Impact of Nanoparticulates On Respiratory Health Effects: Toxicity Is Not Always Dependent Solely Upon Particle Size and Surface Area

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DuPont Haskell Laboratory
Newark, DE

Nanotechnology and OSWER: New Opportunities and Challenges
July 12, 2006

Outline

• Lung structure and particle deposition
• Pulmonary bioassay as a measure of lung toxicity- Hazard Assessment
• Pulmonary bioassay with Fine/Nanoscale TiO₂ dots and rods; Fine/Nanoscale Quartz particles, and Fine/Nanoscale ZnO particles
• Impacts of Particle Surface Coatings
• Summary
Definitions- Particle Size

- Nano = Ultrafine = < 100 nm
- Fine = 100 nm - 3 μm
- Respirable (rat) = < 3 μm (max = 5 μm)
- Respirable (human) = < 5 μm (max = 10 μm)
- Inhalable (human) = ~ 10 - 100 μm

Rat Lung Microdissection
Rat Lung Tissue Dissected to Demonstrate the Junction of the Terminal Airway and Proximal Alveolar Region

Iron Particle Deposition at Bronchoalveolar Junction
Iron Particle Deposition at Bronchoalveolar Junction
(Backscatter Image)

Alveolar Macrophage Clearance of Inhaled Iron Particles
Alveolar Macrophage Clearance of Inhaled Iron Particles
(Backscatter Image)

Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis
Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis

(Backscatter Image)

Clearance of Iron Particles on the Airway Mucociliary Escalator
Clearance of Iron Particles on the Airway Mucociliary Escalator

Morphometry at Bronchoalveolar Junctions
Common Perceptions on Pulmonary Toxicity of Nanoparticles

- Nanoparticles are more toxic (inflammogenic, tumorigenic) than fine-sized particles of identical composition.
- Concept generally based on 3 particle-types:
  - Titanium Dioxide particles
  - Carbon Black particles
  - Diesel Particles

Complications related to the Dogma of Nanoparticulate Toxicology

- Not all Nanoparticles are more toxic
- Surface coatings of particles
  - Coatings - passivated or dispersion
- Species Differences in Lung Responses
  - Rat is the most sensitive species
- Particle aggregation/disaggregation potential
- Fumed vs. precipitated Nanoparticles
- Surface charge of particles
The Key Issue: Risk

Health Risk is a product of
• Hazard and Exposure

Studies to Assess Pulmonary Hazards to Nanoparticulates
Pulmonary Bioassay Studies

- Working hypothesis
- Four factors influence the development of pulmonary fibrosis
  1) inhaled materials which cause cell/lung injury
  2) inhaled materials which promote ongoing inflammation
  3) inhaled materials which reduce alveolar macrophage function
  4) inhaled materials which persist in the lung

Pulmonary Bioassay Components

**Bronchoalveolar Lavage Assessments**

- Lung Inflammation & Cytotoxicity
  - Cell Differential Analysis
  - BAL Fluid Lactate Dehydrogenase (cytotoxicity)
  - BAL Fluid Alkaline Phosphatase (epithelial cell toxicity)
  - BAL Fluid Protein (lung permeability)

**Lung Tissue Analysis**

- Lung Weights
- Lung Cell Proliferation (BrdU)
  - Parenchymal
  - Airway
- Lung Histopathology
Cytocentrifuge Preparation of BAL – Recovered Cells From a Sham – Exposed Rat

Cytocentrifuge Preparation of BAL – Recovered Cells From a Quartz (Crystalline Silica) – Exposed Rat
Cytocentrifuge Preparation of BAL – Recovered Cells From a Carbonyl Iron – Exposed Rat

Parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALF Cells and Differentials</td>
<td>Lung Inflammation</td>
</tr>
<tr>
<td>BALF Lactate Dehydrogenase</td>
<td>Non-specific cytotoxicity</td>
</tr>
<tr>
<td>BALF Alkaline Phosphatase</td>
<td>Type 2 cell epithelial toxicity</td>
</tr>
<tr>
<td>BALF Protein</td>
<td>Permeability ↑ of alveolar/capillary barrier</td>
</tr>
<tr>
<td>Lung Weights</td>
<td>Pulmonary edema or fibrosis</td>
</tr>
<tr>
<td>Macrophage phagocytosis</td>
<td>Lung clearance functions</td>
</tr>
<tr>
<td>Cell Proliferation</td>
<td>Inflammation/lung fibrosis and tumor potential</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Evaluation of lung tissue responses</td>
</tr>
</tbody>
</table>
**Pulmonary Bioassay Bridging Studies**

![Diagram of studies](image)

