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

David B. Warheit, Ph.D.
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 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

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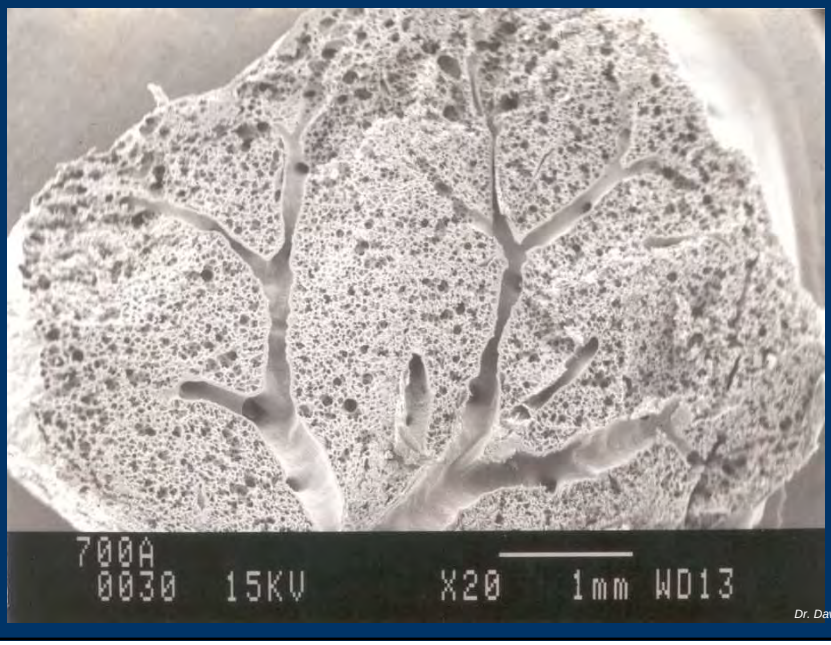
3 of 79

Rat Lung Microdissection



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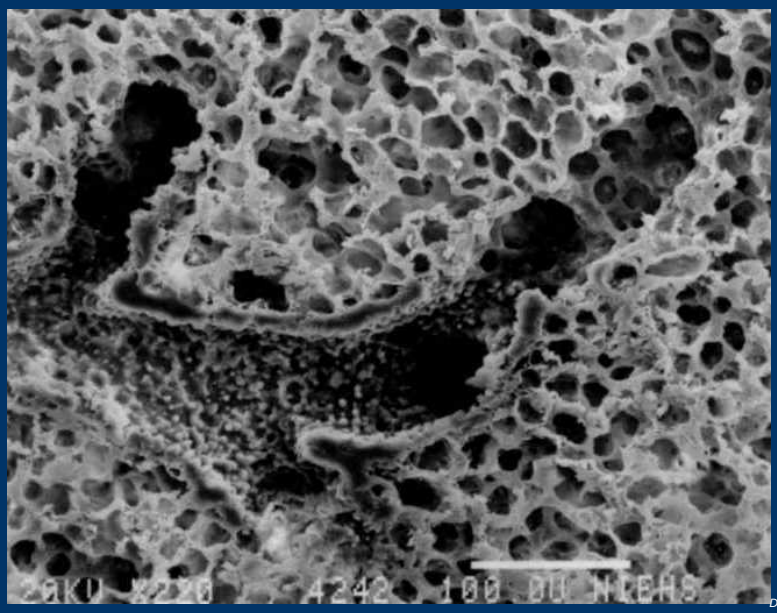
4 of 79

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



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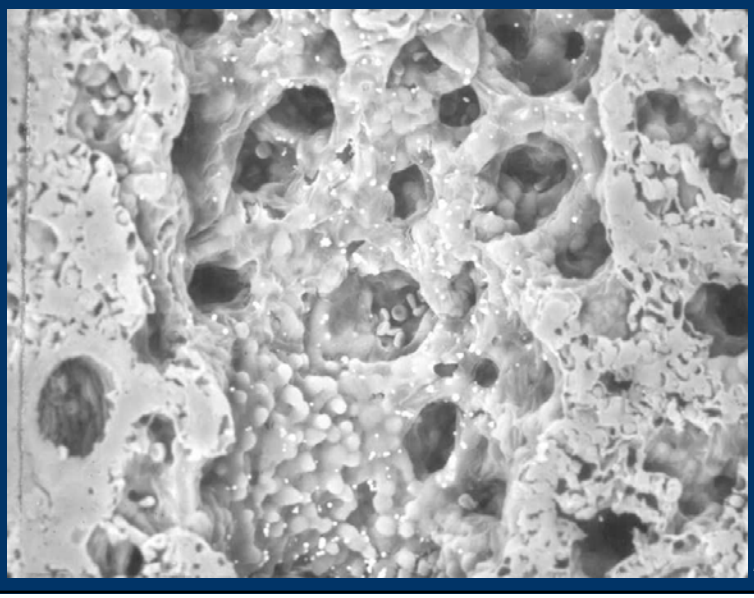
5 of 79

Iron Particle Deposition at Bronchoalveolar Junction



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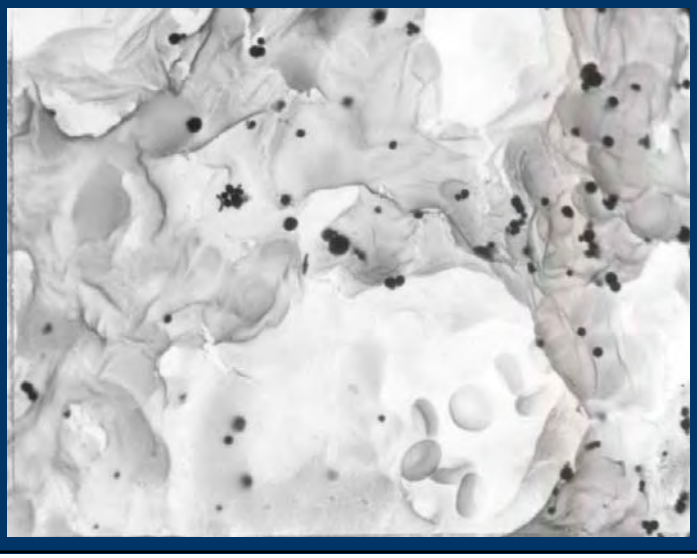
6 of 79

Iron Particle Deposition at Bronchoalveolar Junction

(Backscatter Image)



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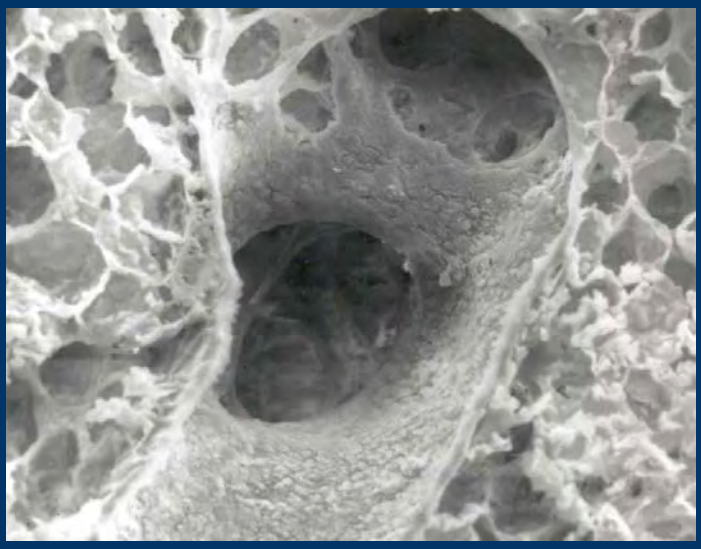
7 of 79

Alveolar Macrophage Clearance of Inhaled Iron Particles



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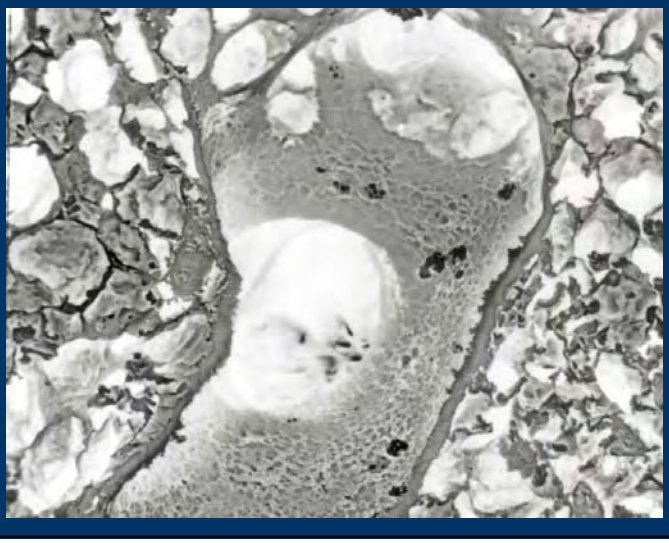
8 of 79

