[S-3]

Impact of Nanoparticulates on Respiratory Health Effects

David B. Warheit

DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, Delaware, USA

Published pulmonary toxicology studies in rats have demonstrated thatultrafine or nanoparticles (generally defined as particles in the size range < 100nm) administered to the lung cause a greater inflammatory response when compared to larger particles of identical chemical composition at equivalent mass concentrations. However, this common perception that all nanoparticles are more toxic than fine-sized particles is based upon a systematic comparison of only 3 particle-types (titanium dioxide particles, carbon black particles and diesel exhaust particles). Additional factors, other than particle size, may play more important roles in modifying pulmonary toxicity of nanoparticles. These include: surface coatings of particles; the tendency of aerosolized particles to aggregate/disaggregate; whether the particle was generated in the gas or liquid phase (i.e., fumed vs. colloidal/precipitated); and surface charge. Results of pulmonary bioassay hazard studies will be presented demonstrating that fine-sized quartz particles (1.6μm) may produce greater pulmonary toxicity in rats when compared to nanoscale quartz particles (50nm) but not when compared to smaller nanoquartz sizes (e.g., < 30nm). In addition, other studies have demonstrated no difference in pulmonary toxicity between fine-sized TiO_2 particles (300nm) and TiO_2 nanodots (25nm) and nanorods. Finally, studies will be presented which demonstrate that surface coatings on particles can modify lung inflammatory effects. In summary, these are the most important conclusions:

1) Risk is a product of Hazard and Exposure;
2) one cannot assume that nanomaterials have the same toxicity as their microscale or macroscale counterparts (i.e., either greater than or less than);
3) therefore, each particle-type should be tested on a case-by-case basis.

Impact of Nanoparticulates on Respiratory Health Effects

David B. Warheit, Ph.D.
DuPont Haskell Laboratory
Korean Society of Toxicology (KSOT)
Toxicology of Nanoparticles
Seoul National University
May 13, 2005

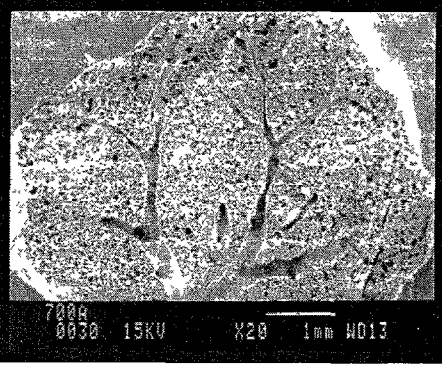
Outline

- Lung structure and particle deposition
- Pulmonary bioassay as a measure of lung toxicity- Hazard Assessment
- Pulmonary bioassay with Fine/Nanoscale TiO_2 dots and rods; Fine/Nanoscale Quartz particles, and Fine/Nanoscale ZnO particles
- Impacts of Particle Surface Coatings
- Summary

