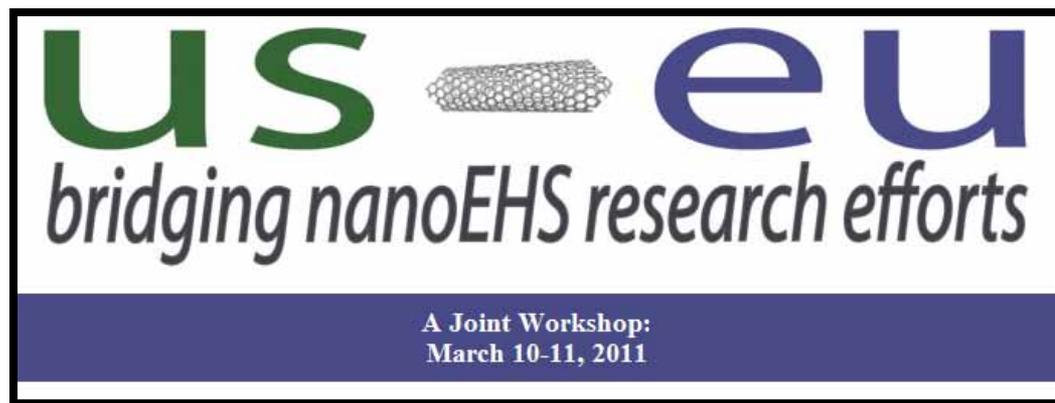


# Experiences with Assessing the Risks of Nanomaterials, and Implications for Research



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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

# Regulatory Authorities, and Associated Data Needs

- **The Toxic Substances Control Act (EPA) -- “Industrial Chemicals”**
  - NMs 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

# 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
- **Industrial Chemicals** (from *Sayre, et al. In Press: J. Occupat. & Env. Med.*):

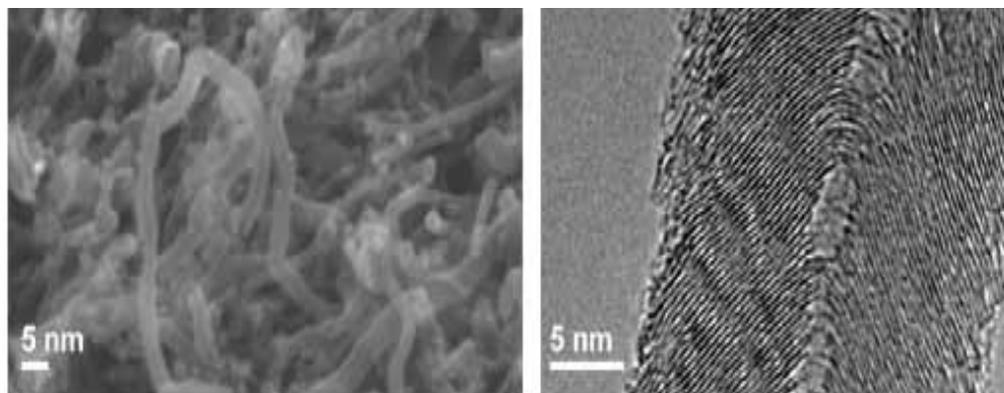
<u>Nanomaterial Classes</u>	<u>General Uses</u>	<u>Approximate Range of Nanomaterials, per Class</u>
Single-walled, and multi-walled carbon nanotubes; carbon nanofibers; and other carbon particles	Enhanced electrical conductivity, mechanical reinforcement, and/or color additives	Less than 50
Fullerenes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

**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

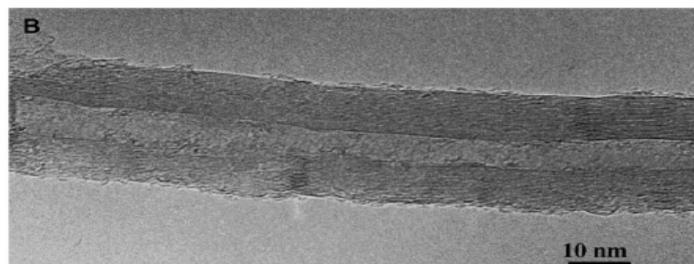
Leah A. Mitchell,\*† Jun Gao,\* Randy Vander Wal,‡ Andrew Gigliotti,† Scott W. Burchiel,\* and Jacob D. McDonald†<sup>1</sup>

\*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.

