Strategies for Setting Occupational Exposure Limits for Engineered Nanomaterials

Setting OELs Using an In Vitro Parallelogram Approach

Mark S. Maier, PhD, MSEH, DABT
Valspar, Pittsburgh PA
What is the only thing that most nanomaterials have in common?

The "NANO" BRAND
This talk is really about...

Using In Vitro Read-Across to Set OELs for Nano-scale Thingamajigs & Doohickies

Doohicky
With Mounting Bracket

Thingamajig
With Mounting Brackets
Occupational Risk Assessment Requires the Entire Dataset

- Biomarkers
- Functional Genomics
- Bioinformatics
- Systems biology
- PK & PBPK models
- OEB / OEL
- Hazard + Dose-Response
- High-throughput screens
- Stem cell biology
- In silico Systems Biology
- Structure Activity
How much toxicity data are there?

Does hazard and dose-response information exist for the intermediate?

Yes

Develop OEL

Limited

No

Identify hazard using structure-activity and computer methods

Choose relevant in vitro assay

Compare in vitro responses of data-rich and unstudied molecules using parallelogram approach

Develop OEB
**Usually the best you can do is an OEB**

**Occupational Exposure Bands (OEBs)**

**EXPOSURE CONTROL BAND FRAMEWORK**

<table>
<thead>
<tr>
<th>ECB 1</th>
<th>ECB 2</th>
<th>ECB 3</th>
<th>ECB 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONTROL CATEGORY 1</strong></td>
<td><strong>CONTROL CATEGORY 2</strong></td>
<td><strong>CONTROL CATEGORY 3</strong></td>
<td><strong>CONTROL CATEGORY 4</strong></td>
</tr>
<tr>
<td>Low Bioactivity or Toxicity</td>
<td>Moderate Bioactivity or Toxicity</td>
<td>High Bioactivity or Toxicity</td>
<td>Very High Bioactivity or Toxicity</td>
</tr>
<tr>
<td>Open handling</td>
<td>Special hazard</td>
<td>Low dose effects</td>
<td>Very low dose effects</td>
</tr>
<tr>
<td>Standard lab PPE</td>
<td>Additional PPE</td>
<td>Reversible effects</td>
<td>Life threatening</td>
</tr>
<tr>
<td>Exposure monitoring</td>
<td></td>
<td>Non-life threatening</td>
<td>Non-reversible effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Compounds without data</td>
<td>Closed handling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Handling restrictions</td>
<td>Destruction method required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ventilation requirements</td>
<td></td>
</tr>
</tbody>
</table>
In Cerebro Assessment

- Define the nano widget
- Define the hazard
- Identify a positive control
- Define relevant test doses
Define the Nano-Widget

- Well Characterized?
- Inorganic?
- Metal?
- Organic?
- Biological?
- Behavior in air?
- Filterability?
- Fiber shape/ratios?
- Inert?
- Metabolized?
- Immunogenic?
- Electrophilic?
- Nucleophilic?
- Lipophilic?
- Water soluble?
- Bioaccumulate?
- Biopersist?
Test Methods to Define the Hazard

Which test systems are good enough?

Human Relevance/ Cost/Complexity

Throughput/ Simplicity
Identify a Positive Control

Data-rich Compound

In vitro

In vivo

Unstudied Intermediate

OEL

Exposure Limit

OEB

Read-Across Parallelogram
Identify a Positive Control

Straight fiber or particle: Amphibole asbestos may be a good positive control

Ludwig Limbach et al. Swiss Federal Institute of Technology
In Vitro Cytotoxicity Studies of Oxides Nanoparticles and Comparison to Asbestos.
Human mesothelioma and rodent fibroblasts.
For modified nanomaterials, the starting material may be a good positive control.
Define Relevant Dose

In vitro concentration = f (exposure limit)

\[ NC = \frac{OEL \times BR \times ET}{MW \times ASL \times 1000} \]

NC = Nominal media concentration (mM)
OEL = Desired occupational exposure limit (µg/m3)
BR = Breathing rate (25 L/min)
ET = Exposure time (480 min)
MW = Molecular weight of test material (mg/mmol)
ASL = Airway surface liquid volume (47.3 mL)
In vitro test for direct lung cytotoxicity

\[ NC = \frac{OEL \times BR \times ET}{MW \times ASL \times 1000} \]