- **Inhalation Studies**
  - Fine TiO2 Particles
  - Quartz Particles

- **Intratracheal Instillation Studies**
  - PBS Tween Sham
  - Fine TiO2 Particles vs Quartz Particles
  - TiO2 Nanorods/Nanodots

**Collaborative Studies with Rice University – CBEN - Vicki Colvin and Christie Sayes on the Pulmonary Toxicity of Nanoscale TiO2 and Quartz Particle-types**
Pulmonary Instillation Studies with Nanoscale TiO$_2$ Rods and Dots in Rats: Toxicity is not Dependent upon Particle Size and Surface Area

DB Warheit, TR Webb CM Sayes, VL Colvin and KL Reed

• Toxicological Sciences 91:227-236, 2006
Protocol for Nanoscale TiO2 Pulmonary Bioassay Study

Intratracheal Instillation Exposure Doses of 1 and 5 mg/kg

Exposure Groups
- PBS (control)
- Particulate Types (1 and 5 mg/kg)
  - Fine-sized TiO2 particles
  - Nanoscale TiO2 rods
  - Nanoscale TiO2 dots
  - Quartz Particles (positive control)

Postexposure Evaluation via BAL and Lung Tissue

24 hr  1 wk  1 mo  3 mo

TiO2 Nanoscale Dots

TiO2 Dots ("RHT-137")
**TiO2 Nanoscale Rods**

![TiO2 Rods (Chem_EE)](image)

- **200 nm**

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**TiPure® R-100**

![TiPure R-100](image)

- **0.20 µ**
Characterization of Nanoscale TiO\(_2\) and Quartz Particles

<table>
<thead>
<tr>
<th>XRD</th>
<th>particle size</th>
<th>Surface Area</th>
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<tbody>
<tr>
<td>Fine TiO(_2) rutile</td>
<td>(d_{50} = 300) nm</td>
<td>6.0 m(^2)/g</td>
</tr>
<tr>
<td>TiO(_2) Nanorods anatase</td>
<td>length= 90 - 233 nm &lt;br&gt;width = 20 – 35 nm</td>
<td>26.5 m(^2)/g</td>
</tr>
<tr>
<td>TiO(_2) Nanodots anatase</td>
<td>(d_{50} = 6) nm</td>
<td>169.4 m(^2)/g</td>
</tr>
<tr>
<td>Min-U-Sil (\alpha Q)</td>
<td>(d_{50} = 1.3) (\mu)m</td>
<td>4.0 m(^2)/g</td>
</tr>
</tbody>
</table>

RESULTS

Biomarkers =

Pulmonary Inflammation
Pulmonary Cytotoxicity
Collaborative Studies with Rice University: TiO₂

Percent Neutrophils in BAL Fluids of Rats Exposed to TiO₂ Particles, Nano-rods, and Nano-dots

Pigmentary & Nano-TiO₂ are not different

BAL Fluid LDH Values In Rats Exposed to TiO₂ Particles, Nano-rods, and Nano-dots
Second Nanoscale TiO2 Study

Pulmonary Inflammation

Percent Neutrophils in BAL Fluids of Rats exposed to Fine and Nano-sized TiO2 Particulates
(Second Study)

Exposure Groups
- 24 Hour
- 1 Week
- 1 Month
- 3 Month
Characterization of Nanoscale TiO$_2$ and Quartz Particles

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<td>$d_{50} = 1.3$ µm</td>
<td>$4.0$ m$^2$/g</td>
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Relationship of Pulmonary Inflammation to TiO$_2$
Particle Mass dose at 24 hrs PE
(Oberdorster$^3$ EHP, 2005)
Relationship of Pulmonary Inflammation to TiO$_2$
Particle Surface Area at 24 hrs PE Oberdorster$^3$
(EHP, 2005)

Relationship of Pulmonary Inflammation to TiO$_2$
Particle Mass dose at 24 hrs PE
(Oberdorster$^3$ EHP, 2005) + (Haskell)
Hypothesis and a Question

- **Hypothesis:** At similar doses - Ultrafine (Nano) particles have greater pulmonary toxicity than fine-sized particles of identical composition.

- **Question** – generally this dogma applies to low toxicity particulates. However, in considering a cytotoxic particle such as crystalline silica – would nanoquartz particles be even more toxic than fine-sized Min-U-Sil quartz particles?
Nanoscale Quartz

Physicochemical Characterization of Quartz Particulates

A, B, C: Images of nanoquartz I, nanoquartz II, and fine quartz, respectively, showing the size distribution.

D: Graph showing the 2θ values for nanoquartz I and nanoquartz II.

