The National Nanotechnology Initiative presents

A Joint US-EU Workshop

March 10 - 11, 2011
Washington, DC

In Vitro - In Vivo Correlations of Dose- and Response-Metrics: Concepts for OEL Extrapolation

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University of Rochester
March 11, 2011
Data Needs for ENM Induced Environmental and Health Effects and Associated Risks

Sources

Exposure Pathways

Receptor Interactions

Risk Assessment

Predictive Models

ENM Synthesis

Characterization – Properties

Life Cycle Releases

From Cradle to Grave

Dispersion, Transformation

Air, Water, Soil, Sediment

Exposure Assessment

Environment; Human

Hazard Characterization

Eco/Human Toxicity

Risk Characterization

Bioinformatics

Predicting nanostructure-induced responses
Concepts of Nanomaterial Toxicity Testing: Risk Assessment as Function of Exposure and Hazard

**Exposure**
- *in vivo*
  - Humans
    - Workplace
    - Laboratory
    - Consumer
  - Phys-chem. Properties
  - Target Organs
  - Respirability
- Phys-chem. Properties
- NOAELs; OELs;
- HECs
- Inhalation; oral; dermal
doosimetry

**Hazard (hazard scale)**
- *in vitro*
  - Bolus; ALI
  - Target cells, Tissues
  - Dose-Response
- Phys-chem. Properties
- Endpoints; Ref. Material
- Mechanisms
- Reproducibility
- pristine; dispersed
doosimetry

**Risk Assessment**
- Long-term
- In silico models
- Long-term goal
- prediction
- extrapolation
- prediction
- prediction
- validation

- *in vivo* Animals (Inhal/Bolus)
  - Biokinetics
  - (translocation; corona formation)
  - Dose-Response

- *in vivo* Animals
  - Phys-chem. Properties
  - Endpoints; Ref. Material
  - Hi-Lo Dose; Relevancy
  - Mechanisms
  - Reproducibility

- *in vitro* Bolus; ALI
  - Target cells, Tissues
  - Dose-Response

- Long-term
- In silico models
“Best” Dosemetric?

Exposure $\longrightarrow$ Dose $\longrightarrow$ Response

$\text{metric?}$  $\text{metric?}$  $\text{metric?}$
## Mass/Number/surface Area Correlations for Selected NPs

<table>
<thead>
<tr>
<th>Particle Type</th>
<th>Diameter (nm)</th>
<th>Density (g/cm³)</th>
<th>Spec. Srf. Area (m²/g)</th>
<th>Specific # number/g</th>
<th>Airborne Conc. of 100 µg/m³</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Surface cm²/m³</td>
</tr>
<tr>
<td>Pt</td>
<td>50</td>
<td>21.09</td>
<td>5.69</td>
<td>7.24 x 10¹⁴</td>
<td>5.69</td>
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<tr>
<td>Gold</td>
<td>50</td>
<td>19.3</td>
<td>6.22</td>
<td>7.91 x 10¹⁴</td>
<td>6.22</td>
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<tr>
<td>Ag</td>
<td>50</td>
<td>7.2</td>
<td>11.43</td>
<td>1.46 x 10¹⁵</td>
<td>11.43</td>
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<tr>
<td>Cu</td>
<td>50</td>
<td>8.9</td>
<td>13.48</td>
<td>1.72 x 10¹⁵</td>
<td>13.48</td>
</tr>
<tr>
<td>Al</td>
<td>50</td>
<td>2.7</td>
<td>44.44</td>
<td>5.65 x 10¹⁵</td>
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<tr>
<td>TiO₂(R)</td>
<td>50</td>
<td>4.23</td>
<td>28.37</td>
<td>3.61 x 10¹⁵</td>
<td>28.37</td>
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<tr>
<td>TiO₂(A)</td>
<td>50</td>
<td>3.9</td>
<td>30.77</td>
<td>3.92 x 10¹⁵</td>
<td>30.77</td>
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<tr>
<td>C</td>
<td>50</td>
<td>2.26</td>
<td>53.10</td>
<td>6.76 x 10¹⁵</td>
<td>53.10</td>
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<tr>
<td>Polystyrene</td>
<td>50</td>
<td>1.05</td>
<td>114.3</td>
<td>1.46 x 10¹⁶</td>
<td>114.3</td>
</tr>
</tbody>
</table>
Usual Physical Dose-Metrics for NPs that Correlate with Biol./Toxicol. Effects:

