What Should Trigger the Need for Setting an OEL for an Engineered Nanomaterial (if an OEL Already Exists for the Bulk Form)?

Which nanomaterial properties and dose metrics are relevant for setting OELs?

Gunter Oberdörster
University of Rochester
September 10, 2012
Risk Assessment and Risk Management Paradigm
For Engineered Nanoparticles (NPs)

Hazard Identification

Physico-chemical Parameters!

Adverse NP Effect:
at portal of entry and remote organs

Experimental Animals

Humans

Biokinetics!

Exposure Assessment

Inhalation
Ingestion, Dermal

Biological Monitoring
(markers of exposure)

Occupational/Environmental Monitoring

Risk Management

Public health/social/economical/political consequences

Regulations
Expos. Standards

Prevention/Intervention Measures
Biomed./Engineering

Risk Calculation

Susceptibility Extrapolation Models
(high → low)
(animal → human)

Mechanistic Data

Exposure-Dose-Response Data

In Vivo Studies
(acute; chronic)

In Vitro Studies
(non-cellular)
(animal/human cells)
(subcellular distribution)

Dose-Metric!

Modified from Oberdörster et al., 2005
Risk Assessment and Risk Management Paradigm
For Engineered Nanoparticles (NPs)

Hazard Identification

Adverse NP Effect: at portal of entry and remote organs

Experimental Animals

Humans

Physico-chemical Parameters!

Biokinetics!

Exposure Assessment

Inhalation
Ingestion, Dermal

Biological Monitoring
(markers of exposure)

Occupational/Environmental Monitoring

Risk Management

Expos. Standards

Public health/social/economical/political consequences

Regulations

Prevention/Intervention Measures
Biomed./Engineering

Risk Characterization

Dose-Metric!

Exposure-Dose-Response Data

In Vivo Studies
(acute; chronic)

In Vitro Studies
(non-cellular)
(animal/human cells)
(subcellular distribution)

Risk Calculation

Susceptibility Extrapolation Models
(high → low)
(animal → human)

Mechanistic Data

Modified from Oberdörster et al., 2005
Physico-chemical NP Properties of Relevance for Toxicology

Size \((aerodynamic, \ hydrodynamic)\)
Size distribution
Shape
Agglomeration/aggregation
Density \((material, \ bulk)\)
Surface properties:
- area \((porosity)\)
- charge
- reactivity
- chemistry \((coatings, \ contaminants)\)
- defects
Solubility/Sol-Rate \((lipid, \ aqueous, \ in \ vivo)\)
Crystallinity

Properties can change
- with: method of production preparation process storage
- when introduced into physiol. media, organism

Key parameter: Dose!
Physical Dose-Metrics for NPs that Correlate with Biol./Toxicol. Effects:

Mass
Number
Surface (area; reactivity)
Volume

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: Definition of bioavailable SA

Also: BET equivalent particle size:
to characterize agglomeration and aggregation
In Addition: “Chemical” Dose-Metric,
e.g., ROS inducing potential in cell-free medium:

- **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]*)
## 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>
</tr>
</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>7.24x10¹⁴</td>
<td>5.69</td>
</tr>
<tr>
<td>Gold</td>
<td>50</td>
<td>19.3</td>
<td>6.22</td>
<td>7.91x10¹⁴</td>
<td>6.22</td>
</tr>
<tr>
<td>Ag</td>
<td>50</td>
<td>7.2</td>
<td>11.43</td>
<td>1.46x10¹⁵</td>
<td>11.43</td>
</tr>
<tr>
<td>Cu</td>
<td>50</td>
<td>8.9</td>
<td>13.48</td>
<td>1.72x10¹⁵</td>
<td>13.48</td>
</tr>
<tr>
<td>Al</td>
<td>50</td>
<td>2.7</td>
<td>44.44</td>
<td>5.65x10¹⁵</td>
<td>44.44</td>
</tr>
<tr>
<td>TiO₂(R)</td>
<td>50</td>
<td>4.23</td>
<td>28.37</td>
<td>3.61x10¹⁵</td>
<td>28.37</td>
</tr>
<tr>
<td>TiO₂(A)</td>
<td>50</td>
<td>3.9</td>
<td>30.77</td>
<td>3.92x10¹⁵</td>
<td>30.77</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>2.26</td>
<td>53.10</td>
<td>6.76x10¹⁵</td>
<td>53.10</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>50</td>
<td>1.05</td>
<td>114.3</td>
<td>1.46x10¹⁶</td>
<td>114.3</td>
</tr>
</tbody>
</table>

*consider also packing or bulk density
Morrow Hypothesis: Lung particle overload associated impairment of AM clearance function correlates with phagocytized particle volume.
10.3 μm particle phagocytised in vivo by rat alveolar macrophage
Lung Retention of Intratracheally-Instilled $3.3 \, \mu m$ and $10.3 \, \mu m$ Particles in the Rat

($^{141}$Ce-labelled and $^{95}$Nb-labelled)