E: Graph showing the weight loss as a function of temperature for nanoquartz I and nanoquartz II.
Physicochemical Characterization of Quartz Particulates (cont.)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average Size (nm)</th>
<th>Size Range (nm)</th>
<th>Surface Area (m²/g)</th>
<th>Crystallinity</th>
<th>ICP-AES (% Fe content)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nano quartz I</td>
<td>50</td>
<td>30-65</td>
<td>31.4</td>
<td>α-quartz</td>
<td>0.080%</td>
</tr>
<tr>
<td>nano quartz II</td>
<td>12</td>
<td>10-20</td>
<td>90.5</td>
<td>α-quartz</td>
<td>0.034%</td>
</tr>
<tr>
<td>fine quartz</td>
<td>300</td>
<td>100-500</td>
<td>4.2</td>
<td>α-quartz</td>
<td>0.011%</td>
</tr>
<tr>
<td>Min-U Sil</td>
<td>534</td>
<td>300-700</td>
<td>5.1</td>
<td>α-quartz</td>
<td>0.042%</td>
</tr>
</tbody>
</table>

Nanoscale Quartz Particles

Q1 Characterization

Nanoquartz
Diameter: 50 nm

Intensity (arbitrary units)

2-theta (degrees)
Preliminary Collaborative Studies with Rice University: SiO₂

Percent Neutrophils In BAL Fluids of Rats Exposed to Min-U-Sil and Nano Quartz Particles

Nano-SiO₂ is lower than Min-U-Sil

BAL Fluid LDH Values In Rats Exposed to Min-U-Sil and Nano Quartz Particles
**Characterization of Quartz Particles**

<table>
<thead>
<tr>
<th>XRD particle size</th>
<th>Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoscale-Q I</td>
<td>αQ d₅₀ = 50 nm</td>
</tr>
<tr>
<td>Min-U-Sil</td>
<td>αQ d₅₀ = 534 µm</td>
</tr>
</tbody>
</table>

**Second Nanoscale Quartz Study**
**Protocol for Second Nano quartz Pulmonary Bioassay Study**

Intratracheal Instillation Exposure Doses of 1 and 5 mg/kg

**Exposure Groups**
- PBS (vehicle control)
- Particulate Types (1 and 5 mg/kg)
  - Carbonyl Iron Particles (negative control)
  - Min-U-Sil Quartz Particles (534 nm)
  - Nano Quartz II Particles (12 nm)
  - Fine Quartz Particles (300 nm)

**Instillation Exposure**

**Postexposure Evaluation via BAL and Lung Tissue**

- 24 hr
- 1 wk
- 1 mo
- 3 mo

**Follow-up Collaborative Studies with Rice University: SiO₂**

BAL Fluid LDH Values in Rats Exposed to Fine and Nanoquartz Particulates (Rice 2)

Nano-SiO₂ is ≥ or = to Min-U-Sil
Pulmonary Inflammation

Percent Neutrophils in BAL Fluids of Rats exposed to Fine and Nano-sized Quartz Particles (Study #2)

BAL Fluid LDH Values (cytotoxicity)
Characterization of Quartz Particles

<table>
<thead>
<tr>
<th>XRD particle size</th>
<th>Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine Quartz</td>
<td>$\alpha Q \ d_{50} = 300 \text{ nm}$</td>
</tr>
<tr>
<td>Nanoscale-Q II</td>
<td>$\alpha Q \ d_{50} = 12 \text{ nm}$</td>
</tr>
<tr>
<td>Min-U-Sil</td>
<td>$\alpha Q \ d_{50} = 534 \text{ nm}$</td>
</tr>
</tbody>
</table>

CI – 2B-3M – 2aab – 20x
Lung Section of Rat exposed to Nanoquartz Particles (3M pe)

Hemolytic Potential of Quartz Samples (Surface Reactivity)

Session 2: Potential Exposure Scenarios and Potential Toxicity of Nanomaterials
Dr. David B. Warheit -- Presentation Slides
NANOTECHNOLOGY AND OSWER
New opportunities and challenges
Dr. David B. Warheit -- Presentation Slides
108    July 12-13, 2006     Washington DC
Summary of α-Quartz Results

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Nano quartz I</th>
<th>Nano quartz II</th>
<th>Fine quartz</th>
<th>Min-U-Sil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Surface area</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Fe content</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Crystallinity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Radical content</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Hemolytic potential</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Airway BrdU</td>
<td>NA</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Lung paren. BrdU</td>
<td>NA</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Histopathology</td>
<td>NA</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Protocol for Fine and Nanoscale ZnO Pulmonary Bioassay Studies

Inhalation Exposure at concs of 25, 35 or 50 mg/m³ for 1 or 3 hours
### Aerosol Generation Equipment and Set-up

![Image of aerosol generation equipment](image)