LISON AND MULLER



Inhalation of multiwalled carbon nanotubes (MWCNTs) at particle concentrations ranging from 0.3 to 5 mg/m<sup>3</sup> 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

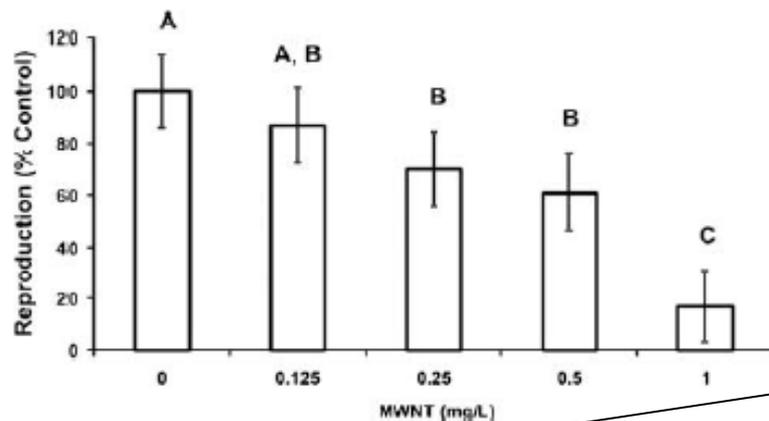
No.	Group of organisms	Inorganic nanoparticles				Organic nanoparticles			Most toxic NP
		mg TiO <sub>2</sub> /l Nano TiO <sub>2</sub>	mg ZnO/l Nano ZnO	mg CuO/l Nano CuO	mg Ag/l Nano Ag	mg/l SWCNT	mg/l MWCNT	mg/l C60	
1	Crustaceans	67.7 (10)	0.62 (3)	2.65 (2)	<b>0.040</b> (1)	15.0 (3)	<b>8.7</b> (1)	35.0 (5)	Nano Ag
2	Bacteria	603 (4)	20 (3)	71 (2)	7.60 (5)	163 (2)	500 (1)	0.81 (4)	C60
3	Algae	<b>65.5</b> (4)	<b>0.068</b> (2)	<b>0.87</b> (1)	0.23 (2)	<b>1.04</b> (1)	NF	100.0 (1)	Nano ZnO
4	Fish	300 (4)	1.9 (2)	NF	7.1 (1)	NF	NF	1.0 (3)	C60
5	Ciliates	NF	5.4 (1)	156.5 (1)	39.0 (1)	6.8 (1)	NF	<b>0.25</b> (1)	C60
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	<b>65.5</b>	<b>0.068</b>	<b>0.87</b>	<b>0.040</b>	<b>1.04</b>	<b>8.7</b>	<b>0.25</b>	
1-7	Most sensitive organisms	Algae	Algae	Algae	Crustaceans	Algae	Crustaceans	Ciliates	
1-7	Classification (1-7) <sup>b</sup>	Harmful	Extremely toxic	Very toxic	Extremely toxic	Toxic	Toxic	Very toxic	
1-3	Classification (1-3) <sup>c</sup>	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<sup>a</sup>

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



*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: Weinich, 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/m<sup>3</sup> (*R. Landsiedel et al, Tox. Sci., 2009*)
  - Second available subchronic study for MWCNTs, with the NOAEL = 0.1 mg/m<sup>3</sup> (*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/m<sup>3</sup> (*N. Walker et al, SOT Poster, 2009*)
  - Few Chronic inhalation studies on Manufactured Nanomaterials
  - Emerging concerns for other organ systems: cardiovascular effects due to inhalation exposure (*Nurkiewicz, et al 2008 Particle & Fibre Toxicology, 5:1 / Erdely, et al 2008 Nano Letters*)
  - 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*).
- General Trends: Increased concern for metal and metal oxide nanoparticles at sizes below 30 nm?  
10 March 2011 (*Auffan, et al. 2009, Nature Nanotechnol.*)