$3.3 \, \mu m$: $T_{1/2} = 80$ days

$10.3 \, \mu m$: $T_{1/2} = 1020$ days

Oberdörster et al, 1992
**Lung Particle Overload: Which dosemetric?**

*AM-mediated Particle Clearance*

**12-Week Inhalation Exposure, Ultrafine and Fine TiO₂ and Cristobalite**

<table>
<thead>
<tr>
<th>Test Particle Retention</th>
<th>Mass (µg)</th>
<th>Volume (nl)</th>
<th>% of AM Volume (%)</th>
<th>Surface (cm²)</th>
<th>Number x 10⁹</th>
<th>Retained dose/10⁶ AM at end of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TiO₂ fine (250 nm)</td>
<td>340</td>
<td>90</td>
<td>9</td>
<td>21.9</td>
<td>10.9</td>
<td>1.8*</td>
</tr>
<tr>
<td>TiO₂ ultrafine (25 nm)</td>
<td>99.8</td>
<td>26</td>
<td>2.6</td>
<td>49.9</td>
<td>5420</td>
<td>8.2*</td>
</tr>
<tr>
<td>Cristobalite</td>
<td>~20</td>
<td>7.6</td>
<td>0.76</td>
<td>2.4</td>
<td></td>
<td>28.8*</td>
</tr>
</tbody>
</table>

*Significantly different from control

*Oberdörster et al, 1994*
Lung Particle Overload: Which dosemetric?

*AM-mediated Particle Clearance*

12-Week Inhalation Exposure, Ultrafine and Fine TiO$_2$ and Cristobalite

<table>
<thead>
<tr>
<th>Retained dose/10$^6$ AM at end of exposure</th>
<th>Mass</th>
<th>Volume</th>
<th>Surface</th>
<th>Number</th>
<th>Test Particle Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg</td>
<td>nl</td>
<td>% of AM volume</td>
<td>cm$^2$</td>
<td>x 10$^{-9}$</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TiO$_2$ fine (250 nm)</td>
<td>340</td>
<td>90</td>
<td>9</td>
<td>21.9</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>(578)</td>
<td>(58)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TiO$_2$ ultrafine (25 nm)</td>
<td>99.8</td>
<td>26</td>
<td>2.6</td>
<td>49.9</td>
<td>5420</td>
</tr>
<tr>
<td></td>
<td>(768)</td>
<td>(77)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cristobalite</td>
<td>~20</td>
<td>7.6</td>
<td>0.76</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(24)</td>
<td>(2.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from control (packing density volume)

Oberdörster et al, 1994
Correlation between surface area of TiO$_2$ particles phagocytized by AM and pulmonary retention half-time of inhaled polystyrene test particles

From: Oberdörster et al., 1994
**In vitro** *(A549Luc1 cells)* Luciferase vs In Vivo Rat PMN *(Intratrach. Instill.)*

Response to Nanoparticles
Normalized to Particle Surface Area

\[ R^2 = 0.76 \]
\[ p = 0.005 \]

Rushton et al. 2010

---

**Graph:**
- **Y-axis:** In vivo inflammation
- **X-axis:** Relative Light Units/cm²
- **Legend:**
  - TiO₂D-25
  - TiO₂M-20
  - TiO₂F-200
  - PS-NH₃-65
  - Ag-30
  - Carbon-4
  - Cu-22
  - Au-50

---

**In vitro** A549 cells
### 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>
<thead>
<tr>
<th>NP-TYPE</th>
<th>SIZE</th>
<th>HAZARD CATEGORY</th>
<th>HAZARD RANKING per cm² (Carbon Black = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon black</td>
<td>41 nm (aggregated)</td>
<td>Very low</td>
<td>1</td>
</tr>
<tr>
<td>TiO₂ (anat.)</td>
<td>20 nm (aggregated)</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>60 nm (positive charge)</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>TiO₂ (anat./rut.)</td>
<td>25 nm (aggreg/agglomer)</td>
<td>Low</td>
<td>11</td>
</tr>
<tr>
<td>Au</td>
<td>50 nm</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Ag</td>
<td>35 nm (aggreg/agglomer)</td>
<td>High</td>
<td>80</td>
</tr>
<tr>
<td>Cu</td>
<td>40 nm (aggreg/agglomer)</td>
<td>Very high</td>
<td>620</td>
</tr>
</tbody>
</table>