- Mass
- Number
- Surface Area
- (Volume)

\[ \text{correlation between these should be part of NP characterization} \]

BET Surface Area:

- Which one?
  - N; Kr; Ar; others?

Need for standardization: Most common is use of nitrogen.

- Most desirable: Measure of bioavailable SA

Also: BET equivalent particle size:

- to characterize/estimate agglomeration/aggregation
## Physico-Chemical Properties of Investigated Nanoparticles

<table>
<thead>
<tr>
<th>Particle</th>
<th>Origin</th>
<th>Primary Particle Size (nm) (a)</th>
<th>Crystal Phase</th>
<th>Specific Surface Area (m²/gm)</th>
<th>BET Equivalent Diameter (nm) (b)</th>
<th>Hydrodynamic Diameter (nm) (b)</th>
<th>Zeta Potential (mV) (c)</th>
<th>Agglommm/Aggregation Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elemental Carbon (EC)</td>
<td>Electric Spark Generated (Rochester)</td>
<td>41</td>
<td>Amorphous</td>
<td>767.9</td>
<td>3.4</td>
<td>204</td>
<td>-50.5</td>
<td>High</td>
</tr>
<tr>
<td>TiO₂(F)</td>
<td>Fischer Scientific</td>
<td>250</td>
<td>Anatase</td>
<td>8.0</td>
<td>195</td>
<td>1287</td>
<td>-14.7</td>
<td>Medium</td>
</tr>
<tr>
<td>TiO₂(M)</td>
<td>Millenium Chemical Corp.</td>
<td>~20</td>
<td>Anatase</td>
<td>86.1</td>
<td>18.3</td>
<td>1608</td>
<td>14</td>
<td>High</td>
</tr>
<tr>
<td>TiO₂(D)</td>
<td>Evonik Chemicals</td>
<td>~25</td>
<td>80% anatase/20% rutile</td>
<td>57.4</td>
<td>27</td>
<td>576</td>
<td>27.3</td>
<td>Medium</td>
</tr>
<tr>
<td>Copper-40</td>
<td>Nanotechnologies</td>
<td>40</td>
<td>FCC crystal</td>
<td>30.6</td>
<td>21.9</td>
<td>850</td>
<td>-0.6</td>
<td>Medium</td>
</tr>
<tr>
<td>Silver-35</td>
<td>Nanotechnologies</td>
<td>35</td>
<td>FCC crystal</td>
<td>21.0</td>
<td>27.3</td>
<td>483</td>
<td>-47.0</td>
<td>Medium</td>
</tr>
<tr>
<td>Au₅₀</td>
<td>Ted Pella (Ca)</td>
<td>50</td>
<td>FCC crystal</td>
<td>6</td>
<td>51.8</td>
<td>93</td>
<td>-33.8</td>
<td>Low</td>
</tr>
<tr>
<td>PS-NH₃</td>
<td>Bangs Laboratories</td>
<td>65</td>
<td>Amorphous</td>
<td>88</td>
<td>65</td>
<td>72</td>
<td>83.0</td>
<td>Very low</td>
</tr>
</tbody>
</table>

(a) Size as reported by manufacturer; (b) measured by dynamic light scattering (DLS) in water; (c) measured by electrophoretic light scattering (ELS) in water.
Which Dose-Metric?