Alveolar Macrophage Clearance of Inhaled Iron Particles
(Backscatter Image)



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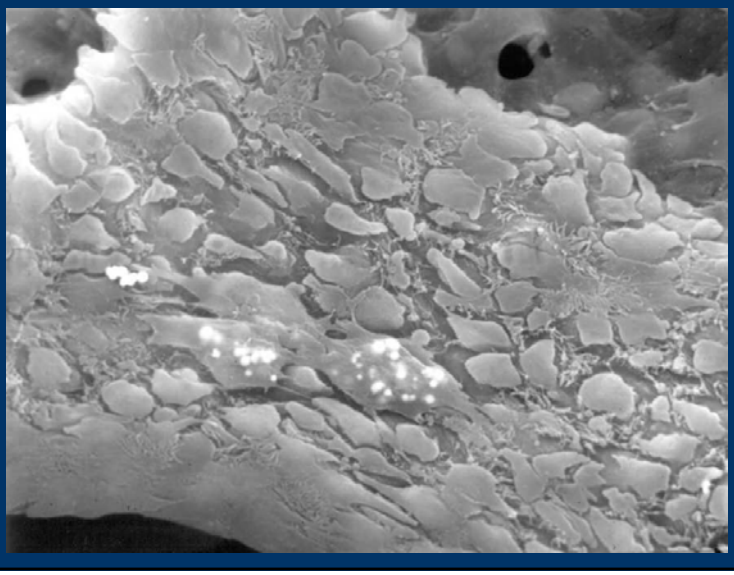
9 of 79

Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis



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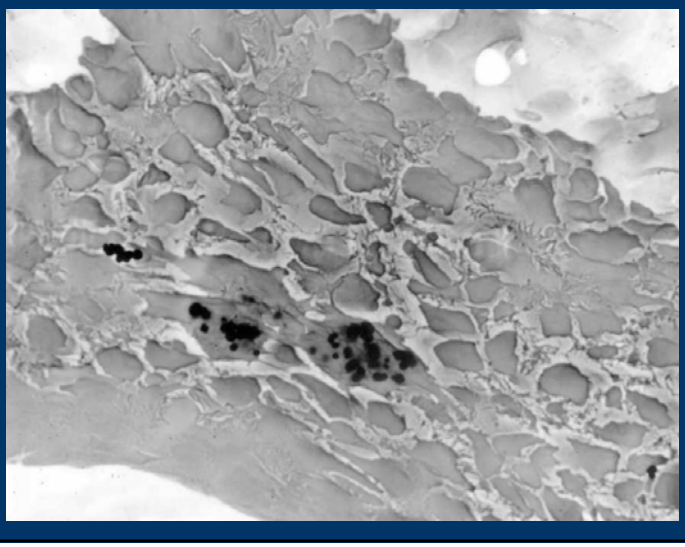
10 of 79

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



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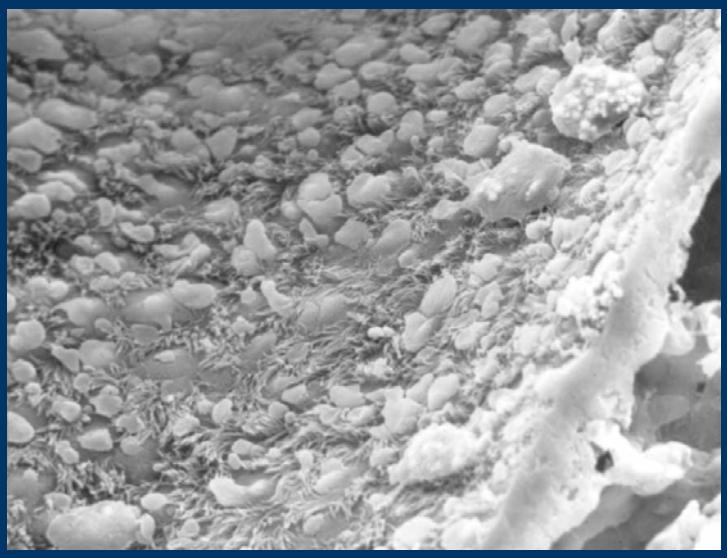
11 of 79

Clearance of Iron Particles on the Airway Mucociliary Escalator



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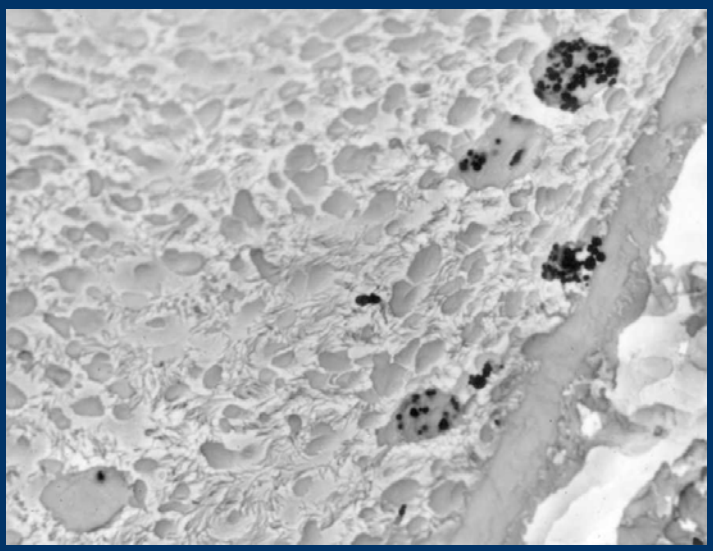
12 of 79

Clearance of Iron Particles on the Airway Mucociliary Escalator



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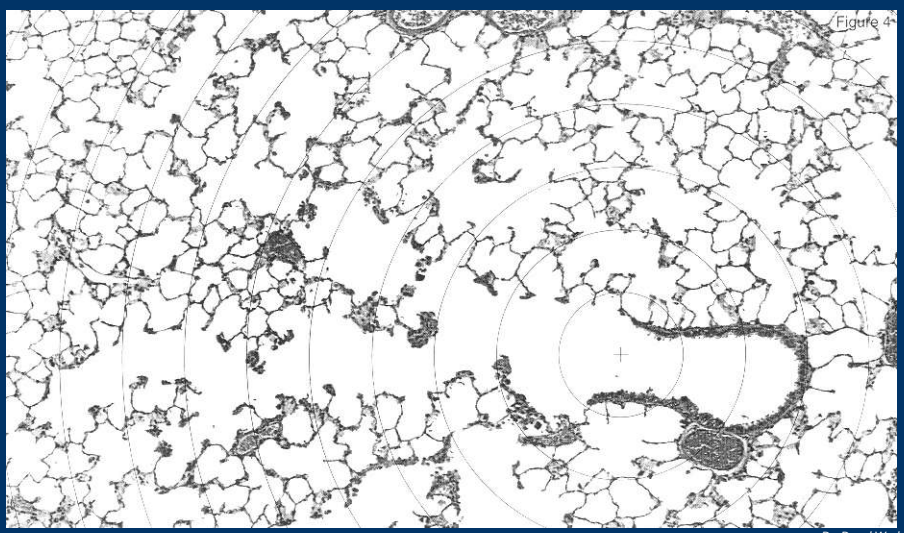
13 of 79

Morphometry at Bronchoalveolar Junctions



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14 of 79

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

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15 of 79

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

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16 of 79

The Key Issue: Risk

Health Risk is a product of

- **Hazard and Exposure**

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17 of 79

Studies to Assess Pulmonary Hazards to Nanoparticulates

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18 of 79

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

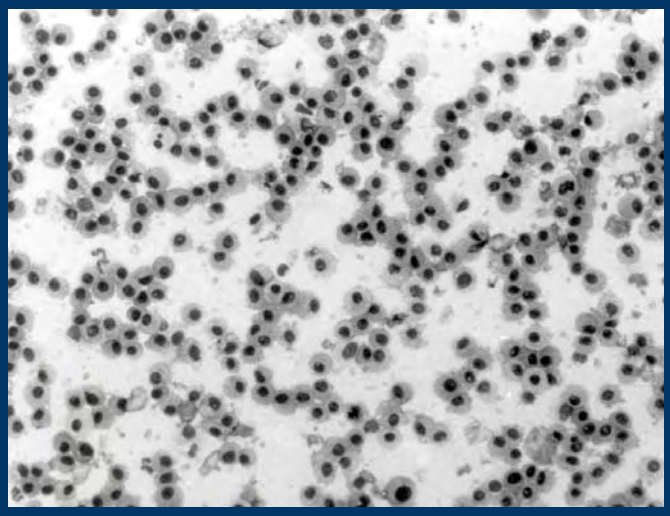
Bronchoalveolar Lavage Assessments

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



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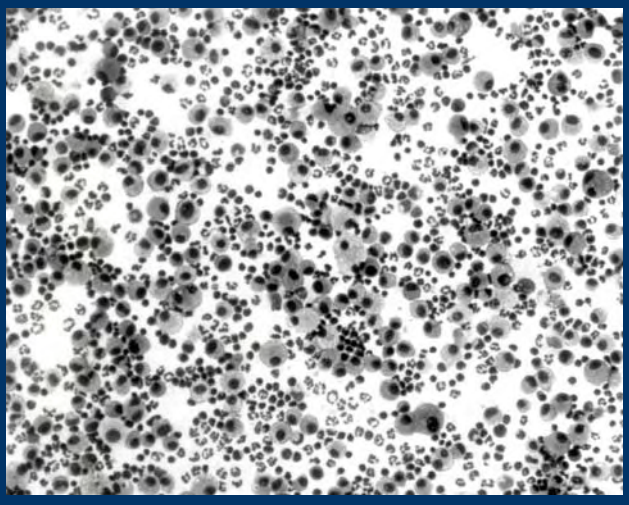
21 of 79