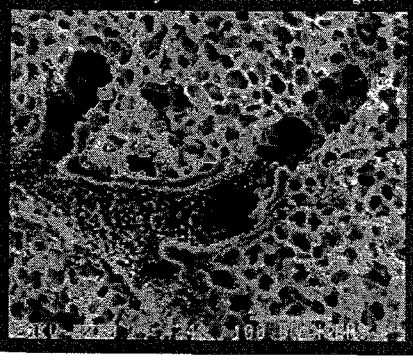
Definitions- Particle Size

- 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

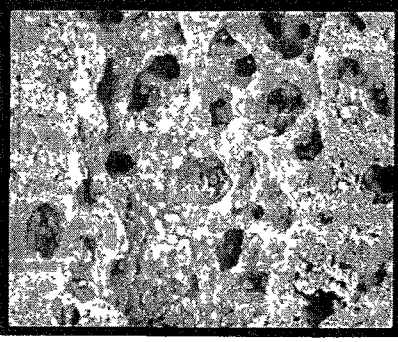
Rat Lung Microdissection

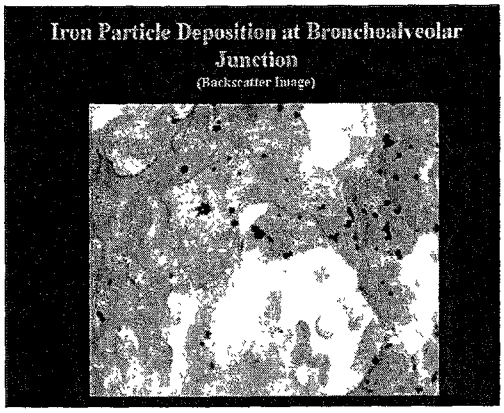


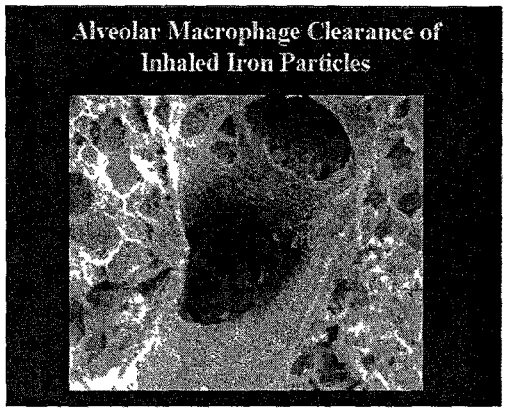
Rat Lung Tissue Dissected to Demonstrate the Junction of the Terminal Airway and Proximal Alveolar Region

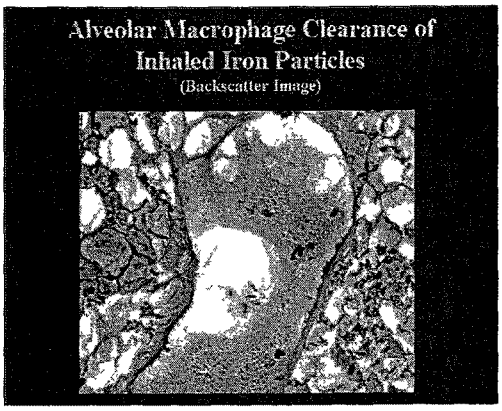


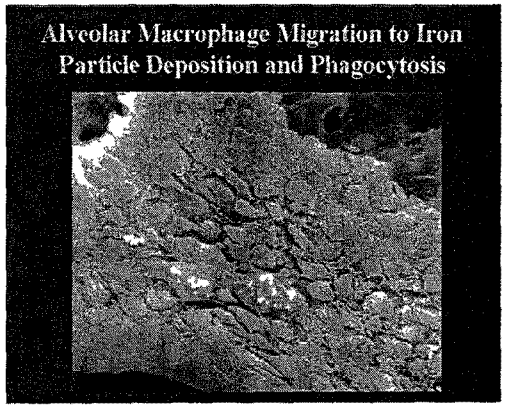
Iron Particle Deposition at Bronchoalveolar Junction

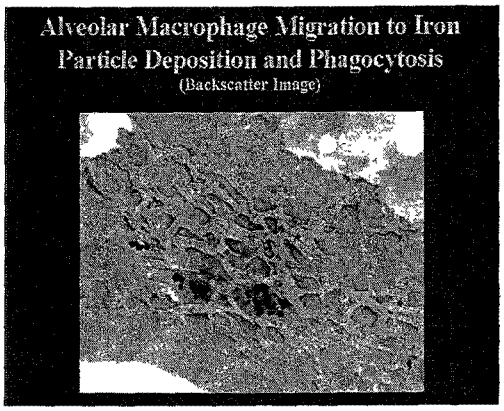


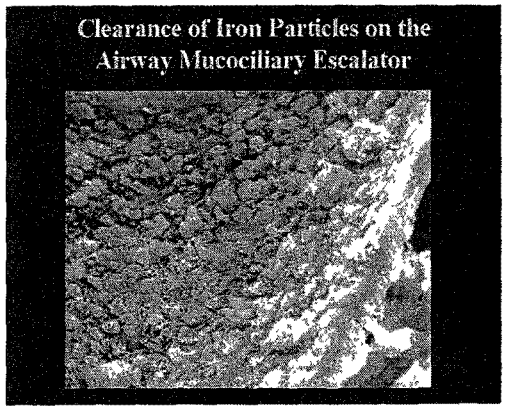

Iron Particle Deposition at Bronchoalveolar Junction
(Backscatter Image)


Alveolar Macrophage Clearance of Inhaled Iron Particles

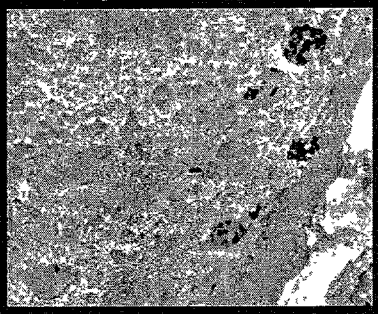

Alveolar Macrophage Clearance of Inhaled Iron Particles
(Backscatter Image)


Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis

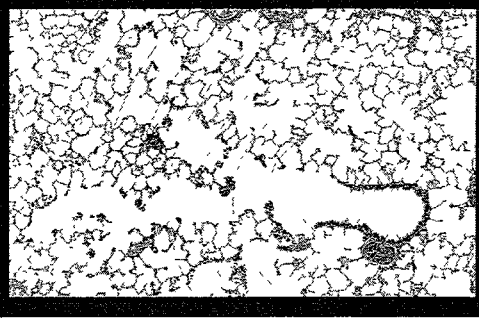

Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis
(Backscatter Image)


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

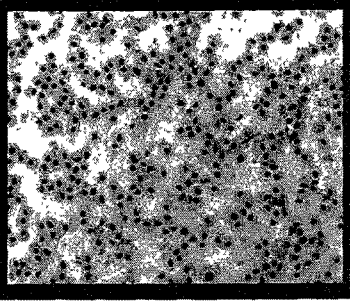
Bronchoalveolar Lavage Assessments

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

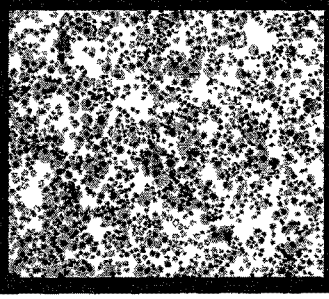
Lung Tissue Analysis

- Lung Weights
- Lung Cell Proliferation (BrdU)
  - Parenchymal
  - Airway
- Lung Histopathology

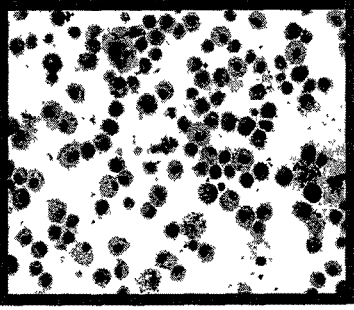
Cytocentrifuge Preparation of BAL – Recovered Cells From a Sham – Exposed Rat



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

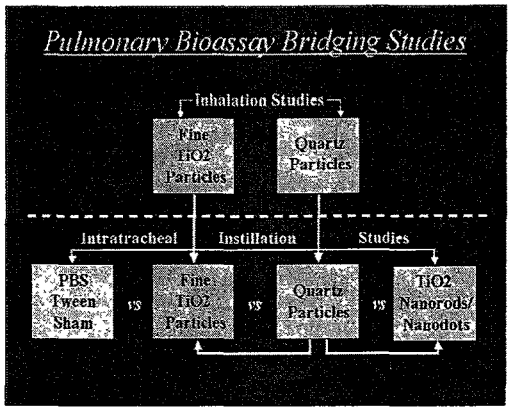


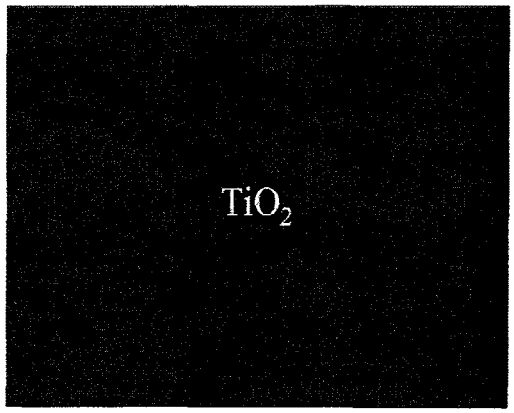
Cytocentrifuge Preparation of BAL – Recovered Cells From a Carbonyl Iron – Exposed Rat

