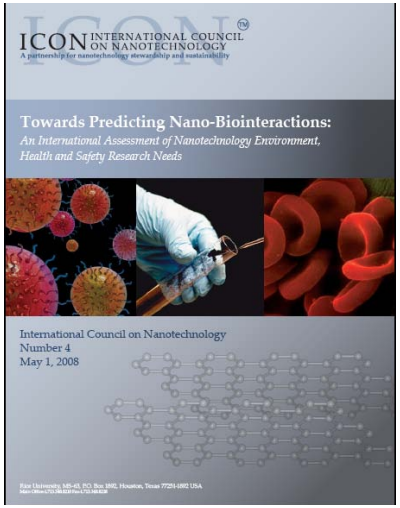
***NM = Nanomaterial***

# Quality Information about Risks & Benefits

Knowledge Base

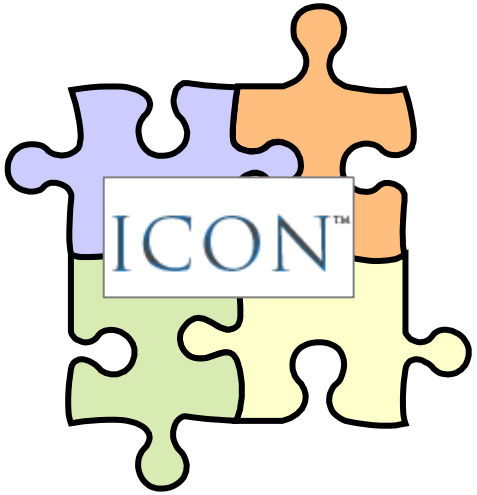



**Database/VJ on nanoEHS research**

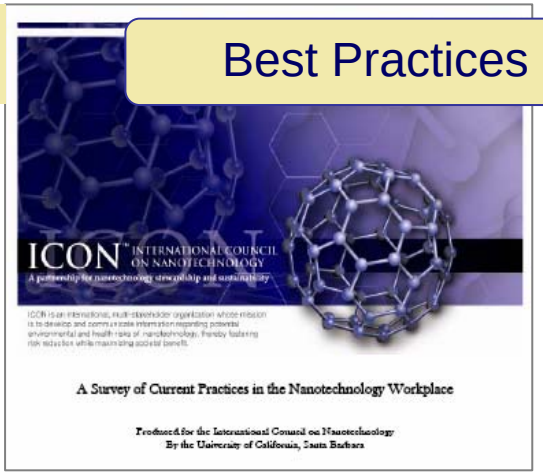



**International nanoEHS research needs assessment**

**New ES&T paper:**
**DOI: 10.1021/es702158q**




Best Practices




**Survey of current workplace practices**

Communications

**ICONsultations with diverse stakeholders**

ICON Working Groups

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# Acknowledgments

**Workshop Steering Team Members**

- Cate Alexander Brennan, National Nanotechnology Coordination Office
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- Kristen Kulinowski, Rice University
- Andrew Maynard, Woodrow Wilson International Center for Scholars
- Günter Oberdörster, University of Rochester
- Jennifer Sass, Natural Resources Defense Council
- Hideo Shindo, NEDO Japan
- Vicki Stone, Napier University
- Sally Tinkle, National Institute of Environmental Health Sciences

- David Johnson, Rice University

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NSF Grant BES-0646107

# E2456-06 Standard Terminology Relating to Nanotechnology

- Agglomerate
- Aggregate
- Fine particle
- Nano
- Nanoparticle
- Nanoscale
- Nanoscience
- Nanostructured
- Nanotechnology
- Non-transitive nanoparticle
- Particle
- Transitive nanoparticle
- Ultrafine particle

**nanoparticle,** *n—in nanotechnology*, a sub-classification of ultrafine particle with lengths in two or three dimensions greater than 0.001 micrometer (1 nanometer) and smaller than about 0.1 micrometer (100 nanometers) and which may or may not exhibit a size-related intensive property.

DISCUSSION—This term is a subject of controversy regarding the size range and the presence of a size-related property. Current usage emphasizes size and not properties in the definition. The length scale may be a hydrodynamic diameter or a geometric length appropriate to the intended use of the nanoparticle.

# Std Occupational Guide

## E2535: Standard Guide for Handling Unbound Engineered Nanoparticles in Occupational Settings

**Standard Guide for**
**Handling Unbound Engineered Nanoparticles in Occupational Settings[1]**

This standard is issued under the fixed designation E XXXX; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

**INTRODUCTION**

Nanometer-scale particles (nanoparticles) are encountered in nature and in industry in a variety of forms and materials. Engineered nanoparticles as a class comprise a range of materials differing in shape, size, and chemical composition, and represent a broad range of physical and chemical properties. Workers within some nanotechnology-related industries and operations have the potential to be exposed to these engineered nanoparticles at levels exceeding ambient nanoparticle concentrations through inhalation, dermal contact and ingestion when not contained on or within a matrix (unbound). Occupational health risks associated with manufacturing, processing and handling unbound nanoparticles, agglomerates or aggregates of nanoparticle are not yet clearly understood. Dominant exposure routes, potential exposure levels and any material hazard are expected to vary widely among particular nanoparticle materials and handling contexts. Additional research is needed to understand the impact of these exposures on employee health and how best to devise appropriate exposure monitoring and control strategies. Until clearer understandings emerge, the limited evidence available suggests caution when potential exposures to unbound engineered nanoparticles (UNP) may occur.

- Technical Contact: Steven Brown (Intel)
- Passed final ballot 9/15/2007