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



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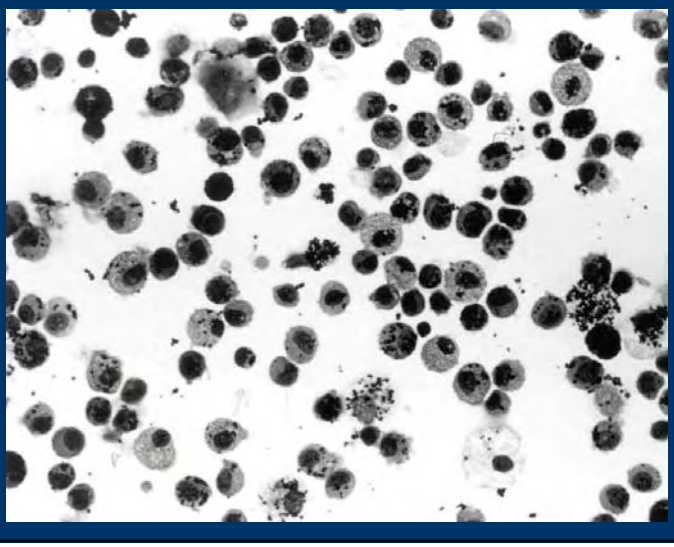
22 of 79

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



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23 of 79

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

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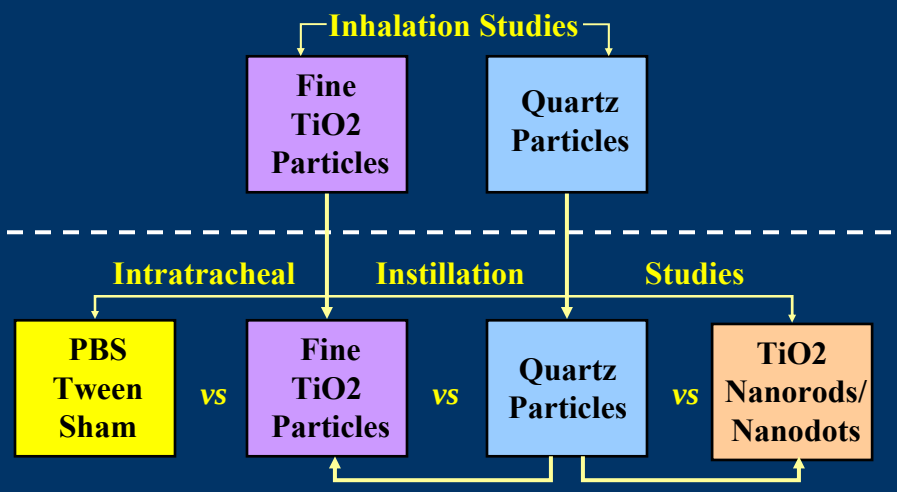
24 of 79

Pulmonary Bioassay Bridging Studies



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25 of 79

Collaborative Studies with Rice University – CBEN - Vicki Colvin and Christie Sayes on the Pulmonary Toxicity of Nanoscale TiO_2 and Quartz Particle-types

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26 of 79

TiO_2

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)

Instillation Exposure

Postexposure Evaluation via BAL and Lung Tissue

24 hr | 1 wk | 1 mo | 3 mo

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29 of 79

TiO2 Nanoscale Dots

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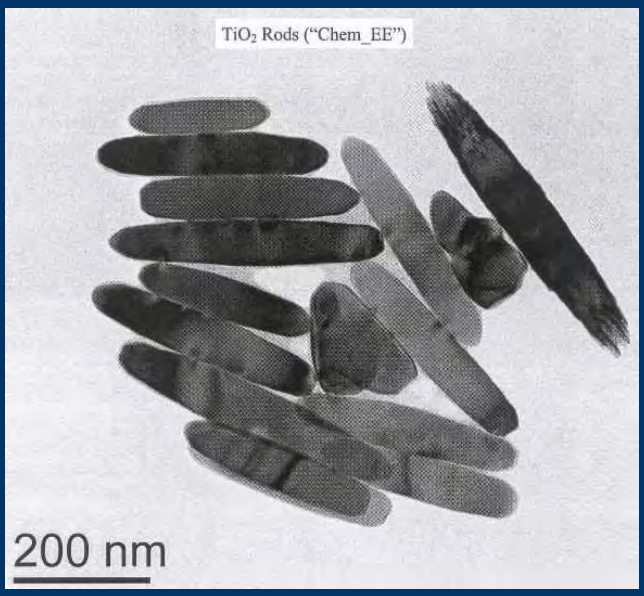
30 of 79

TiO2 Nanoscale Rods



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31 of 79

TiPure® R-100

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32 of 79

Characterization of Nanoscale TiO_2 and Quartz Particles

	XRD	particle size	Surface Area
• **Fine TiO_2**	rutile	d_{50} = 300 nm	**6.0 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**
• **Min-U-Sil**	αQ	d_{50} = 1.3 µm	**4.0 m^2/g**

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33 of 79

RESULTS

Biomarkers = Pulmonary Inflammation Pulmonary Cytotoxicity

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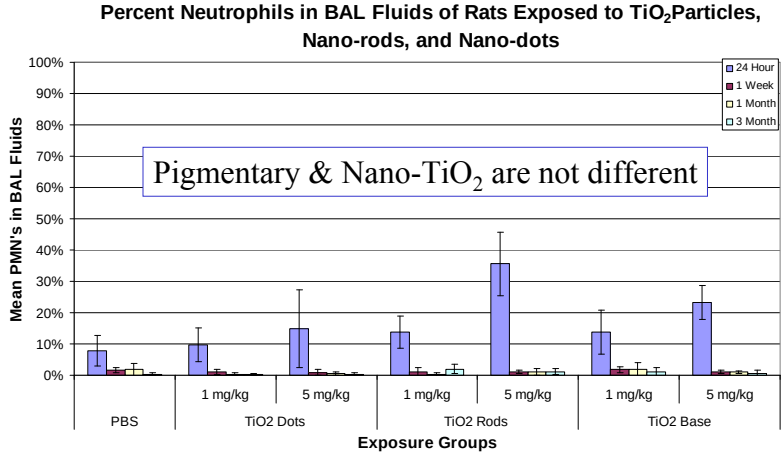
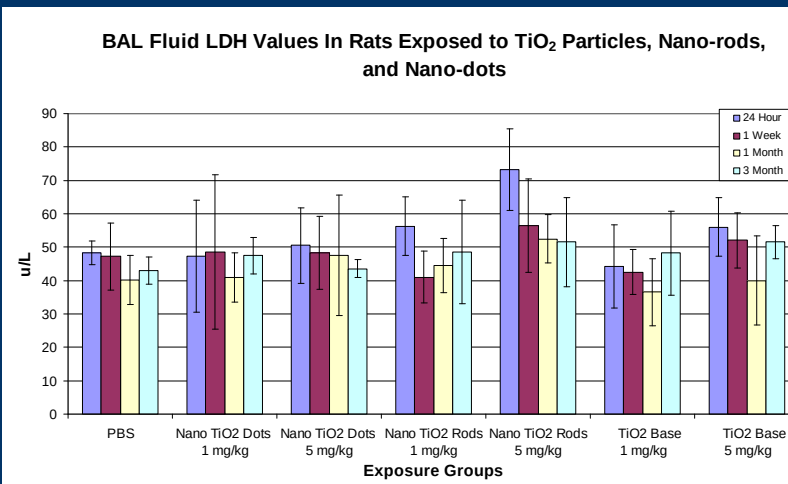
34 of 79