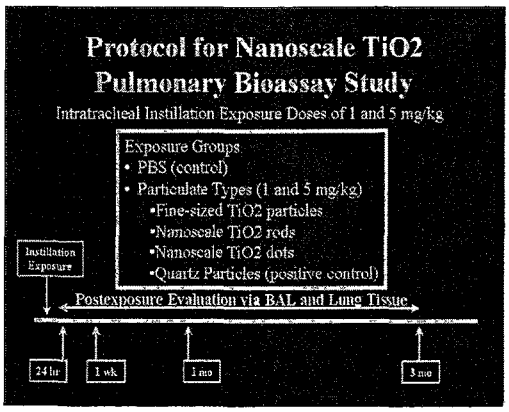


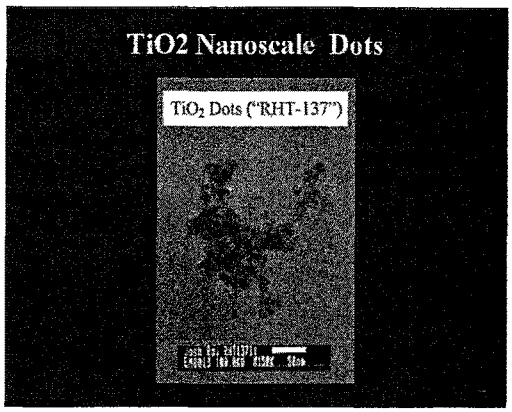
Use of Bronchoalveolar Lavage, Cell Proliferation, and Histopathology to Assess the Lung Toxicity of Particulate samples

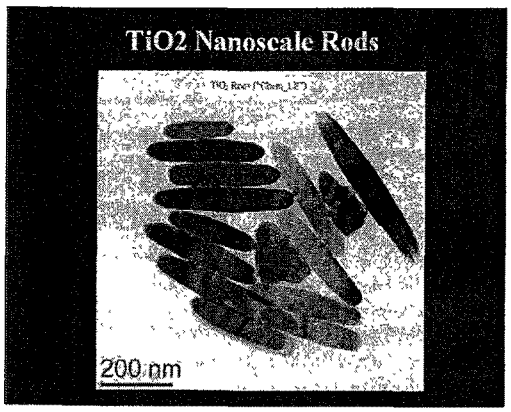
Parameter	Indicator
(BALF = Bronchoalveolar Lavage Fluid Analysis)	
BALF Cells and Differentials	Lung Inflammation
BALF Lactate Dehydrogenase	Non-specific cytotoxicity
BALF Alkaline Phosphatase	Type 2 cell epithelial toxicity
BALF Protein	Permeability ↑ of alveolar capillary barrier
Lung Weights	Pulmonary edema or fibrosis
Macrophage phagocytosis	Lung clearance functions
Cell Proliferation	Inflammation/lung fibrosis and tumor potential
Histopathology	Evaluation of lung tissue responses

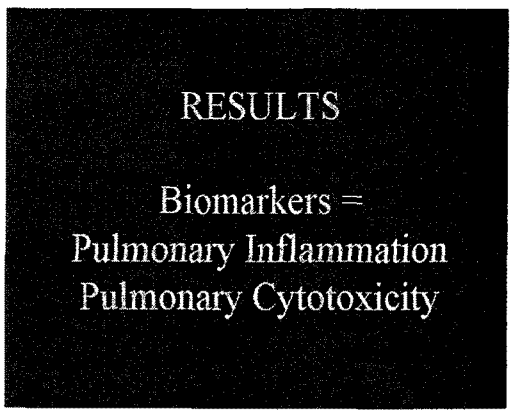

Pulmonary Bioassay Bridging Studies
Inhalation Studies
Fine TiO2 Particles
Quartz Particles
Intratracheal Instillation Studies
PBS Tween Sham
vs
Fine TiO2 Particles
vs
Quartz Particles
vs
TiO2 Nanorods/ Nanodots


TiO_2

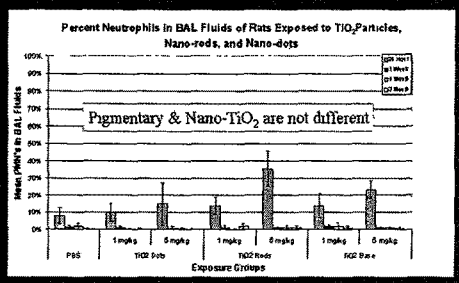

Protocol for Nanoscale TiO2 Pulmonary Bioassay Study
Intratracheal Instillation Exposure Doses of 1 and 5 mg/kg
Exposure Groups
• PBS (control)
• Particulate Types (1 and 5 mg/kg)
•Fine-sized TiO2 particles
•Nanoscale TiO2 rods
•Nanoscale TiO2 dots
•Quartz Particles (positive control)
Instillation Exposure
Postexposure Evaluation via BAL and Lung Tissue
24 hr
1 wk
1 mo
3 mo

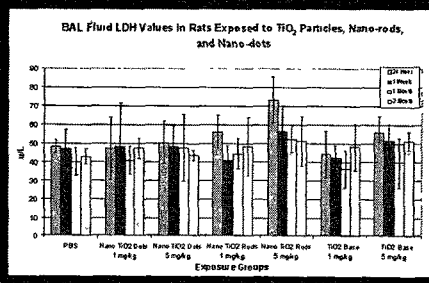

TiO2 Nanoscale Dots
TiO_2 Dots ("RHT-137")