Percent of Neutrophils in Lung Lavage 24 hrs after Intratrachael Dosing of Ultrafine and Fine TiO$_2$ in Rats

- fine TiO$_2$ (200nm)
- ultrafine TiO$_2$ (25nm)
- saline

Particle Mass, ug vs Particle Number

Particle Mass vs Particle Number
Which Dose-Metric?

Percent of Neutrophils in BAL 24 hrs after Instillation of TiO$_2$ in Rats

Correlation with Particle Surface Area

- **ultrafine TiO$_2$** (≈25nm)
- **fine TiO$_2$** (≈200nm)
- **saline**

![Graph showing the correlation between particle surface area and percent of neutrophils](image-url)
Percent of Neutrophils in BAL 24 hrs after Instillation

closed symbols: rat  
open symbols: mice

- ultrafine TiO$_2$
- fine TiO$_2$

Particle Surface Area/ gram Lung [cm$^2$/g]
In addition: “Chemical” Dose-Metric, 
*e.g.*, ROS inducing potential:

- **DCFH-DA** *(2’-7’ dichlorofluorescin-diacetate) assay*
- **FRAS** *(ferric reducing ability of serum) assay*
- **Vit C assay**
- others…

*(as screening tool for categorization of NPs based on reactivity [Bello et al., 2009; Rushton et al., 2010]*)
ROS-Inducing Capacity of NanoTiO$_2$ depends on Crystalline State:

- amorphous >
  - anatase >
  - anatase/rutile mixture >
  - rutile
ROS /cm² Response of Anatase TiO₂ in Cell Free Assay as a Function of Particle Size:
Response is size dependent

![Graph showing the equivalent H₂O₂ per surface area as a function of particle size, with a response that increases with decreasing particle size. The graph is labeled with the source: Jiang et al, 2008.]
Maximal ROS /cm² Response to Anatase TiO₂ in Cell Free Assay as a Function of Particle Size

Cell free ROS

Maximal PMN /cm² Response to Anatase TiO₂ in Rats 24 Hours after Intratrachael Instillation as a Function of Particle Size

In vivo inflammation
Cell-free ROS (DCFH oxidation) Response vs In Vivo Rat PMN (Intratracheal Instill)
Response to Nanoparticles
Normalized to Particle Surface Area

**In-vivo inflammation**

**PMN Number/cm²**

- TiO2-D
- TiO2-F
- TiO2-M
- PS-NH3
- Carbon
- Ag-35
- Cu-40
- Au-50

**In vitro Cell-free ROS**

*Rushton et al. 2010*

\[ R^2 = 0.74 \]
\[ P = 0.006 \]
Cell-free ESR (DMPO Spin Trapping) Response vs In Vivo Rat PMN (Intratrach. Instill.)
Response to Nanoparticles
Normalized to Particle Surface Area

In vivo inflammation

# PMN/ cm²

10⁷

10⁶

10⁵

10⁴

10³

10²

10⁰

0.0001 0.001 0.01 0.1 1 10

rel. signal strength (# of spins)/cm²

R²=0.79
p=0.021

TiO₂D-25  Ag-30
TiO₂F-200  Carbon-4
TiO₂M-20   Cu-22
PS-NH₃-65  Au-50
ASSESSING TOXICITY OF FINE AND NANOPARTICLES
Crystalline Silica; Amorphous Silica; Nano Zinc Oxide; Fine Zinc Oxide

in vitro system, lung epithelial cells (Sayes et al., 2007)
In Vivo/In Vitro Correlation, Highest Measured Responses
(Sayes et al, 2007)

In vivo inflammation

In vitro macrophages

MIP-2 (pg/ml)

# PMN x 10^6

NZO

R^2=0.12
P=0.66

FZO

CS

AS

Crystalline Silica

Amorphous Silica

Nano Zinc Oxide

Fine Zinc Oxide

Rushton et al, 2010
Slope (response per unit dose) is Dose Dependent

Rushton et al, 2010

Steepest slope = \frac{y_{i+1} - y_i}{x_{i+1} - x_i}
Comparing responses from different assays:

Select response at corresponding points of dose response curves of assays, normalized to a unit of dose.