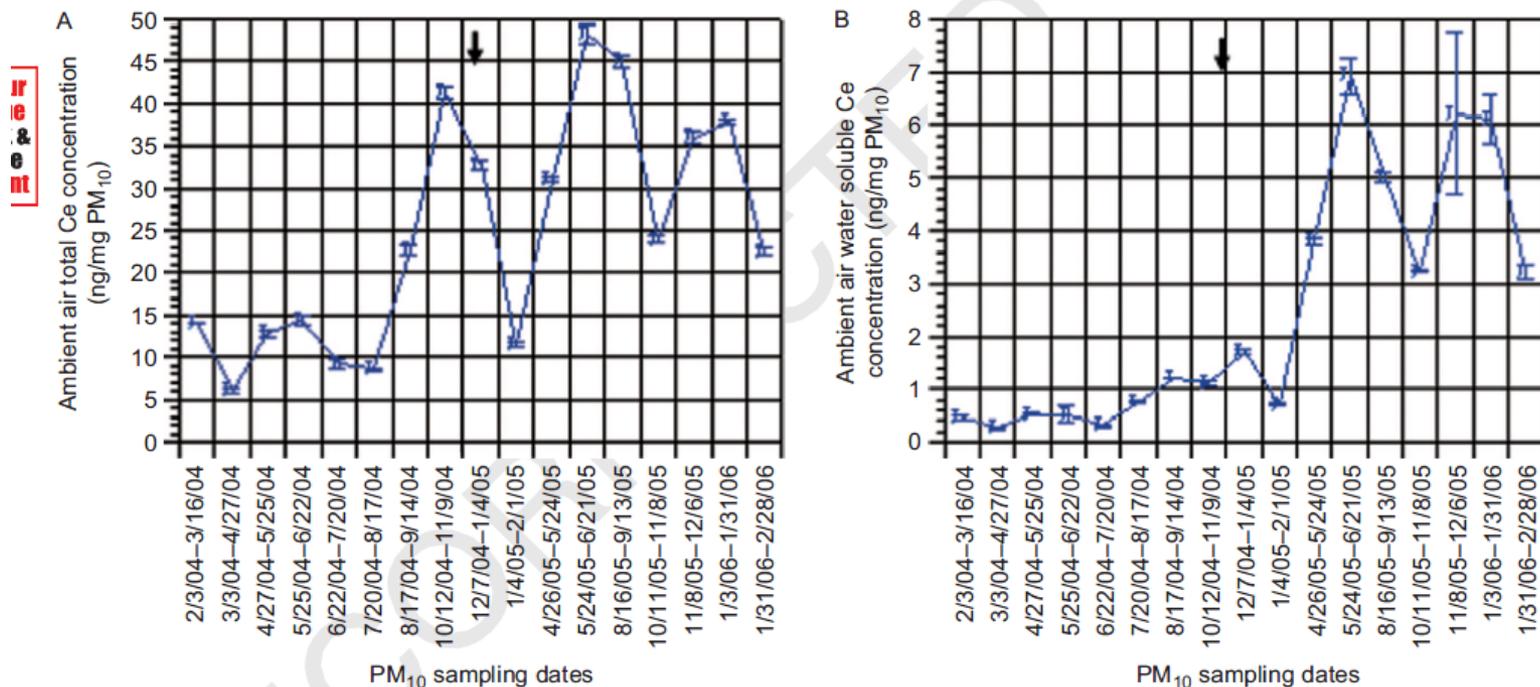
## Ability to Bridge Data for Nanomaterials