TiO2 Nanoscale Rods
200 nm


RESULTS
Biomarkers =
Pulmonary Inflammation
Pulmonary Cytotoxicity

Collaborative Studies with Rice University: TiO_2



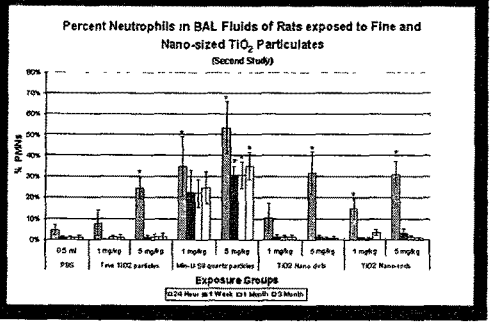


Characterization of Nanoscale TiO_2 Particles

	XRD particle size	Surface Area
• Fine TiO_2	rutile d_{50} = 300 nm	6 m^2/g
• TiO_2 Nanorods	anatase length= 90 - 233 nm width = 20 – 35 nm	26.5 m^2/g
• TiO_2 Nanodots	anatase d_{50} = 6 nm	169.4 m^2/g

Second Nanoscale TiO2 Study

Pulmonary Inflammation



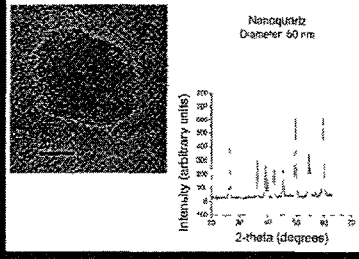
Hypothesis and a Question

- 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?

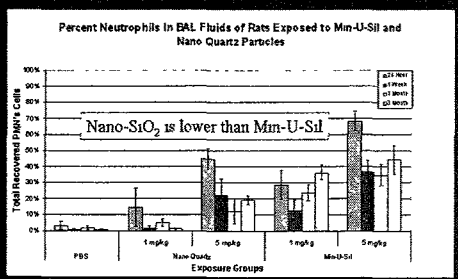
Nanoscale Quartz

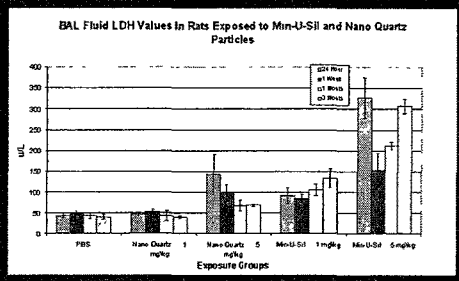
Nanoscale Quartz Particles

Q1 Characterization



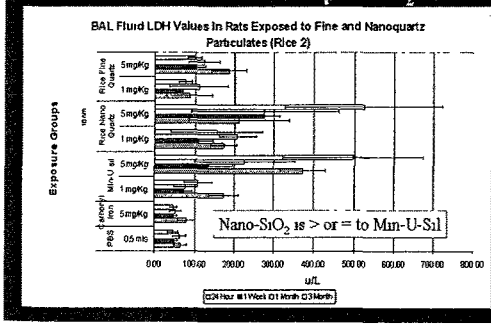
Preliminary Collaborative Studies with Rice University: SiO_2





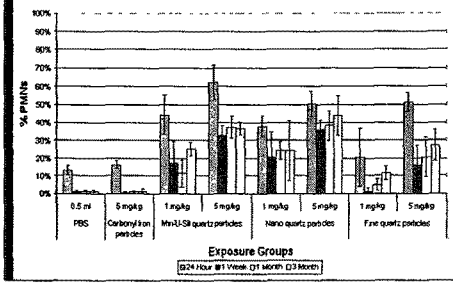
Second Nanoscale Quartz Study

Follow-up Collaborative Studies with Rice University: SiO_2



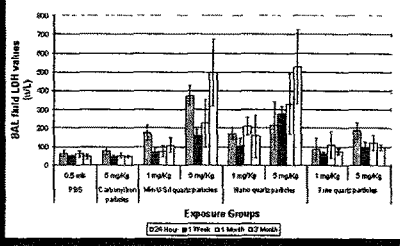
Pulmonary Inflammation

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

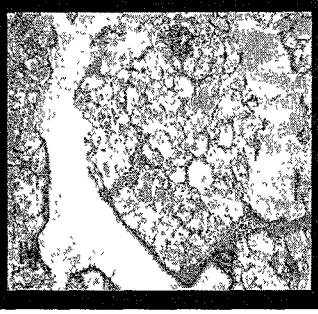


BAL Fluid LDH Values (cytotoxicity)