### Mean Particle Size Determinations in the ZnO and MgO Inhalation Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>MMAD (cascade impactor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO 25 mg/m³</td>
<td>3.3 μm</td>
</tr>
<tr>
<td>ZnO 35 mg/m³</td>
<td>2.7 – 3.2 μm</td>
</tr>
<tr>
<td>ZnO 50 mg/m³</td>
<td>3.2 μm</td>
</tr>
<tr>
<td>MgO 50 mg/m³</td>
<td>3.0 μm</td>
</tr>
<tr>
<td>Nano ZnO 25 mg/m³</td>
<td>2.8 μm</td>
</tr>
</tbody>
</table>
Preliminary Studies with Fine and Nano Zinc Oxide particles

**Percent Neutrophils in BAL Fluids of Rats**
Inhaling Fine ZnO or Nano ZnO Particles (25 mg/m³)

<table>
<thead>
<tr>
<th>Exposure Groups</th>
<th>Mean % PMN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO</td>
<td>100</td>
</tr>
<tr>
<td>N-ZnO</td>
<td>98</td>
</tr>
<tr>
<td>0%</td>
<td>96</td>
</tr>
<tr>
<td>1 hr Exposure</td>
<td>94</td>
</tr>
<tr>
<td>3 hr Exposure</td>
<td>92</td>
</tr>
</tbody>
</table>

Fine ZnO & Nano ZnO are not different

**Mean LDH Values in BAL Fluids of Rats**
Inhaling Fine ZnO or Nano ZnO Particles (25 mg/m³)

<table>
<thead>
<tr>
<th>Exposure Groups</th>
<th>uL/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO</td>
<td>110</td>
</tr>
<tr>
<td>N-ZnO</td>
<td>108</td>
</tr>
<tr>
<td>0%</td>
<td>106</td>
</tr>
<tr>
<td>1 hr Exposure</td>
<td>104</td>
</tr>
<tr>
<td>3 hr Exposure</td>
<td>102</td>
</tr>
</tbody>
</table>

Fine ZnO & Nano ZnO are not different
Impact of Surface Treatments/Coatings on TiO$_2$ Particles

- Inhalation Studies
- Pulmonary Bioassay Intratracheal Instillation Studies

Comparative Pulmonary Toxicity Inhalation and Instillation Studies with Different TiO$_2$ Particle Formulations: Impact of Surface Treatments on Particle Toxicity

DB Warheit, WJ Brock, KP Lee, TR Webb, and KL Reed

- Toxicological Sciences 88:514-524, 2005
**TiO$_2$ Coatings Formulations**

- **TiO$_2$ base** - 99% TiO$_2$ - 1% alumina
- **TiO$_2$ I** - 99% TiO$_2$ - 1% alumina + organic grinding aid
- **TiO$_2$ II** - 96% TiO$_2$ - 4% alumina
- **TiO$_2$ III** - 83% TiO$_2$ - 6% alumina - 11% amorphous silica
- **TiO$_2$ IV** - 91% TiO$_2$ - 3% alumina - 6% amorphous silica
- **TiO$_2$ V** - 94% TiO$_2$ - 3% alumina - 3% amorphous silica

---

**Protocol for TiO$_2$ Coatings Bioassay Study**

**Instillation Study**

- **Exposure Groups**
  - Sham (Air Controls)
  - Base TiO$_2$ formulation
  - TiO$_2$-1 formulation
  - TiO$_2$-2 formulation
  - TiO$_2$-3 formulation
  - TiO$_2$-4 formulation
  - TiO$_2$-5 formulation

**Postexposure Evaluation via BAL and Lung Tissue**

- 24 hr
- 1 wk
- 1 mo
- 3 mo
% Neutrophils in BAL Fluids From Rats Exposed to TiO2 Coatings

TiO2 Coatings - BAL Fluid LDH Values
**Important Particle Characteristics**

- Primary particle size
- Particle shape (SEM)
- Surface area
- Surface charge
- Composition - e.g. crystalline vs. amorphous
- Surface Coatings
- Aggregation status
- Particle number
- Method of synthesis (gas vs. liquid phase)

**Summary**

- Risk is a product of Hazard and Exposure
- Cannot assume that nanomaterials are the same as their bulk counterpart
- Each particle-type should be tested on a case-by-case basis
- A variety of factors (in addition to particle size/surface area) influence toxicity of nanoparticulates
Acknowledgments

- Tom Webb and Ken Reed provided the pulmonary toxicology technical expertise for the study. Denise Hoban, Elizabeth Wilkinson and Rachel Cushwa conducted the BAL fluid biomarker assessments. Carolyn Lloyd, Lisa Lewis, John Barr prepared lung tissue sections and conducted the BrdU cell proliferation staining methods. Don Hildabrandt provided animal resource care. Dr. Christie Sayes and Dr. Vicki Colvin – collaborators.