- 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

# Exposure Data are Limited

- **Exposures in the Workplace**
  - CNTs:
    - SWCNTs: 53 ug/m<sup>3</sup> *Maynard et al. 2004. J. Toxl. & Envir. Health,*
    - MWCNTs: 0.018 – 194 tubes/cc *Han, et al. 2008 Inhalat. Tox.*
  - Fullerenes: Measurements confounded by background particle counts/lack of specificity in detection methods (*Yeganeh, et al. 2008. Environ. Sci. Technol., 42:12; Fujitani, et al, 2008, JEOH, 5:6*)
  - 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/m<sup>3</sup> 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*)

# General Population Exposures: Ceria from Combustion of Diesel Fuels



**Figure 3.** Ambient air PM<sub>10</sub> cerium levels before and after deployment of Envirox nanoCeO<sub>2</sub> fuel additive in England. (A) total PM<sub>10</sub> cerium levels in ambient air collected from 2/3/04 to 2/28/06 from the Newcastle, England air quality monitoring site. (B) Water soluble cerium levels in ambient air collected from 2/3/04 to 2/28/06 from the Newcastle, England air quality monitoring site. Arrow indicates the time period of transition from testing to full deployment of Envirox nanoCeO<sub>2</sub> fuel additive into diesel powered buses. Methods: Ambient PM<sub>10</sub> was collected at the Newcastle, England air

At the moment, in vivo or clinical studies evaluating effects of nanoscale CeO<sub>2</sub> 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<sub>2</sub> caused pronounced effects at a mass concentration of 0.14 mg/m<sup>3</sup> (increased levels of biochemical parameters and leukocytes in bronchoalveolar lavage fluid).

Cassee, et al. 18 Jan 2011. *Crit Rev. Tox.*

# Consumer Exposures to Spray-Applied Nanomaterials

*Inhalation Toxicology*, 2010; 22(13): 1072-1002

informa  
healthcare

RESEARCH ARTICLE

## Nanoparticles-containing spray can aerosol: characterization, exposure assessment, and generator design

Bean T. Chen<sup>1</sup>, Aliakbar Afshari<sup>1</sup>, Samuel Stone<sup>1</sup>, Mark Jackson<sup>1</sup>, Diane Schwegler-Berry<sup>1</sup>, David G. Frazer<sup>1</sup>, Vincent Castranova<sup>1</sup>, and Treye A. Thomas<sup>2</sup>

In order to assess an exposure, the mass of the TiO<sub>2</sub> particles that deposits in the pulmonary region of the lung needs to be estimated. Assuming a peak TiO<sub>2</sub> aerosol concentration of 3.4 mg/m<sup>3</sup> 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<sup>2</sup> (Stone et al., 1992), the approximate lung burden after 1 min of spray application would be ~0.075 µg TiO<sub>2</sub> per m<sup>2</sup> alveolar epithelium. This is equivalent to a pulmonary dose of about 0.03 µg TiO<sub>2</sub> in a rat (Stone et al., 1992).