Collaborative Studies with Rice University: TiO_2





Second Nanoscale TiO2 Study

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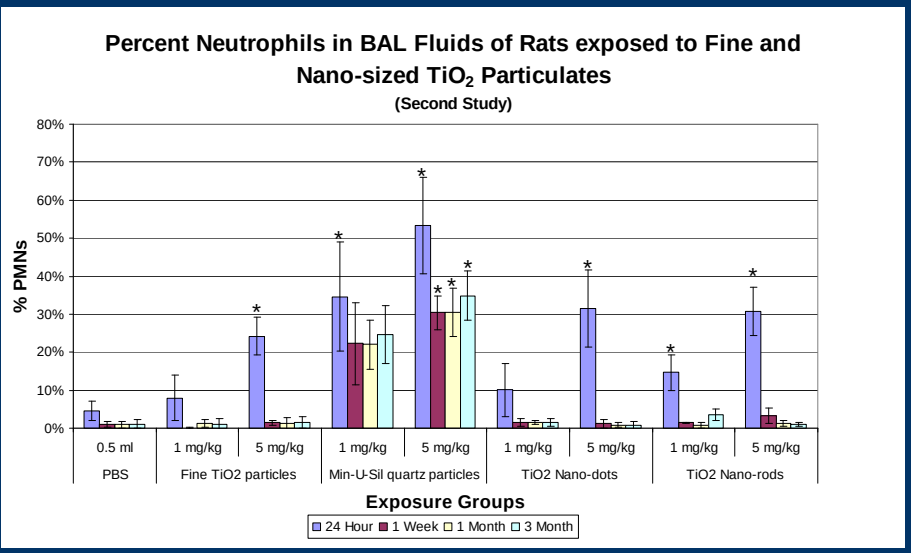
37 of 79

Pulmonary Inflammation



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38 of 79

Characterization of Nanoscale TiO_2 and Quartz Particles

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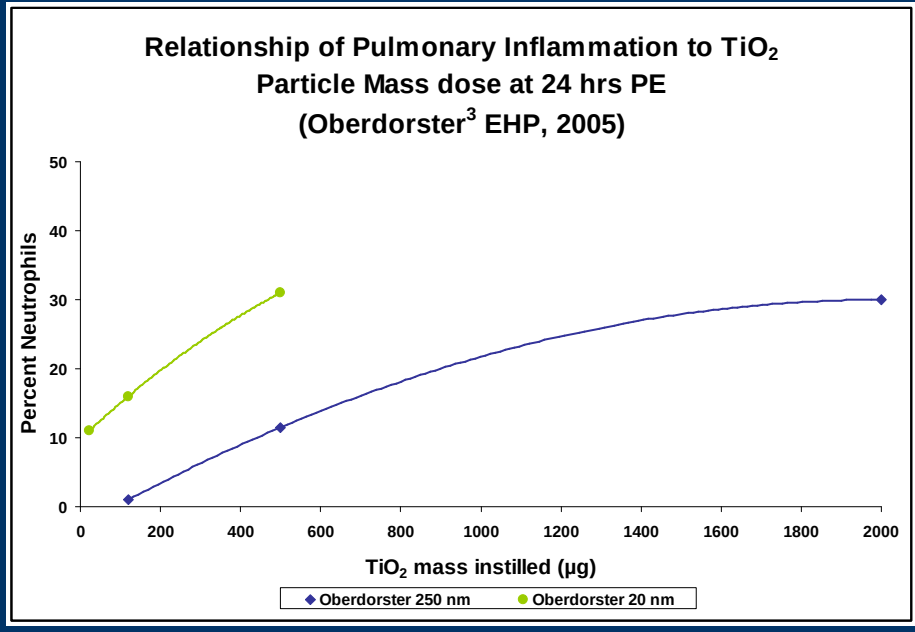
39 of 79



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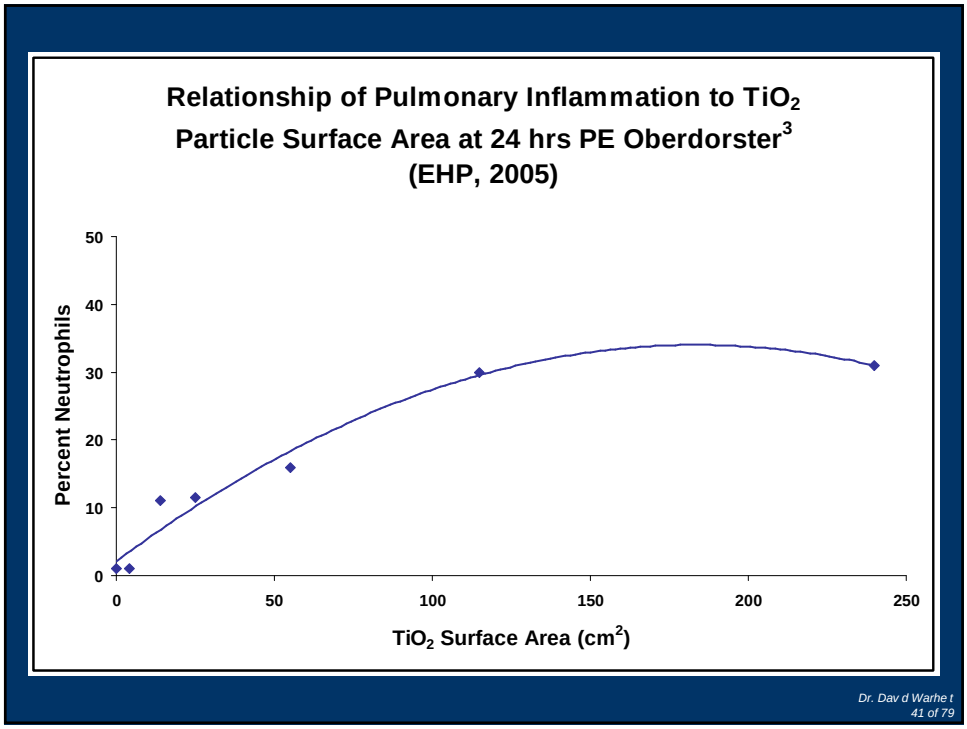
40 of 79


Relationship of Pulmonary Inflammation to TiO_2
Particle Surface Area at 24 hrs PE Oberdorster[3]
(EHP, 2005)
Percent Neutrophils
0
10
20
30
40
50
0
50
100
150
200
250
TiO_2 Surface Area (cm^2)
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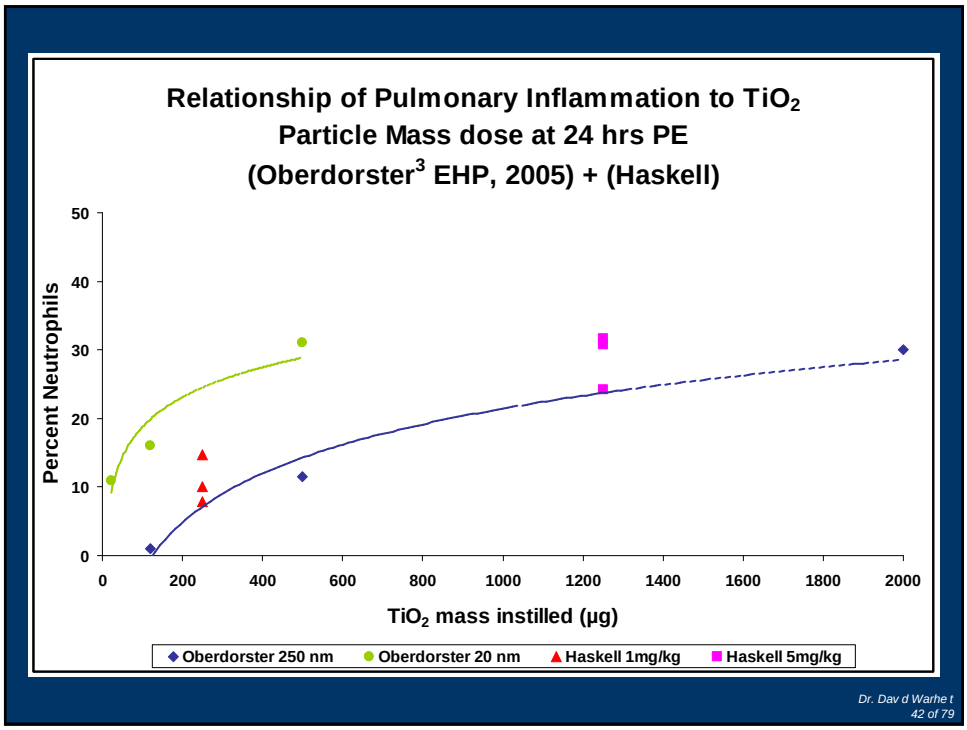
41 of 79