BAL Fluid LDH Values in Rats exposed to Fine and Nano-sized Quartz Particles



Lung Section of Rat exposed to Nanoquartz Particles (3M pe)

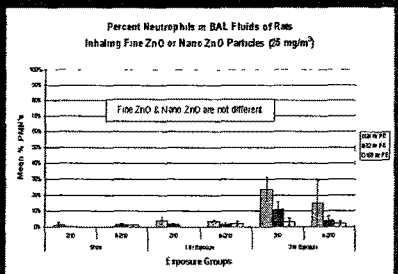


Characterization of Nanoscale Quartz Particles

		XRD particle size	Surface Area
• Fine Quartz	αQ	d_{50} = 179 nm	4.2 m^2/g
• Nanoscale Q	αQ	d_{50} = <30 nm	31.4 m^2/g
• Min-U-Sil	αQ	d_{50} = 1.3 μm	4.0 m^2/g

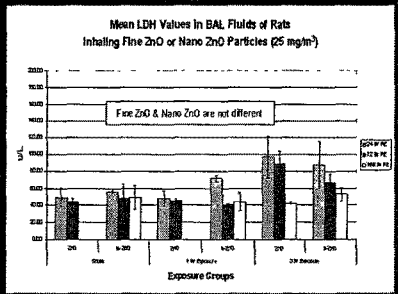
Preliminary Studies with Fine and Nano Zinc Oxide particles

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



Preliminary Studies with Fine and Nano Zinc Oxide particles

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



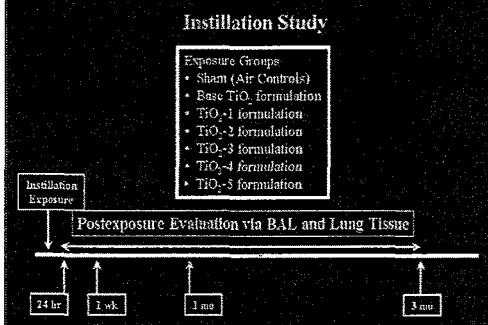
Impact of Surface Treatments/Coatings on TiO_2 Particles

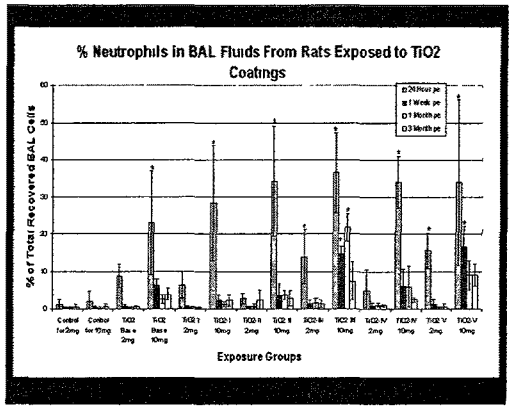
- Inhalation Studies
- Pulmonary Bioassay Intratracheal Instillation Studies

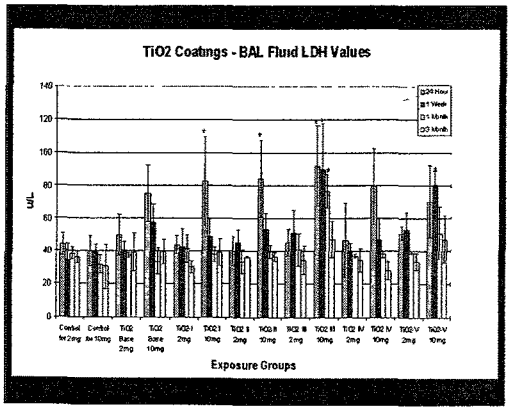
TiO_2 Coatings Formulations

- TiO2 base - 99% TiO2 - 1% alumina
- TiO2 I - 99% TiO2 - 1% alumina + organic grinding aid
- TiO2 II - 96% TiO2 - 4% alumina
- TiO2 III - 83% TiO2 - 6% alumina - 11% amorphous silica
- TiO2 IV - 91% TiO2 - 3% alumina - 6% amorphous silica
- TiO2 V - 94% TiO2 - 3% alumina - 3% amorphous silica

Protocol for TiO_2 Coatings Bioassay Study







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

Acknowledgments

- This study was supported by DuPont Central Research and Development.
- 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.