Possibilities:

- Steepest slope of linear dose response relationship (Max of derivative)
- \( ED_{50} \) equivalent of log dose response
ASSESSING TOXICITY OF FINE AND NANOPARTICLES
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in vitro system, lung epithelial cells (Sayes et al., 2007)
In Vivo/In Vitro Correlation, Highest Responses Per NP Surface Area

(Sayes et al, 2007)

PMN Number x 10^5/cm^2

In vivo inflammation

MIP-2 x 10^5 (pg/ml)/cm^2

In vitro macrophages

R^2 = 0.92
P = 0.04

Sayes et al, 2007

Rushton et al, 2010

Crystalline Silica
Amorphous Silica
Nano Zinc Oxide
Fine Zinc Oxide
In vitro (A549Luc1 cells) Luciferase vs In Vivo Rat PMN (Intratrach. Instill.) Response to Nanoparticles Normalized to Particle Surface Area

In vivo inflammation

In vitro A549 cells

PMN/cm²

rel. Light Units/cm²

Rushton et al, 2010

R²=0.88
p=0.004
Example of categorizing NPs by a hazard scale or by Reference Particle Equivalent
(based on maximum effect per NP surface, mass or number, as derived from dose-response curves)

<table>
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<th>NP-TYPE</th>
<th>SIZE</th>
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<td>Carbon black</td>
<td>41 nm (aggregated)</td>
<td>Very low</td>
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Example is based on pulmonary inflammatory response in rats (elicited PMN/cm²).
Other in vitro or in vivo endpoints can be selected, e.g., ROS/cm²; LDH/cm²; MN/cm²; Prot.Aggr./cm² …
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Comparing Pulmonary Response to TiO$_2$ NPs in Rats with same dose administered by Instillation or Inhalation:

- *bolus type vs aerosol delivery*

- *high vs. low dose rate*

- “*prepared*” vs. “*pristine*” NPs

- *more central vs. more peripheral lung deposition*
Dispersion as essential step when preparing NPs for toxicity testing using bolus type delivery in vitro and in vivo:

But:

*Does it affect NP properties?*

*Which dispersant?*

*Which sonication system, time and power?*
Deposition per Unit Surface Area (cm$^2$) over 8 Hour Exposure at 100 $\mu$g/m$^3$ (nasal breathing, resting conditions)

Nanoparticle Size: CMD = 20 nm; GSD = 1.0
Density: $\rho = 1$ g/cm$^3$
Dosimetric Extrapolation of Inhaled Particles from Rats to Humans

**Rat**

- Exposure \( [mg(m^3)^{-1}] \)
- Inhaled Dose \( [mg(kg)^{-1}] \)
- Deposited Dose
  - \( [\mu g(cm^2)^{-1}; \mu g(g)^{-1}] \)

**Human**

- Exposure (HEC) \( [mg(m^3)^{-1}] \)
- Inhaled Dose \( [mg(kg)^{-1}] \)
- Deposited Dose
  - \( [\mu g(cm^2)^{-1}; \mu g(g)^{-1}] \)

**Box:**

- Breathing
  - Minute Volume

- Tidal Volume, Resp. Rate
  - Resp. Pause
  - Particle characteristics
  - Anatomy

- Clearance
  - Retention
  - Regional Uptake
    - \( (Metabolism, T_{1/2}) \)

**Retained (Accumulated) Dose**

- \( [\mu g(g)^{-1}; \mu g(cm^2)^{-1}] \)

**Effects**

Assumption: *If retained dose is the same in rats and humans, then effects will be the same*

Oberdörster, 1989
Dosimetric Extrapolation of Inhaled Particles from Rats to Humans

Assumption: If retained dose is the same in rats and humans, then effects will be the same

Oberdörster, 1989
Risk = \( f (hazard; exposure) \)