Relationship of Pulmonary Inflammation to TiO_2
Particle Mass dose at 24 hrs PE
(Oberdorster[3] EHP, 2005) + (Haskell)
Percent Neutrophils
0
10
20
30
40
50
0
200
400
600
800
1000
1200
1400
1600
1800
2000
TiO_2 mass instilled (µg)
Oberdorster 250 nm
Oberdorster 20 nm
Haskell 1mg/kg
Haskell 5mg/kg
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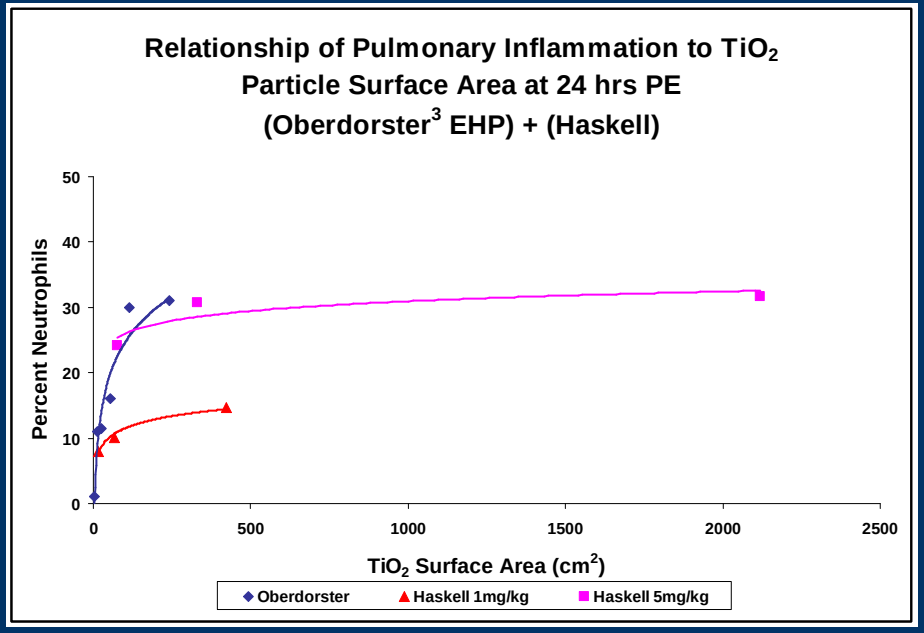
42 of 79



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43 of 79

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?

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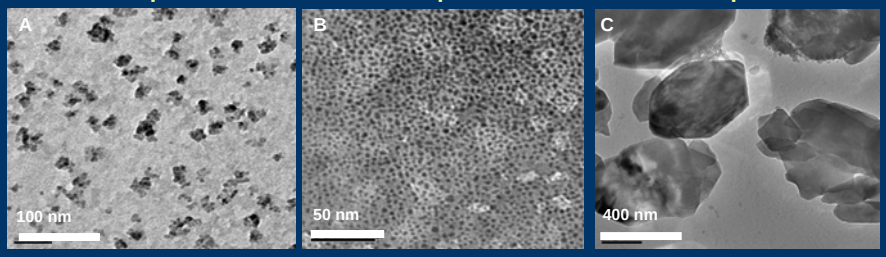
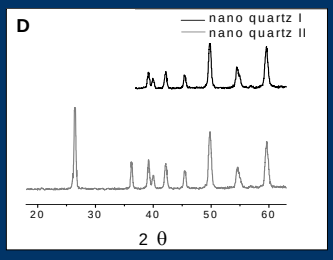
44 of 79

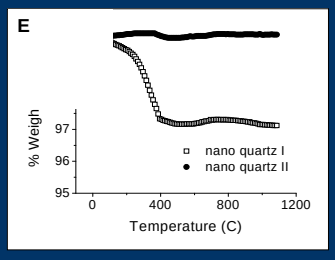
Nanoscale Quartz

Physicochemical Characterization of Quartz Particulates

nano quartz I | nano quartz II | fine quartz



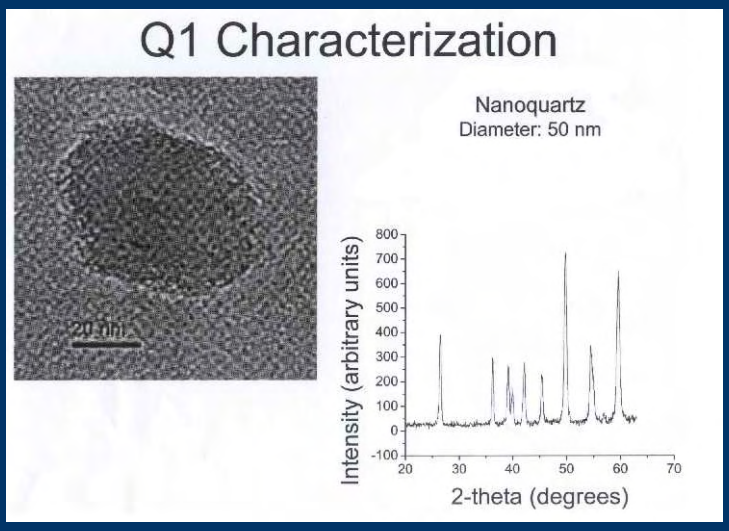




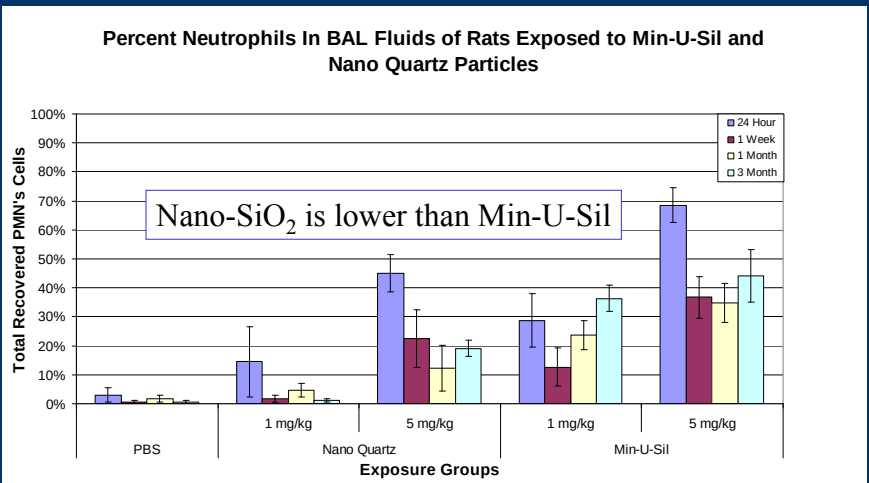
Physicochemical Characterization of Quartz Particulates (cont.)

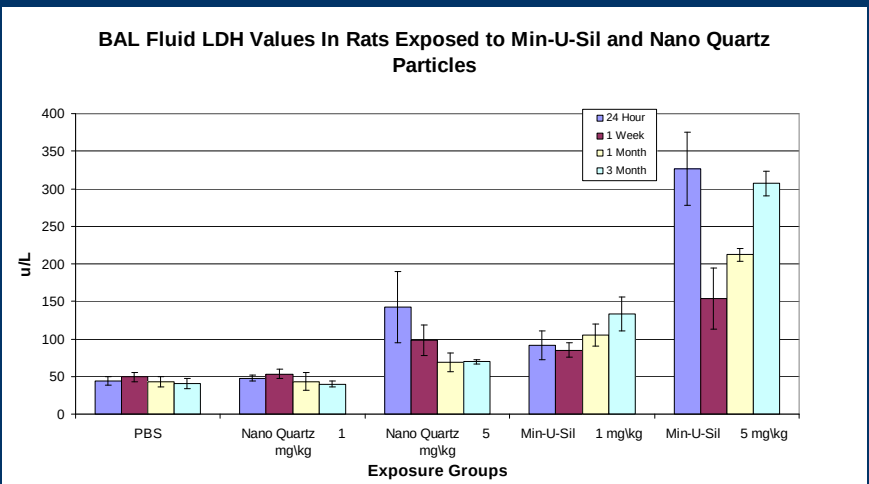
Sample	Average Size (nm)	Size Range (nm)	Surface Area (m^2/g)	Crystallinity	ICP-AES (% Fe content)
nano quartz I	50	30-65	31.4	α-quartz	0.080%
nano quartz II	12	10-20	90.5	α-quartz	0.034%
fine quartz	300	100-500	4.2	α-quartz	0.011%
Min-U Sil	534	300-700	5.1	α-quartz	0.042%

Nanoscale Quartz Particles



Preliminary Collaborative Studies with Rice University: SiO_2





Characterization of Quartz Particles

	XRD particle size	Surface Area
• **Nanoscale-Q I**	αQ d_{50} = 50 nm	**31.4 m^2/g**
• **Min-U-Sil**	αQ d_{50} = 534 μm	**5.1 m^2/g**