<table>
<thead>
<tr>
<th>OEL (ug/m³)</th>
<th>Quantity inhaled (ug)</th>
<th>Concentration in lung fluid (ug/ml)</th>
<th>(\beta)-CD MW 1135</th>
<th>(\beta)-CD Int 1 MW 1413</th>
<th>(\beta)-CD Int 2 MW 1355</th>
<th>(\beta)-CD Int 3 MW 1253</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>2.54E-01</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.0002</td>
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<tr>
<td>5</td>
<td>60</td>
<td>1.27E+00</td>
<td>0.0011</td>
<td>0.0009</td>
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<tr>
<td>10</td>
<td>120</td>
<td>2.54E+00</td>
<td>0.0022</td>
<td>0.0018</td>
<td>0.0019</td>
<td>0.0020</td>
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<tr>
<td>30</td>
<td>360</td>
<td>7.61E+00</td>
<td>0.0067</td>
<td>0.0054</td>
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<td>0.0061</td>
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<tr>
<td>50</td>
<td>600</td>
<td>1.27E+01</td>
<td>0.0112</td>
<td>0.0090</td>
<td>0.0094</td>
<td>0.0101</td>
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<tr>
<td>70</td>
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<tr>
<td>100</td>
<td>1200</td>
<td>2.54E+01</td>
<td>0.0224</td>
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<td>0.0187</td>
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</tr>
<tr>
<td>500</td>
<td>6000</td>
<td>1.27E+02</td>
<td>0.1118</td>
<td>0.0898</td>
<td>0.0936</td>
<td>0.1012</td>
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<tr>
<td>800</td>
<td>9600</td>
<td>2.03E+02</td>
<td>0.1788</td>
<td>0.1436</td>
<td>0.1498</td>
<td>0.1619</td>
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<tr>
<td>3000</td>
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<td>7.61E+02</td>
<td>0.6706</td>
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<td>0.5618</td>
<td>0.6073</td>
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<tr>
<td>5000</td>
<td>60000</td>
<td>1.27E+03</td>
<td>1.1176</td>
<td>0.8976</td>
<td>0.9363</td>
<td>1.0122</td>
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<tr>
<td>8000</td>
<td>96000</td>
<td>2.03E+03</td>
<td>1.7882</td>
<td>1.4361</td>
<td>1.4981</td>
<td>1.6195</td>
</tr>
</tbody>
</table>
Human Engineered Tissue

Test compounds No
DMSO needed

1.5 months in culture

MucilAir™
Reconstructed tracheal-bronchial epithelium
In vitro test for cell membrane integrity

Trans-Epithelial Electrical Resistance

TEER (ohm cm²)

- β-CD
- Int 1
- Int 2
- Int 3

Concentrations: 0.01 μM, 0.1 μM, 1 μM, 10 μM, 100 μM
In vitro test for cell viability
**In vitro test for observed lung toxicity**

<table>
<thead>
<tr>
<th></th>
<th>Estimated EC$_{50}$ (Resazurin test)</th>
<th>Concentration that affect the Tissue Integrity (Tli)</th>
<th>Concentration that affect the Morphology</th>
<th>Concentration that affect the cilia beating</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD</td>
<td>100 mM &lt; EC$_{50}$</td>
<td>10 mM &lt; Tli &lt; 100 mM</td>
<td>10 mM &lt;</td>
<td>100 mM &lt;</td>
</tr>
<tr>
<td>Int 1 CDD</td>
<td>EC$_{50}$ = 100 mM</td>
<td>10 mM &lt; Tli &lt; 100 mM</td>
<td>10 mM &lt;</td>
<td>100 mM &lt;</td>
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<tr>
<td>Int 2 CDDI</td>
<td>10 mM &lt; EC$_{50}$ &lt; 100 mM</td>
<td>1 mM &lt; Tli &lt; 10 mM</td>
<td>1 mM &lt;</td>
<td>10 mM &lt;</td>
</tr>
<tr>
<td>Int 3 CDDA</td>
<td>EC$_{50}$ ≈ 100 mM</td>
<td>10 mM &lt; Tli &lt; 100 mM</td>
<td>10 mM &lt;</td>
<td>100 mM &lt;</td>
</tr>
</tbody>
</table>

**Conclusion:**

*Int 2 is toxic at a concentration one-order-of-magnitude lower than β-CD*
Read Across

\[
\text{NOEL 10 \mu M} \quad \text{NDC} \quad \text{OEL = 500 \mu m}^3
\]

\[
\text{NOEL 1 \mu M} \quad \text{10X < NDC} \quad \text{OEB 10 - 100 \mu m}^3
\]

\text{Intermediate 3}

\text{In vitro} \quad \text{In vivo} \quad \text{Exposure Limit}

\beta\text{-cyclodextrin}
OEB Assignment

OEB 2 vs OEB 3
Asthmagenicity

Fluorescent antibodies
Anti-Interleukin 8