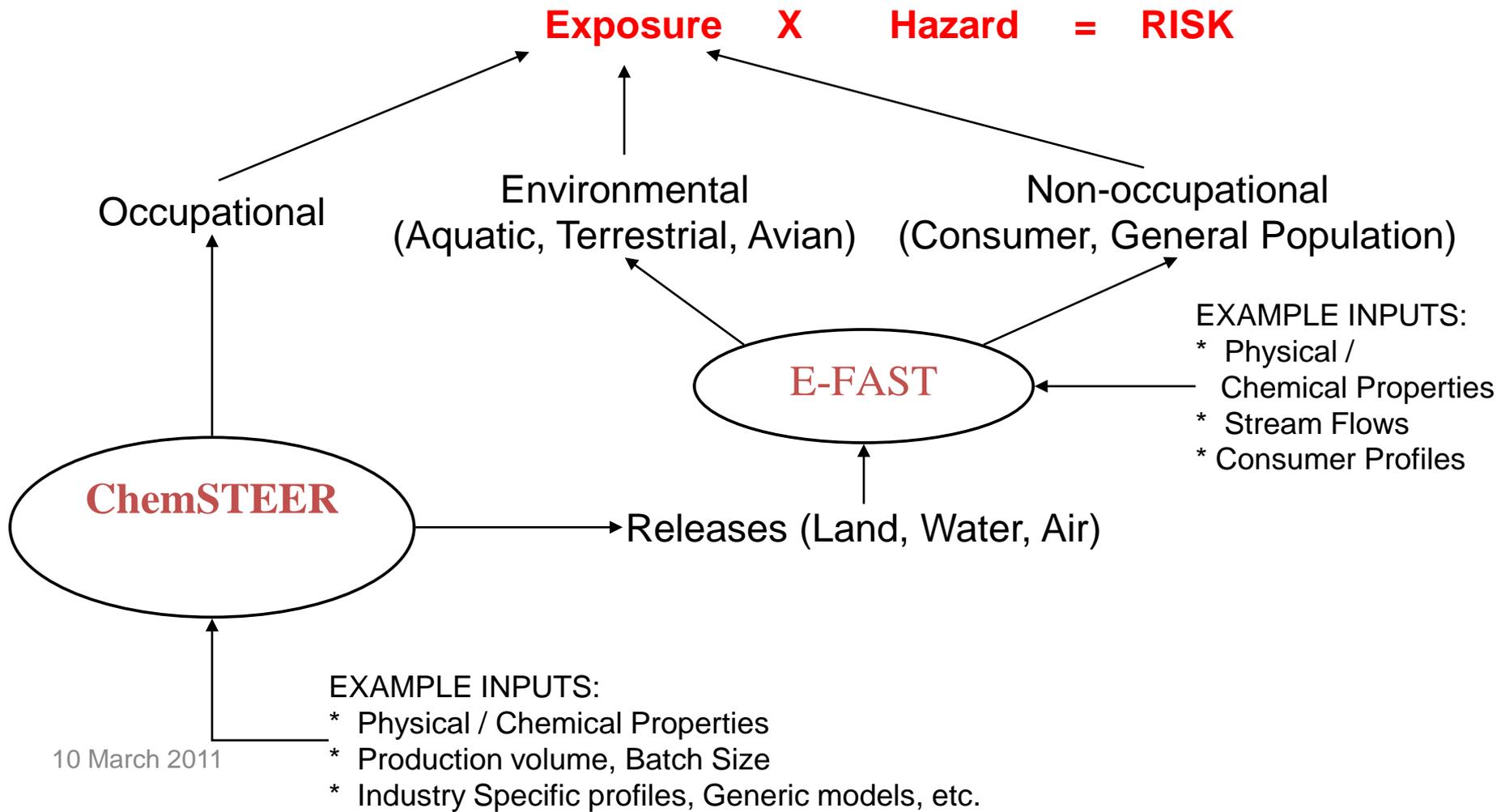
Besides modeling, *in vivo* toxicity studies have been conducted in our laboratory by exposing Sprague–Dawley rats to ultrafine TiO<sub>2</sub> (Degussa, P25) aerosol via inhalation. A dose dependent, systemic microvascular dysfunction was found in rats following the exposure (Nurkiewicz et al., 2008) and the lung burden that produced 50% impairment (ED<sub>50</sub>) was about 10 µg (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<sub>2</sub> 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:**

US EPA <http://www.epa.gov/oppt/exposure/pubs/chemsteer.htm>



# 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

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

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

National Cancer Institute  
U.S. National Institutes of Health | www.cancer.gov



ToxCast™



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



ARTICLE

pubs.acs.org/JACS

Physical–Chemical Aspects of Protein Corona: Relevance to *in Vitro* and *in Vivo* Biological Impacts of Nanoparticles

Marco P. Monopoli,<sup>†‡</sup> Dorota Walczyk,<sup>†</sup> Abigail Campbell,<sup>†</sup> Giuliano Elia,<sup>†</sup> Iseult Lynch,<sup>†</sup> Francesca Baldelli Bombelli,<sup>\*†</sup> and Kenneth A. Dawson<sup>\*†</sup>



LETTERS

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ECOSAR

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

# Charge to Breakout Groups

- Go to either the Human Health or Environment Breakout Groups
  - Health: Remains in Plenary Room
  - Environment: Linder Conference Room, 6<sup>th</sup> 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)