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51 of 79

Second Nanoscale Quartz Study

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52 of 79

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

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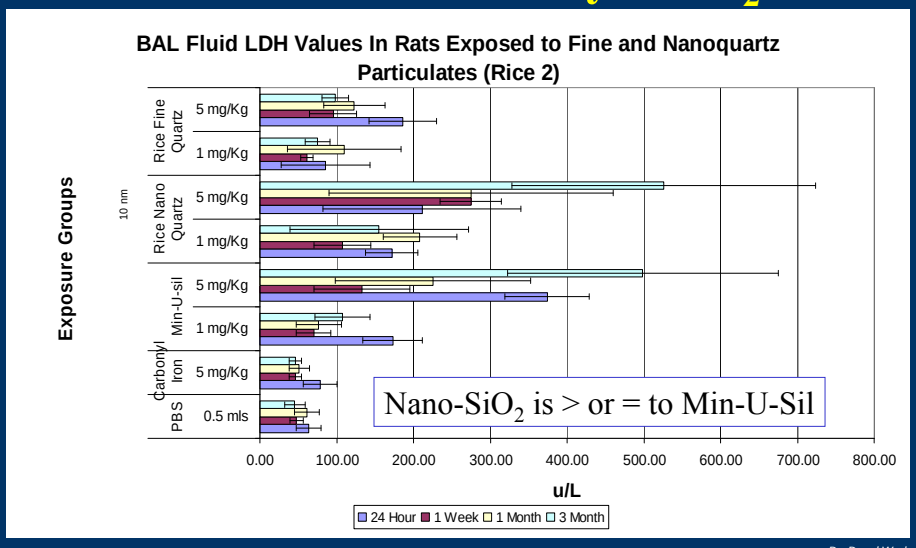
53 of 79

Follow-up Collaborative Studies with Rice University: SiO_2



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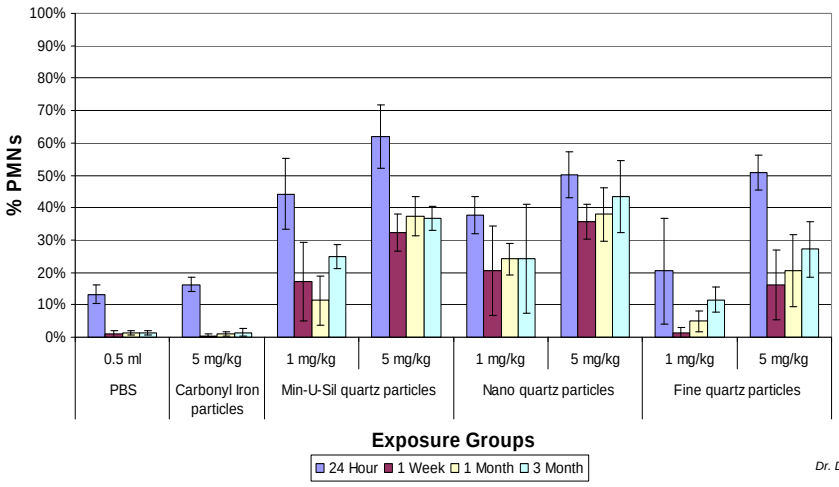
54 of 79

Pulmonary Inflammation

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



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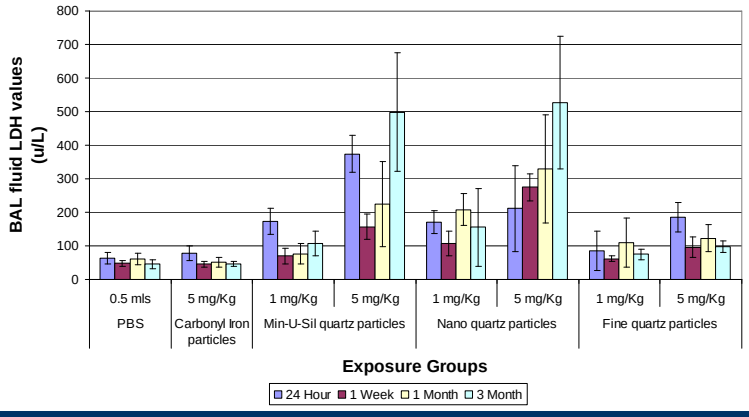
55 of 79

BAL Fluid LDH Values (cytotoxicity)

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



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56 of 79

Characterization of Quartz Particles

	XRD particle size	Surface Area
• **Fine Quartz**	αQ d_{50} = 300 nm	**4.2 m^2/g**
• **Nanoscale-Q II**	αQ d_{50} = 12 nm	**90.5 m^2/g**
• **Min-U-Sil**	αQ d_{50} = 534 nm	**5.1 m^2/g**

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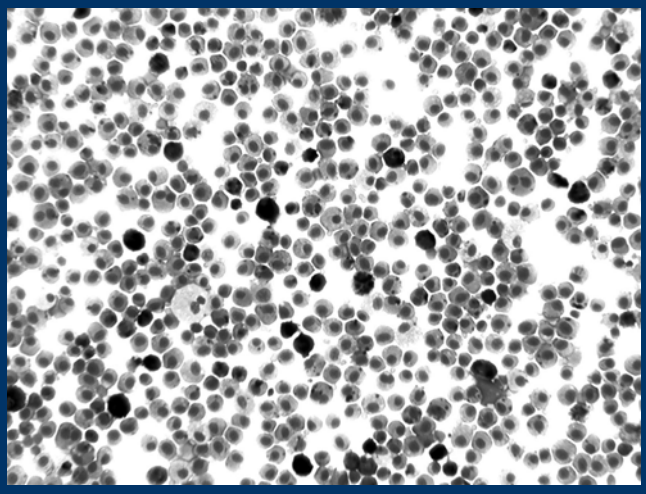
57 of 79

CI – 2B-3M – 2aab – 20x



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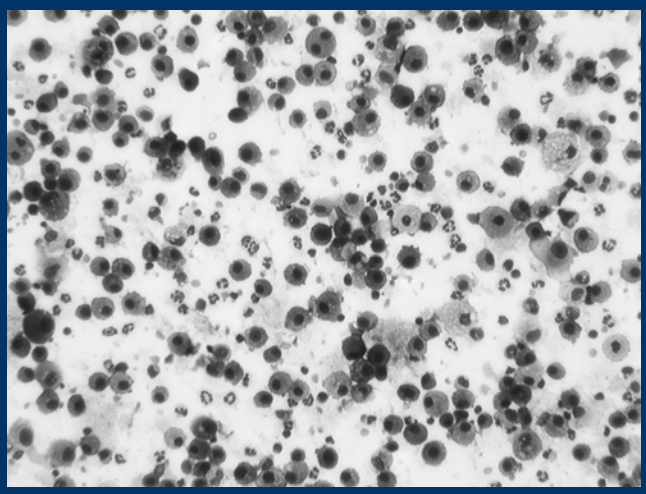
58 of 79

FineQ-5B-3M-2a-20x



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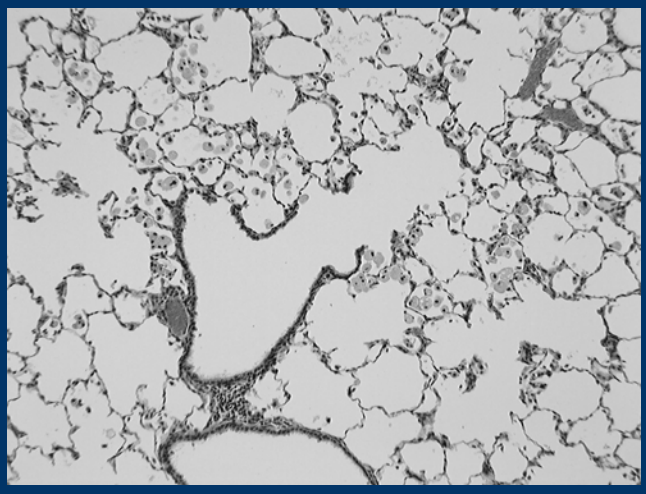
59 of 79

FineQ-5B-3M-5-10x



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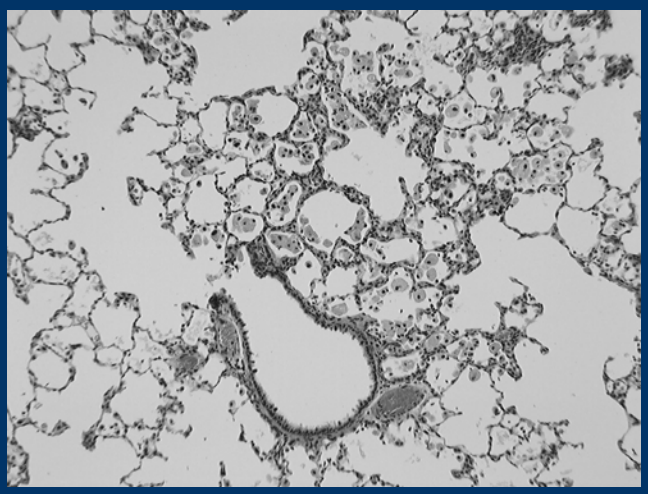
60 of 79