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 …
### 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>
<thead>
<tr>
<th>NP-TYPE</th>
<th>SIZE</th>
<th>HAZARD CATEGORY</th>
<th>HAZARD RANKING per cm² (Carbon Black = 1)</th>
<th>per gram</th>
<th>per 1.33 x 10¹⁵ particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon black</td>
<td>41 nm (aggregated)</td>
<td>Very low</td>
<td>1</td>
<td>6.1</td>
<td>19.3</td>
</tr>
<tr>
<td>TiO₂ (anat.)</td>
<td>20 nm (aggregated)</td>
<td></td>
<td>5</td>
<td>3.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>60 nm (positive charge)</td>
<td></td>
<td>9</td>
<td>6.3</td>
<td><strong>1</strong></td>
</tr>
<tr>
<td>TiO₂ (anat./rut.)</td>
<td>25 nm (aggreg/agglom)</td>
<td>Low</td>
<td>11</td>
<td>5.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Au</td>
<td>50 nm</td>
<td></td>
<td>21</td>
<td><strong>1.0</strong></td>
<td>2.1</td>
</tr>
<tr>
<td>Ag</td>
<td>35 nm (aggreg/agglom)</td>
<td>High</td>
<td>80</td>
<td>13.3</td>
<td>12.2</td>
</tr>
<tr>
<td>Cu</td>
<td>40 nm (aggreg/agglom)</td>
<td>Very high</td>
<td>620</td>
<td>151</td>
<td>508</td>
</tr>
</tbody>
</table>

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 …
Two Subchronic MWCNT Inhalation Studies in Rats

Inhalation Toxicity of Multiwall Carbon Nanotubes in Rats Exposed for 3 Months

Lan Ma-Hock,* Silke Treumann,* Volker Strauss,* Sandra Brill,* Frederic Luizi,† Michael Mertler,‡ Karin Wiench,* Armin O. Gamer,* Bennard van Ravenzwaay,* and Robert Landsiedel*

*Product Safety, BASF SE, 67056 Ludwigshafen, Germany; †Nanocyl S. A., 5060 Sambreville, Belgium; and ‡Process Engineering, BASF SE, 67056 Ludwigshafen, Germany
TOXICOLOGICAL SCIENCES 112(2), 468–481 (2009)

Subchronic 13-Week Inhalation Exposure of Rats to Multiwalled Carbon Nanotubes: Toxic Effects Are Determined by Density of Agglomerate Structures, Not Fibrillar Structures

Jürgen Pauluhn¹

Department of Inhalation Toxicology, Institute of Toxicology, Bayer Schering Pharma, Building Number 514, 42096 Wuppertal, Germany

TOXICOLOGICAL SCIENCES 113(1), 226–242 (2010)
90 - Day Inhalation, Rats: MWCNT, CB, SiO₂, Ni₃S₂, TiO₂

Percent Increase of Lung Weight Above Controls

MWCNT (Pauluhn 2010)
MWCNT (MaHock et al 2009)
Carbon Black (Elder, et al, 2005)
Ni₃S₂ (Oberdörster, unpub.data)

SiO₂ (Crist)
nano TiO₂ (Oberdörster, et al, 1994)
micro TiO₂

As Function of Exposure Concentration

Exposure Concentration, mg/m³
Lung weight, % increase

As Function of Retained Lung Burden (Mass)
Retained Lung Burden, mg
Lung weight, % increase

As Function of Retained Particle Surface Area
Retained Particle Surface Area, cm²
Lung weight, % increase

As Function of Retained Lung Burden (Volume)
(based on bulk density)
Retained Particle Volume, nl
Lung weight, % increase

Carbon Black 77%
@ 32,440 nl
nano TiO₂ 16%
@ 40,000 nl
Hazard Ranking of Different (Nano)-Materials Based on Different Metrics and Steepest Slope of Exposure-Dose-Response Relationships from Subchronic Rat Inhalation Studies (endpoint: lungweight increase)

Three Hazard Groupings:

**Low:** $CB; TiO_2 \rightarrow < 0.3 \% \ lungwt. \ incr./cm^2$

**Medium:** $MWCNT \rightarrow 0.3 – 1 \% \ lungwt. \ incr./cm^2$

**High:** $SiO_2; Ni_3S_2 \rightarrow > 1 \% \ lungwt. \ incr./cm^2$
Risk = f (hazard; exposure)
Multiple Path Particle Deposition Model (MPPD)

B. Asgharian et al. (ARA Inc./Hamner/CIIT) and
F. Cassee et al. (RIVM, Netherlands)

Human Model and Rat Model

5-Lobar Yeh-Schum Model; Stochastic Model; Age Specific Model,
Asymmetric Multiple Path

Particle Parameter: MMAD; MMD; CMD; GSD; Concentration

Inhalability Adjustment; Nanoparticle Model; Gravity

Different Body Orientations

Tidal Volume; Breathing Frequency; Inspiratory Fraction and Pause

Breathing Scenario: Nasal; Oral; Oro-Nasal; Endotracheal

Deposition only; Deposition and Clearance

Bronchial and slow, medium, fast Alveolar Clearance

Exposure Times and Duration; Post Exposure Time
“Best” Dose-Metric…
…may depend on objective and practicality:

Exposure:
monitoring, workplace (OEL) \(\rightarrow\) number concentration, mass concentration \(\begin{cases} \text{size and} \\ \text{distribution} \end{cases} \)

and experimental

Toxicology:
dosimetry \(\rightarrow\) mass/organ, or /tissue
microdosimetry \(\rightarrow\) number, mass, surface, volume/cell

Comparing responses, mechanisms, hazard:
surface-related metric:
area, reactivity, charge ....

Consider also Response-Metric,
activity or response per unit dose – e.g., surface area