Min-U-Sil-3B-3M - 2-10x



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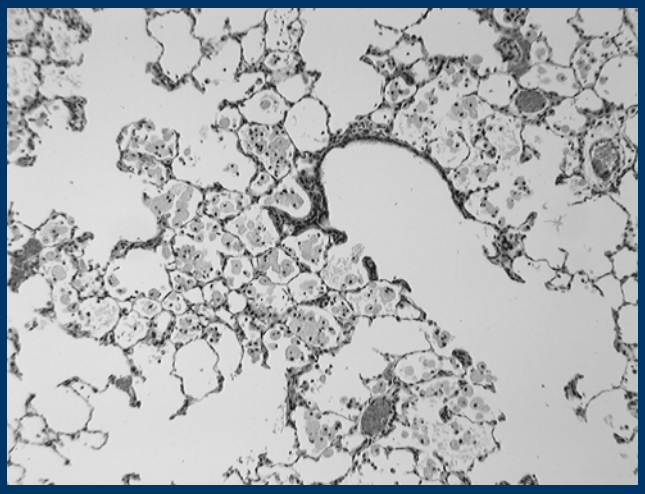
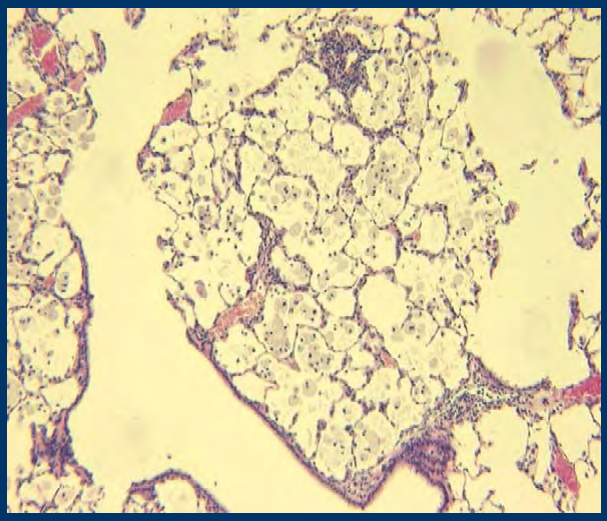
61 of 79

NanoQ-4B-3M-5



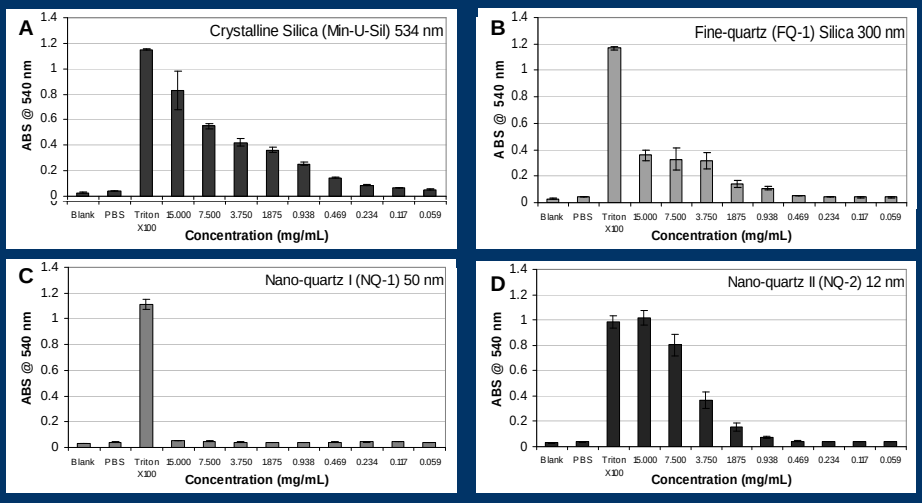
Dr. Dav d Warhe t
62 of 79

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



Dr. Dav d Warhe t
63 of 79

Hemolytic Potential of Quartz Samples (Surface Reactivity)



Dr. Dav d Warhe t
64 of 79

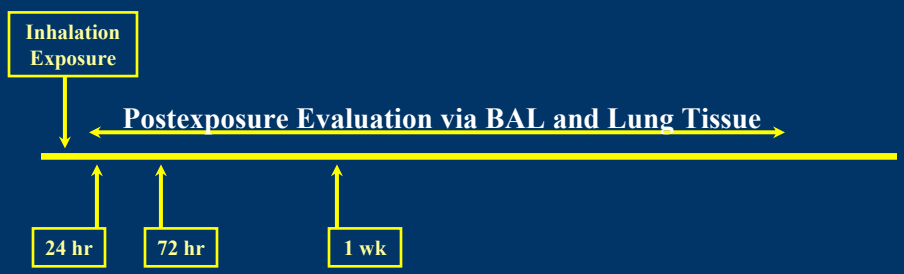
Summary of α-Quartz Results

Endpoint	Nano quartz I	Nano quartz II	Fine quartz	Min-U-Sil
Particle size	++	+	+++	++++
Surface area	+++	++++	++	+
Fe content	+++	++	+	++
Crystallinity	++++	++++	++++	++++
Radical content	+	++	+	+++
Hemolytic potential	+	+++	++	+++
Lung inflammation	++	+++	++	+++
Cytotoxicity	++	+++	+	+++
Airway BrdU	NA	++	+	++
Lung paren. BrdU	NA	++	+	++
Histopathology	NA	++++	++	+++

Dr. Dav d Warhe t
65 of 79

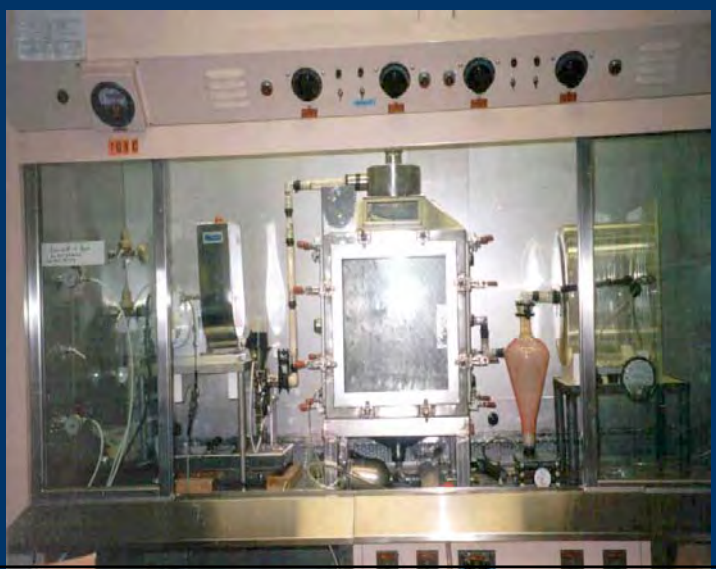
Protocol for Fine and Nanoscale ZnO Pulmonary Bioassay Studies

Inhalation Exposure at concs of 25, 35 or 50 mg/m^3 for 1 or 3 hours



Dr. Dav d Warhe t
66 of 79

Aerosol Generation Equipment and Set-up



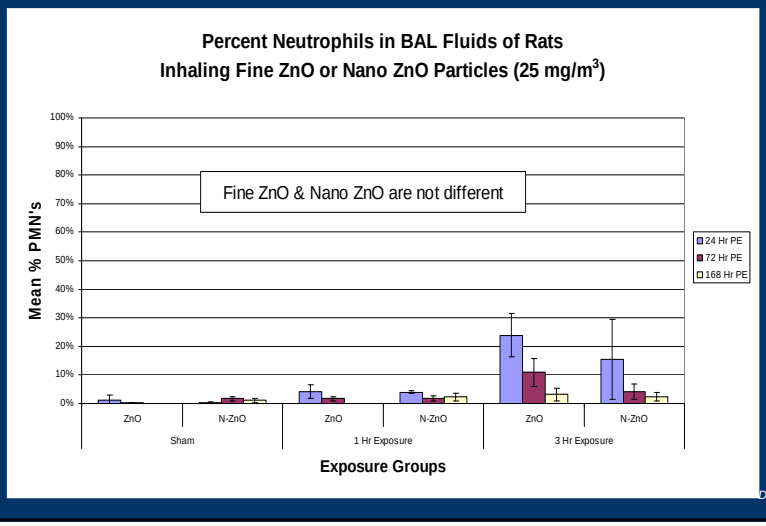
Dr. Dav d Warhe t
67 of 79

Mean Particle Size Determinations in the ZnO and MgO Inhalation Studies

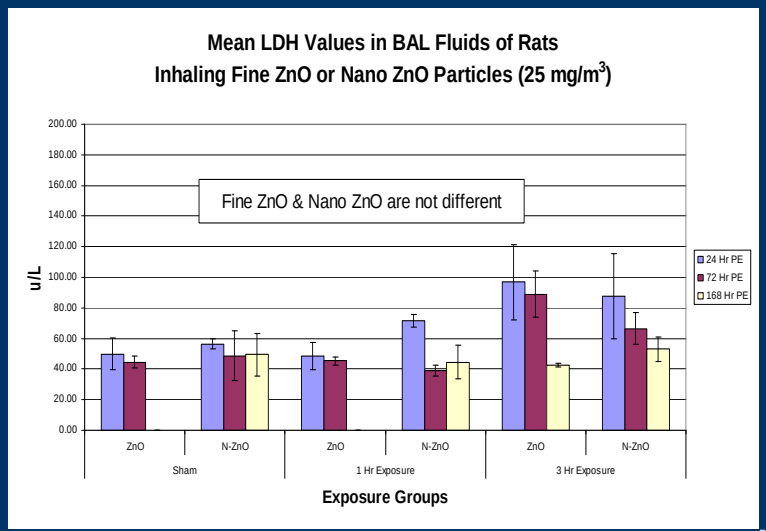
Study	MMAD (cascade impactor)
• ZnO 25 mg/m^3	3.3 µm
• ZnO 35 mg/m^3	2.7 – 3.2 µm
• ZnO 50 mg/m^3	3.2 µm
• MgO 50 mg/m^3	3.0 µm
• Nano ZnO 25 mg/m^3	2.8 µm

Dr. Dav d Warhe t
68 of 79

Preliminary Studies with Fine and Nano Zinc Oxide particles



Preliminary Studies with Fine and Nano Zinc Oxide particles



Impact of Surface Treatments/Coatings on TiO_2 Particles

- Inhalation Studies
- Pulmonary Bioassay Intratracheal Instillation Studies

Dr. Dav d Warhe t
71 of 79

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

Dr. Dav d Warhe t
72 of 79

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

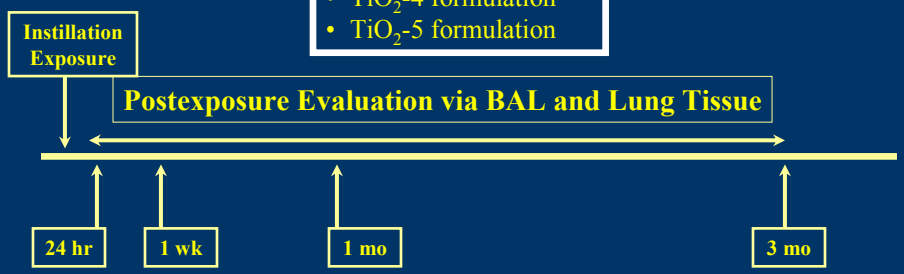
TiO2 V - 94% TiO2 - 3% alumina - 3% amorphous silica

Dr. Dav d Warhe t
73 of 79

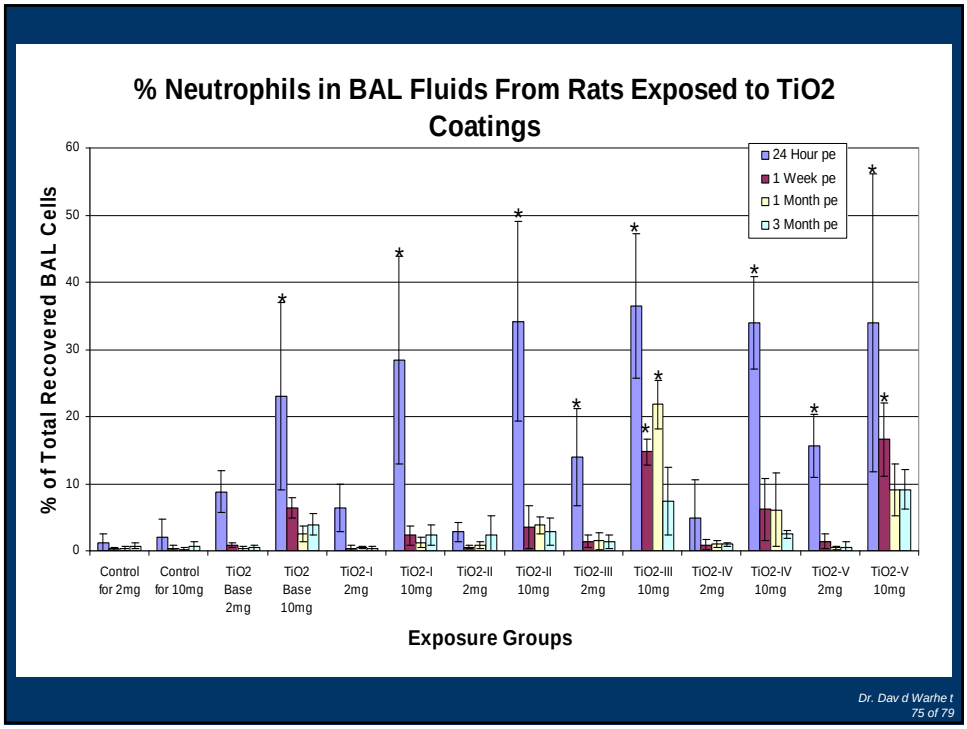
Protocol for TiO_2 Coatings Bioassay Study

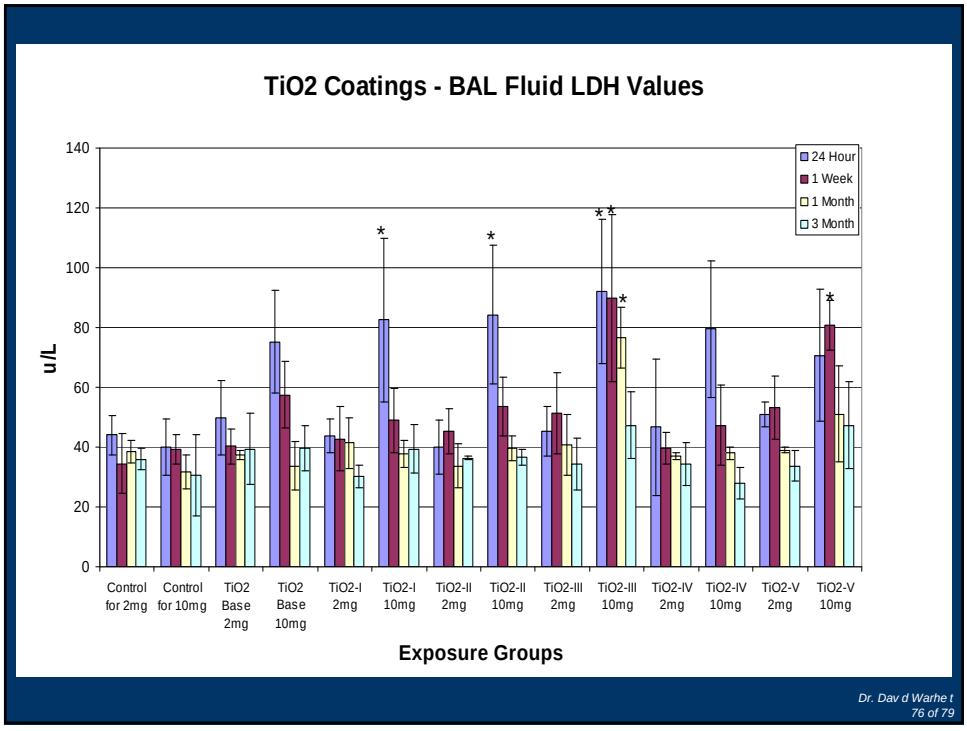
Instillation Study

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



Dr. Dav d Warhe t
74 of 79


% Neutrophils in BAL Fluids From Rats Exposed to TiO2 Coatings
% of Total Recovered BAL Cells
60
50
40
30
20
10
0
24 Hour pe
1 Week pe
1 Month pe
3 Month pe
Control for 2mg
Control for 10mg
TiO2 Base 2mg
TiO2 Base 10mg
TiO2-I 2mg
TiO2-I 10mg
TiO2-II 2mg
TiO2-II 10mg
TiO2-III 2mg
TiO2-III 10mg
TiO2-IV 2mg
TiO2-IV 10mg
TiO2-V 2mg
TiO2-V 10mg
Exposure Groups
Dr. David Warheit
75 of 79


TiO2 Coatings - BAL Fluid LDH Values
u/L
140
120
100
80
60
40
20
0
24 Hour
1 Week
1 Month
3 Month
Control for 2mg
Control for 10mg
TiO2 Base 2mg
TiO2 Base 10mg
TiO2-I 2mg
TiO2-I 10mg
TiO2-II 2mg
TiO2-II 10mg
TiO2-III 2mg
TiO2-III 10mg
TiO2-IV 2mg
TiO2-IV 10mg
TiO2-V 2mg
TiO2-V 10mg
Exposure Groups
Dr. David Warheit
76 of 79

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)

Dr. Dav d Warhe t
77 of 79

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

Dr. Dav d Warhe t
78 of 79

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.